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Poster

113. Postnatal Neurogenesis

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 113.01/A1

Topic: A.02. Postnatal Neurogenesis

Support: Neuroscience Institute

Title: Disruption of cerebellar development by intrauterine growth restriction

Authors: *I. ISKUSNYKH, N. FATTAHOV, R. BUDDINGTON, V. CHIZHIKOV;
Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Intrauterine growth restriction, a condition in which the fetus exhibits poor growth in utero, results in infants born small for gestational age (SGA infants). Most human intrauterine growth restriction cases are caused by placental insufficiency, and infants are born SGA because of sub-optimal delivery of the nutrients and oxygen to the fetus. Being born SGA is associated with impaired cognitive functions and fine motor skills, consistent with disrupted cerebellar development. Despite decades of research, the cellular and molecular mechanisms of cerebellar abnormalities associated with intrauterine growth restriction remain poorly understood. We explored how intrauterine growth restriction affects the development of the cerebellum using the pig as a clinically relevant large animal model and comparing cerebellar samples collected from newborn pigs that were small for gestational age (SGA pigs) and normal weight littermates. The cerebellum of SGA pigs was smaller compared to controls and histological and immunohistochemical analysis revealed an excessive accumulation of differentiating neurons in the cerebellar external granule cell layer (EGL), and particularly in the inner Tag1-positive region. The outer, Tag1-negative, EGL revealed little difference between SGA and control cerebella. While the outer EGL contains proliferating granule cell progenitors, the inner EGL contains granule neurons that exited the cell cycle and initiate migration to the internal granule cell layer (IGL). Thickening of the Tag1+ layer in SGA pigs suggests an accumulation of newly differentiated neurons due to disruption of migration. Using laser capture microdissection of the EGL and qRT-PCR analysis, we found that intrauterine growth restriction compromises expression of several genes that are essential for the radial migration of granule cells. Taken together, our data suggest that an impaired migration in the EGL contributes to disruption of cerebellar development and, as a result, may lead to the long-term motor and cognitive deficits associated with intrauterine growth restriction.

Disclosures: I. Iskusnykh: None. **N. Fattahov:** None. **R. Buddington:** None. **V. Chizhikov:** None.

Poster

113. Postnatal Neurogenesis

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 113.02/A2

Topic: A.02. Postnatal Neurogenesis

Support: Advanced Diagnostics & Therapeutics Discovery Award

Title: Passive physical forces in gyrification lead to characteristic cortical thickness variations

Authors: *M. HOLLAND[1], A. GORIELY[2], E. KUHL[3];
[1]Aerospace & Mechanical Engin., Univ. of Notre Dame, Notre Dame, IN; [2]Mathematical Inst., Univ. of Oxford, Oxford, United Kingdom; [3]Mechanical Engin., Stanford Univ., Stanford, CA

Abstract: While it was long thought that genetic and biochemical signals were solely responsible for the development of the brain, we now know that the brain is subject to the coupled effect of both biological and physical forces in gyrification as our brains' complex wrinkled structure develops. As neurons migrate to the outer layers of the cortex, they generate compressive stresses and, eventually, instabilities that lead to gyrification. We hypothesized that these instabilities generate mechanical asymmetries that lead to thickness variations in the cortex. While cortical thickness is tightly controlled between individuals, it varies markedly and consistently within individuals, with consistently thicker gyri and thinner sulci. In a multi-pronged study, we used medical image analysis, computational finite element simulations, and non-biological physical experiments to test whether biological heterogeneity is necessary to the formation of cortical thickness variations, or if these bifurcations can emerge naturally from the passive forces involved in mechanical instabilities. Using N=564 MRI scans of typically-developing individuals (ages 7-64 years) from the preprocessed Autism Brain Imaging Data Exchange (ABIDE), we considered sulcal and gyral regions separately and found mean thicknesses of 2.47 and 2.87mm, respectively ($p < 10^{-10}$). Additionally, we found that cortical thickness correlates inversely with curvature on the vertex level. In numerical finite element simulations of a thin growing film attached to an elastic substrate and in experiments involving thin polymer layers cured on prestretched polymeric substrates, the layers began with uniform thickness and then bifurcated into thick peaks and thin valleys. These results indicate that thickness variations similar to those seen in the human brain naturally emerge from the passive physical forces generated during instabilities and wrinkling. In addition to their histological characteristics, functional specializations, and axonal trajectories, gyri and sulci appear to differ in their mechanical state as well. Our work predicts that the ratio between gyral and sulcal thicknesses is more pronounced in soft tissues and in thicker layers. This study suggests that physical forces are an important contributer during the process of brain development, and raises

additional questions about their role in both health and neurological disorders, where cortical thickness is known to be affected.

Disclosures: M. Holland: None. **E. Kuhl:** None. **A. Goriely:** None.

Poster

113. Postnatal Neurogenesis

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 113.03/A3

Topic: A.02. Postnatal Neurogenesis

Support: CIHR

Title: Differential effects of neurostimulation therapy on adult hippocampal neurogenesis in animal models

Authors: *T. ZHANG, E. M. GUILHERME, A. KESICI, F. VILA-RODRIGUEZ, J. S. SNYDER;
Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Hippocampal neurogenesis has been shown to play a critical role for antidepressant effect, is negatively regulated by stress, and non-invasive neurostimulation therapy very potently increases neurogenesis. To confirm and further elucidate specific changes in hippocampal neurogenesis following stimulation therapies, we examined the timeline of neurogenesis after electroconvulsive shock (ECS), an animal model of electroconvulsive therapy (ECT), in mice, and compared with neurogenesis changes following repetitive transcranial magnetic stimulation (rTMS, 10 Hz) and intermittent theta burst stimulation (iTBS). These are the three most frequently clinically administered neurostimulation treatments for major depressive disorders. We first assessed new-born cell survival, proliferation, and maturation at 1, 3, or 7 days following a single session of ECS, rTMS, or iTBS neurostimulation. Bromodeoxyuridine (BrdU) was injected to label neurons born two days prior to neuronal stimulation to assess neuronal survival. We found that just one session of ECS increased the number of surviving cells significantly immediately starting on day 1, declining back to baseline on day 3. We saw similar changes with iTBS, but not rTMS. Cell proliferation levels, seen by examining PCNA, following ECS was similar to non-stimulated shams on day 1, peaked on day 3, and declined to basal levels on day 7. One session of iTBS and rTMS did not cause changes in cell proliferation. The data indicates that there is a delayed and transient increase in neuronal proliferation following ECS, and a loss of earlier-born cells that may be due to neuronal death to counteract the proliferating cell increase. For neuronal maturation, labelled by DCX, we did not see differences across days for all forms of neurostimulation.
Since neurostimulation is applied chronically in the clinic, we also investigated whether 10

sessions of iTBS impacted neurogenesis levels and new neuron morphology. Using an Ascl1-CreER mouse, we found that the morphology of adult-born tdTomato+ neurons was also not affected by subsequent chronic iTBS treatment, in terms of dendritic length and spines. Ongoing work is examining the effects of chronic neurostimulation on neurogenesis marker levels. Collectively, these results indicate that different neurostimulation paradigms recruit distinct aspects of the neurogenic process.

Disclosures: T. Zhang: None. **E.M. Guilherme:** None. **A. Kesici:** None. **F. Vila-Rodriguez:** None. **J.S. Snyder:** None.

Poster

113. Postnatal Neurogenesis

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Program #/Poster #: 113.04/A4

Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant R01NS085200
NIH Grant R01MH098003
NIH Grant RF1MH114224

Title: Investigating the development of neurovascular coupling in postnatal mice using fMRi

Authors: *X. HAN, H. UNSAL, N. ZHANG;
The Pennsylvania State Univ., State College, PA

Abstract: Blood Oxygen Level Dependent (BOLD) signal measured by fMRI provide an indirect interpretation of underlying neuron activity. This interpretation is tightly dependent on the neurovascular coupling relationship. We are interested in studying how neurovascular coupling develops in postnatal mice. Previous research in this line was mainly conducted in anesthetized or sedated animals which by themselves can alter the neurovascular relationship. In this study, we scanned awake mice at various ages from neonates to adulthood (P10-P60). Visual or whisker stimulation were used during the fMRI imaging to evoke sensory responses in animals. We compared the changes in BOLD responses at different ages. We found that the mice started to display positive BOLD response from P14, but not before that. During the developmental period to juvenile/adolescence, the animals showed a monotonic increase in BOLD amplitude and shorter response latency. Such results can provide important insight into understanding the development of neurovascular coupling in neonates.

Disclosures: X. Han: None. **H. Unsal:** None. **N. Zhang:** None.

Poster

113. Postnatal Neurogenesis

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Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 113.05/A5

Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant R01MH099114

Title: Development of GABAergic synapses onto adult born dentate granule neurons

Authors: *C. L. REMMERS, C. CASTILLON, A. CONTRACTOR;
Physiol., Northwestern Univ. Dept. of Physiol., Chicago, IL

Abstract: New neurons are continuously produced in the subgranular zone of the dentate gyrus of the hippocampus throughout life. These adult-born dentate granule cells (abDGCs) undergo a stereotyped process of morphological and functional maturation that recapitulates postnatal neuronal development. The inhibitory neurotransmitter GABA is critical for survival, morphological development, and functional maturation of abDGCs. However, little is known about the precise pattern of connectivity between abDGCs and local interneurons, the main source of GABA inputs onto abDGCs. In the present study we focused on two morphologically and chemically distinct populations of dentate interneurons: parvalbumin (PV) and somatostatin (SST) cells. PV interneurons are the most well-studied source of GABA input to abDGCs, but little is known about the development of inputs from SST interneurons onto abDGCs. Previous studies have proposed that GABA released from PV interneurons regulates adult neural stem cell quiescence, survival, and early morphological development of abDGCs. However, abDGCs receive input from multiple other interneuron classes whose function and timing of input remain unknown. This raises the possibility that inputs from specific interneuron populations differentially regulate abDGC maturation. Here we characterize the development of inputs from SST and PV interneurons onto abDGCs in the first four weeks after differentiation using retroviral birthdating combined with optogenetic activation of interneurons. We found that abDGCs receive input from PV interneurons in the first week after differentiation, but do not receive input from SST interneurons until the second week. We also found that the number of synapses from both PV and SST interneurons onto abDGCs increases as the cells mature. Prior studies have demonstrated that voluntary wheel running by mice increases adult hippocampal neurogenesis and improves performance on tasks that rely on this process. We find that voluntary wheel running increases the maximal GABAergic input to abDGCs from both PV and SST interneurons. We propose that the sequential pattern of GABAergic innervation to abDGCs is important to the proper development and integration of these neurons into the hippocampal circuit.

Disclosures: C.L. Remmers: None. **C. Castillon:** None. **A. Contractor:** None.

Poster

113. Postnatal Neurogenesis

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 113.06/A6

Topic: A.02. Postnatal Neurogenesis

Title: Developing a multipotent cell line from the adult mouse suprachiasmatic nucleus for studies of circadian rhythms and myelin formation

Authors: D. H. BELIGALA, A. DE, ***M. E. GEUSZ**;
Dept. of Biol. Sci., Bowling Green State Univ., Bowling Green, OH

Abstract: The suprachiasmatic nucleus (SCN) of the hypothalamus contains a circadian clock that serves an executive function in the circadian timing system of the body. Scattered throughout the SCN and other brain areas are oligodendrocyte progenitor cells (OPCs) with reported abilities to differentiate into neurons and glia. Because the SCN has many cells with characteristics of stem or progenitor cells an exploration of SCN OPCs might also provide insight into apparently undifferentiated SCN cells. OPCs are mitotically active and have an important role in ongoing myelination in the adult brain. One important question concerning fate determination during OPC differentiation is whether the neuronal phenotype it generates is determined by brain location. We used a defined medium to induce OPC proliferation in explant cultures of the SCN, resulting in monolayer cells that included 87% OPCs and suspended cells. The explant was then removed and a second medium was used to induce differentiation into neurons, which were identified by electrical impulses recorded with microelectrode arrays and neuron-specific proteins identified by immunocytochemistry. After differentiation, at least 42% of cells expressed vasoactive intestinal polypeptide (VIP), which is highly expressed in the SCN and suggests site-specific cell fate determination. Time-lapse imaging was used to determine whether the neurons were produced from cells with the OPC morphology rather than other possible progenitor cells in the cultures. In differentiating cultures, a subset of OPCs formed oligodendrocytes positive for myelin oligodendrocyte glycoprotein that appeared to initiate myelination of nascent neurons. These OPC cultures were passaged and expanded in culture repeatedly. OPC cell lines derived from adult SCN cultures may provide a consistent source of rhythmic cells that would enable simpler genetic manipulation of key mammalian clock genes. They may also provide an adequate supply of cells for characterizing low-abundance proteins serving in clock functions, myelination, and possible circadian regulation of OPC differentiation.

Disclosures: **M.E. Geusz:** None. **D.H. Beligala:** None. **A. De:** None.

Poster

113. Postnatal Neurogenesis

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 113.07/A7

Topic: A.02. Postnatal Neurogenesis

Support: Leverhulme Trust

Title: Combining electrophysiology and single-cell transcriptomics reveals a gradient of functional states amongst dopaminergic neurons

Authors: *M. LIPOVSEK[1], L. BROWNE[1], D. BYRNE[1], A. SINGH[1], B. AMEIN[1], I. MACAULAY[2], J. MILL[3], M. GRUBB[1];
[1]Ctr. For Developmental Neurobio., London, United Kingdom; [2]Earlham Inst., Norwich, United Kingdom; [3]Univ. of Exeter, Exeter, United Kingdom

Abstract: Dopaminergic (DA) neurons in the olfactory bulb are inhibitory interneurons that co-release dopamine and GABA to regulate the transmission of information at the earliest stages of sensory processing. The vast majority of bulbar DA neurons are of the anaxonic subtype, and are one of the few neuronal types in the mammalian brain that are continually generated throughout postnatal life. Here, we performed simultaneous electrophysiological recordings and single-cell RNA sequencing (patch-Seq), coupled with immunohistochemical and birth-dating approaches to ask whether this continuous neuronal production results in a gradient of cell states within the resident population of bulbar DA neurons. BrdU based birthdating in 4-week old DAT-IRES-Cre/Floxed-tdT mice revealed that resident DA neurons span an age range of at least 2 weeks. We next collected individual DA neurons by either manual sorting of tdT positive DA neurons, or aspiration after patch-clamp recordings in acute slices. We performed single-cell RNA sequencing using the Smart-Seq2 protocol, obtaining an average of 2.3 million 100bp paired-end reads per cell. Consensus clustering identified 3 putative subpopulations of DA neurons, while cell trajectory analysis identified a single, unbranched, trajectory that closely matched the clusters. Differential gene expression analysis along the identified trajectory revealed 680 differentially expressed genes. This gene set was significantly enriched for GO terms related to neuronal and synaptic function, indicating that the identified trajectory may reflect a maturational gradient of DA cell state. Ongoing analysis of electrophysiological properties along the identified trajectory will reveal whether it describes a gradient of functional states. Finally, combining EdU birth-dating with immunostaining for markers differentially expressed along the trajectory will assign a temporal identity to the single-cell transcriptional profiles. In summary, we have found a hitherto unanticipated gradient of cell state within a specific neuronal subtype, a gradient which could underpin the functional maturation of DA cells in the postnatal brain.

Disclosures: **M. Lipovsek:** None. **L. Browne:** None. **D. Byrne:** None. **A. Singh:** None. **B. Amein:** None. **I. Macaulay:** None. **J. Mill:** None. **M. Grubb:** None.

Poster

113. Postnatal Neurogenesis

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Program #/Poster #: 113.08/A8

Topic: A.02. Postnatal Neurogenesis

Support: BBSRC Grant BB/N014650/1

Title: Input and output connectivity of olfactory bulb dopaminergic neurons

Authors: *M. CHEAH, M. S. GRUBB;
Ctr. for Developmental Neurobio., King's Col. London, London, United Kingdom

Abstract: In the olfactory bulb glomerular layer, dopaminergic (DA) neurons function as inhibitory interneurons that co-release both GABA and dopamine to modulate nose-to-cortex signal processing. Our previous work has shown that embryonic and adult neurogenesis produce two distinct subtypes of DA neurons. The most striking morphological difference between these two subtypes is that the embryonically-generated DA neurons can have an axon, while the adult-generated ones never do. Here we investigated the potential for different connectivity of these two DA subtypes in terms of their synaptic input and output. We used $DAT^{Cre+/-}$ transgenic mice combined with microinjection techniques such as stereotaxic rostral migratory stream (RMS) injection in adult animals and *in utero* intraventricular injection in embryos, to sparsely label the two subtypes with Cre-dependent viral constructs. We utilized monosynaptic retrograde tracing with pseudotyped rabies virus (RV) to examine the different neuronal cell types that have direct synaptic input to these two subtypes. On top of revealing well-known input connections, such as from olfactory sensory neurons, RV tracing revealed yet-to-be-defined neurons both inside and outside of the olfactory bulb that have direct monosynaptic input to the DA neurons. To label putative synaptic output sites, we used a Cre-dependent virus overexpression synaptophysin fused to mRuby. We found that adult-generated axon-negative DA neurons contain a high density of dendritic synaptophysin puncta that co-localize with the vesicular GABA transporter (VGAT), consistent with their ability to perform dendritic release of neurotransmitters. In contrast, the dendrites of embryonically-generated axon-positive DA neurons contained low densities of putative transmitter release sites. However, synaptophysin puncta were clearly present in DA axon terminals, suggestive of a reduced utilization of dendritic release when axonal output connectivity is possible. By investigating how the same neuronal cell type born at different stages in life can have different connectivity within local circuits, this prompts the question of whether these DA neuron subtypes have fundamentally different roles in olfactory processing.

Disclosures: **M. Cheah:** None. **M.S. Grubb:** None.

Poster

113. Postnatal Neurogenesis

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

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Topic: A.02. Postnatal Neurogenesis

Support: FONDECYT 1190848
FONDECYT 1181243
CMA BIOBIO, PIA-Conicyt ECM-12

Title: Characterization of postnatal radial glia and correlation with vitamin C transporter, SVCT2

Authors: *N. SALDIVIA, K. SALAZAR MARTINEZ, F. J. NUALART;
Lab. of Neurobio. and Stem Cells, Neuro CellT, Ctr. for Advanced Microscopy CMA BIOBIO, Univ. de Concepción, Concepción, Chile

Abstract: Introduction: During brain development, the main stem cells of the cerebral cortex is the radial glia (RG), which remain after birth. In rodents, RG are present until the second week of life, maintaining their stem cell properties. Few studies have analyzed the cellular characteristics and molecules that regulate the function of these stem cells in post-natal stages. Here, we have analyzed the (i) expression of different proteins present in RG, (ii) expression of the vitamin C (Vc) transporters, SVCT2 and GLUT1, as Vc regulates pluripotency and differentiation mechanisms in stem cells and (iii) *in vivo* formation of neurons from RG and neuronal migration in the cerebral cortex.
Materials-Methods: Post-natal RG of rat brains from 1 to 20 postnatal days were analyzed by immunohistochemistry coupled to spectral confocal microscopy and two-photon microscopy. Scanning electron microscopy was used for general morphology analysis. *In situ* hybridization was performed to define SVCT2 expression in cerebral ventricular areas. We used laser microdissection to isolate different areas of the cerebral cortex and analyze SVCT2 expression by qrt-PCR. GFP adenoviral labeling was also used in post-natal stages to define normal RG distribution. PN1 rats were electroporated with a GFP- SVCT2 plasmid and analyzed at different days post-electroporation.
Results: Postnatal RG is highly present in the first postnatal week of brain development with polarized morphology. They have similar topographic morphology in the ventricular area as compared with embryonic RG. SVCT2 is expressed in post-natal RG during the first days of PN stages; however, its expression decreased as development decreases. After this period, the elongated morphology of RG transforms to ependymal cells with high GLUT1 expression. Adenovirus-GFP labeled lRGc and showed the presence of migratory neurons in the cerebral

cortex.
Discussion: RG have particular cellular properties; they express ventricularly polarized SVCT2 and form neurons that migrate through the cerebral cortex. SVCT2 expression is maintained in RG upon neuronal differentiation. Before the RG changes morphology and express classical markers, they lose the expression of SVCT2. Thus, Vc may regulate the expression of different genes (Nanog) that define the main functions of these cells.

Disclosures: **N. Saldivia:** None. **K. Salazar Martinez:** None. **F.J. Nualart:** None.

Poster

113. Postnatal Neurogenesis

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 113.10/DP01/A10

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: A.02. Postnatal Neurogenesis

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Grant of RFBR 17-29-01037

Title: Pseudo-4D visualization and analysis of cell division and migration in the whole brain

Authors: *A. LAZUTKIN[1,2,3,4], S. SHUVAEV[5], R. KIRYANOV[1], I. DORONIN[1], K. ANOKHIN[3,2], A. KOULAKOV[5], G. ENIKOLOPOV[4];
[1]Moscow Inst. of Physics and Technol., Moscow, Russian Federation; [2]P. K. Anokhin Res. Inst. of Normal Physiol., Moscow, Russian Federation; [3]Lomonosov Moscow State Univ., Moscow, Russian Federation; [4]Stony Brook Univ., Stony Brook, NY; [5]Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Complex patterns of cell division, migration, elimination, and differentiation define brain structure and function. Ability to image, quantify, and analyze dividing and migrating cells in the whole brain (3D) is critical for revealing the features that are not recognized on conventional flat sections. Moreover, a time series of 3D images (pseudo-4D) may uncover dynamics and hidden patterns of the processes that underlie brain development, aging, disease, and therapy. This holds especially true for the studies of neurogenesis both in the developing and the adult nervous system where neural stem and progenitor cells divide in restricted regions and migrate along intricate trajectories to reach distant areas of the brain. We developed a new histological technique for 3D imaging of proliferating cells in the whole brain of developing and adult mice, based on labeling the dividing cells with 5-ethynyl-2'-deoxyuridine (EdU) and detecting them with fluorescent azide using whole-mount click-reaction (WM-CLICK). We also

developed novel methods for automatic volume registration, cell counting, and morphing of 3D images for pseudo-4D data representation.We have now applied these techniques for visualizing patterns of cell division and migration in the early postnatal and adult mouse brain. We describe 3D patterns of division and migration of cells, most of them neural progenitors, and arrange a 3D time series into a pseudo-4D representation of cell division and migration in the perinatal brain. We also discovered three distinct proliferation/migration streams in the subventricular zone of the adult mouse brain - dorsolateral, dorsomedial and ventral, which merge together into a common rostral migration stream (RMS) and traced the 4D dynamics of their formation. Furthermore, we developed new computational algorithms to reveal the changes in the 3D patterns of cell division induced by pro- or anti-neurogenic factors, such as memantine and gamma-radiation, finding several brain areas affected by memantine treatment, including CA regions and dentate gyrus of the hippocampus, subcallosal zone, postpiriform transition area, and caudal piriform cortex. We also used WM-CLICK and computational algorithms to reveal the differences in early brain development of the wild type vs. autism model mice. Together, these examples demonstrate the utility of our approach for the quantitative and descriptive analysis of neurogenesis.

Disclosures: **A. Lazutkin:** None. **S. Shuvaev:** None. **R. Kiryanov:** None. **I. Doronin:** None. **K. Anokhin:** None. **A. Koulakov:** None. **G. Enikolopov:** None.

Poster

113. Postnatal Neurogenesis

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Topic: A.02. Postnatal Neurogenesis

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University of Connecticut Office of Undergraduate Research Summer Undergraduate Research Fund
University of Connecticut Office of Undergraduate Research Supply Award

Title: Developmental changes to the neural stem cell niche in fetal-onset hydrocephalus

Authors: ***S. KUMAR**[1], A. M. COLETTI[1], D. SINGH[1], M. R. DEL BIGIO[2], K. T. KAHLE[3], J. C. CONOVER[1];

[1]Physiol. & Neurobio., Univ. of Connecticut, Storrs, CT; [2]Dept of Pathology, Univ. of Manitoba, Winnipeg, MB, Canada; [3]Cell. and Mol. Physiol., Yale Univ. Sch. of Med., New Haven, CT

Abstract: Fetal-onset hydrocephalus is a common human birth defect affecting nearly 1 out of every 1000 live births and is characterized by abnormal expansion of the brain's ventricular system. The ventricular-subventricular zone (V-SVZ) stem cell niche, which lies subjacent to the brain's lateral ventricles, is likely implicated in cases of fetal-onset hydrocephalus. Multipotent V-SVZ stem cells are capable of producing neuron precursors and a variety of glia, including ependymal cells that provide epithelial-like functions for the ventricular system. Here, we evaluate the hypothesis that increased ventricular surface area in hydrocephalus increases demand for ependymal cell coverage. Thus, V-SVZ stem cell functions would shift toward ependymogenesis at the expense of neurogenesis - contributing to cognitive deficits experienced in hydrocephalus patients. Our group's novel approach to understanding development in hydrocephalus involves correlating volumetric and curvature data of ventricles from MRI segmentation with V-SVZ histology data from immunofluorescence. We have characterized normal human development of the lateral ventricles (n=125) and cellular organization of the V-SVZ (n=10) at time points from 20-gestational weeks to 10-years. We have shown that during normal brain growth and development: 1) human ependymogenesis proceeds in a posterior to anterior fashion, 2) human lateral ventricle ependymogenesis produces "pinwheel" cytoarchitecture similar to that in other mammals, and 3) lateral ventricle volume and surface area plateau around 1.5-years - when human ependymogenesis is completed. In a case study format, equivalent MRI and immunofluorescence analysis of fetal/postnatal hydrocephalus brains shows: 1) reduced ventricle-surface stem cell counts per unit area, 2) variable degrees of astrogliosis, and 3) extensive warping of the ventricular lining associated with surgical treatment via shunting. In fetal stages, hyperproliferative V-SVZ phenotypes have been observed. This study will provide researchers and clinicians with a foundation to develop enhanced tools for hydrocephalus diagnosis and treatment.

Disclosures: S. Kumar: None. **A.M. Coletti:** None. **D. Singh:** None. **M.R. Del Bigio:** None. **K.T. Kahle:** None. **J.C. Conover:** None.

Poster

113. Postnatal Neurogenesis

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Topic: A.02. Postnatal Neurogenesis

Support: NIH-DC013791
NIH-DC012441
Brown-Coxe Fellowship Yale University

Title: Axon extension and molecular maturation of olfactory sensory neurons in the young adult

Authors: *T. LIBERIA[1], D. J. RODRIGUEZ-GIL[2], E. MARTÍN LÓPEZ[1], S. J. MELLER[1], C. A. GREER[1];
[1]Neurosurg., Yale Univ. Sch. of Med., New Haven, CT; [2]East Tennessee State Univ., Johnson City, TN

Abstract: Mouse olfactory sensory neurons (OSNs) express only 1 of ~1200 types of olfactory receptors (ORs). Although OSNs expressing the same OR are widely distributed within the OE they project their axons specifically into 2-3 molecularly specific olfactory bub glomeruli. This highly organized topographic map must be maintained throughout the life due to the robust ongoing neurogenesis of OSNs. The olfactory system is not fully formed during embryogenesis and high levels of plasticity are evident during perinatal stages. Furthermore, the olfactory nerve is not fully formed during the first 3 postnatal weeks. Although numerous works have used the OE as a model to study neurogenic processes in perinatal stages, little has been done to understand the behavior of those OSNs produced in a mature environment, where the olfactory nerve is fully formed and the axons of new OSNs must navigate established axon tracts and innervate the appropriate glomerulus. In this study we used an *in vivo* fate-mapping strategy to track the spatiotemporal axonal extension of OSNs produced in 25-day-old (P25) mice. Moreover, we used BrdU pulse-labeling to analyze the radial migration of OSNs cell bodies throughout the OE and their molecular maturation beginning at basal cell division to establish a temporal correlation with their axon projection. Our results show that, once the olfactory nerve is fully formed, newborn OSNs require 8 days following basal cell division to sequentially transition from immature to mature OSNs. In parallel, OSNs generated in the young adult extend their axons gradually beginning 2 days following basal cell division and continuing for 8 more days until they innervate the glomerular neuropil 10 days after basal cell division. In summary, this study showed that the overall maturation process of the OSNs occurs sequentially in the young adult OE, once the olfactory system is completely developed.

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Poster

113. Postnatal Neurogenesis

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Program #/Poster #: 113.13/A13

Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant R01MH099114

Title: Kainate receptor regulation of the maturation of adult-born dentate granule cells

Authors: *Y. ZHU[1], A. CONTRACTOR[2];
[1]Physiol., Northwestern Univ. - Chicago, Chicago, IL; [2]Physiol., Northwestern Univ. Dept. of Physiol., Chicago, IL

Abstract: In the adult hippocampus of many mammalian species, populations of newborn dentate granule cells (GCs) are continuously generated and undergo subsequent activity-dependent neuronal maturation and incorporation into the preexisting hippocampal circuitry. Increasing evidence has demonstrated that these young adult-born GCs participate in numerous cognitive and affective processes such as pattern separation, acquisition and retrieval of hippocampal dependent memories, and stress responses. Therefore, the mechanisms that control maturation of adult-born GCs are of relevance to multiple neurocognitive processes. We have found that the GluK2 receptor subunit, a member of the kainate receptor subfamily of glutamate receptors, which are abundantly expressed on adult-born GCs, contributes to the activity-dependent maturation of this important neuronal population. To study the maturation of adult-born GCs we used retroviral birth-date labeling of dividing neural progenitors to track their development during a 2 to 6-week critical period. Using single cell patch clamp recordings, we compared the maturation of GCs in both constitutive GluK2 KO mice and after conditional GluK2 deletion restricted to the adult-born GCs. Functional measures were made including the intrinsic membrane properties, and both inhibitory and excitatory synaptic inputs at various time points after retroviral labeling. We found that these measures of neuronal maturity were consistent with a more rapid maturation of adult-born GCs after ablation of GluK2. Further, we found that this novel role for kainate receptors was mediated by an effect on GABA signaling, which is known to play a critical role in adult-born GC maturation. Finally, using an inducible knockout strategy, we timed GluK2 ablation in adult-born neurons and found that mice had significant deficits in spatial memory tasks known to be dependent on adult-born neurons. In summary, our studies have revealed a novel role of kainate receptors in regulating the maturation of adult-born GCs and consequent adult-neurogenesis dependent cognitive behaviors.

Disclosures: Y. Zhu: None. **A. Contractor:** None.

Poster

113. Postnatal Neurogenesis

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 113.14/A14

Topic: A.02. Postnatal Neurogenesis

Support: NSF GRFP DGE-1656466
NIH Grant MH117459-01

Title: Adult-generated neurons in the dentate gyrus preferentially innervate and affect interneurons surrounded by perineuronal nets

Authors: *B. A. BRIONES[1], T. J. PISANO[1], A. E. HAYE[1], E. J. DIETHORN[1], M. N. PITCHER[1], E. A. TAWA[1], M. J. LOTITO[1], H. A. CAMERON[2], E. GOULD[1];
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Abstract: Adult neurogenesis in the mammalian hippocampus is a dynamic process during which newborn neurons integrate into the preexisting circuitry in an ongoing basis. Adult-generated granule cells in the dentate gyrus extend mossy fiber axons, which are known to form connections with hilar interneurons, mossy cells, and pyramidal neurons of the CA2 and CA3 regions. Studies have shown that a portion of hilar parvalbumin-positive (PV+) interneurons are enwrapped in perineuronal nets (PNNs), specialized extracellular matrix structures known to control plasticty in other systems. We used GFP retrovirus labeling and 3R-Tau immunolabeling to investigate mossy fibers emanating from adult-generated neurons and determine whether they form contacts with PV+PNN+ hilar interneurons. Using high-resolution confocal microscopy and Wisteria floribunda agglutinin labeling, we quantitatively analyzed PNNs associated with PV+ interneurons and compared mossy fiber and bouton numbers across PV+ cells with and without PNNs (PV+PNN+ vs. PV+PNN-), as well as across various PNN intensities. Our data suggest consistently greater mossy fiber and bouton number associated with PV+ cells that had higher PNN intensity. Furthermore, when comparing neighboring PV+PNN+ and PV+PNN- interneurons we found a similar relationship—PV+PNN+ interneurons had more mossy fiber boutons than PV+PNN- interneurons. We also explored whether the presence of new neurons in the dentate gyrus had an influence on PNN expression using GFAP-TK mice in which adult neurogenesis was blocked, and found a decrease in hilar PNN expression around PV+PNN+ interneurons compared to age matched wild-type mice. These results indicate that new neurons preferentially innervate PV+ interneurons surrounded by PNNs and that their activity may further increase PNN expression around these cells.

Disclosures: B.A. Briones: None. **T.J. Pisano:** None. **A.E. Haye:** None. **E.J. Diethorn:** None. **M.N. Pitcher:** None. **E.A. Tawa:** None. **M.J. Lotito:** None. **H.A. Cameron:** None. **E. Gould:** None.

Poster

113. Postnatal Neurogenesis

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

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Topic: A.02. Postnatal Neurogenesis

Support: Funding Program for Next Generation World-Leading Researchers
MEXT/JSPS KAKENHI
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The Takeda Science Foundation

Title: Fyn controls detachment of chain-forming neuroblasts by regulating cell cell adhesion in the postnatal brain

Authors: *K. FUJIKAKE[1,2], M. SAWADA[2], T. HIKITA[2], Y. SETO[2], N. KANEKO[2], V. HERRANZ-PEREZ[3], N. DOHI[2], N. Y. HOMMA[2], S. OSAGA[4], Y. YANAGAWA[5], T. AKAIKE[6], J. MANUEL GARCIA-VERDUGO[3], M. HATTORI[7], K. SOBUE[1], K. SAWAMOTO[2];
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Abstract: In the postnatal brain, neuroblasts are continuously generated from neural stem cells in the ventricular-subventricular zone (V-SVZ). Neuroblasts generated in the V-SVZ form chain-like cell aggregates and migrate toward the olfactory bulb (OB) through the rostral migratory stream (RMS). After reaching the OB, neuroblasts are dissociated from the neuronal chains and start to migrate individually and radially toward their final destination. In this process, the transition from the chain to individual neuron migration is believed to be critical for maintaining the OB structure and function. However, the cellular and molecular mechanisms controlling cell-cell adhesion during this detachment remain unknown. Here, we report the function of Fyn, a nonreceptor tyrosine kinase, in the detachment of neuroblasts from chains in the male and female mouse OB. First, we performed a chemical screen in in vitro cultures of V-SVZ-derived migrating neuroblasts. Of 287 target-known chemical inhibitors, we found that PP2,an Src family tyrosine kinase (SFK) inhibitor, prevented the detachment of neuroblasts from chains in vitro. By a combination of immunohistochemical analysis and in vivo loss- and gain-of-function experiments, we found that Fyn, a member of the SFK, promotes detachment of neuroblasts from the chains, and is involved in neuronal migration from the RMS into the OB. We further show that Fyn and Dab1 (disabled-1) decrease the cell-cell adhesion between chain-forming neuroblasts, which involves adherens junction-like structures. Furthermore, Fyn-mediated promotion of detachment of neuroblasts from chains was canceled by Dab1 deficiency. These results indicate that Dab1 is involved in the Fyn-induced promotion of neuroblast detachment and migration in the OB. We lastly performed Fyn and N-cadherin double-KD experiments. N-cadherin KD rescued the Fyn KD induced suppression of the detachment of neuroblasts, in the double-KD condition.This result indicate that Fyn regulates the N-cadherin-mediated cell adhesion between neuroblasts. Together, our results suggest that Fyn-mediated regulation of the cell-cell adhesion of neuroblasts is critical for their detachment from chains in the postnatal OB.

Disclosures: **K. Fujikake:** None. **M. Sawada:** None. **T. Hikita:** None. **Y. Seto:** None. **N. Kaneko:** None. **V. Herranz-Perez:** None. **N. Dohi:** None. **N.Y. Homma:** None. **S. Osaga:** None. **Y. Yanagawa:** None. **T. Akaike:** None. **J. Manuel Garcia-Verdugo:** None. **M. Hattori:** None. **K. Sobue:** None. **K. Sawamoto:** None.

Poster

113. Postnatal Neurogenesis

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 113.16/A16

Topic: A.02. Postnatal Neurogenesis

Support: University of Notre Dame

Title: Epigenetic remodeling of postnatal neurogenesis and hippocampal function in a mouse model of intellectual disability

Authors: ***M. ALAM**, A. MYSORERAJASHEKARA, K. LEE, V. SANDERS, K. HALDAR; Biol. Sci., Univ. of Notre Dame, Notre Dame, IN

Abstract: Post-natal neurogenesis occurs throughout life in the subventricular zone (SVZ) and subgranular zone (SGZ) of the dentate gyrus (DG) of hippocampus in mouse models. Loss of neurogenesis in the hippocampus affects cognition, memory and intellectual ability, but mechanisms that sustain neurogenesis remain elusive. Kabuki syndrome (KS) is associated with varying degrees of intellectual disability. Around 80% of the cases are caused by the heterozygous loss-of-function mutations in an epigenetic modifier histone lysine-specific methyltransferase 2D (*KMT2D*). A mouse model carrying a heterozygous mutation in *Kmt2d* shows reduced histone H3 lysine 4 (H3K4) trimethylation, decreased hippocampal neurogenesis as well as a deficit in somatosensory function and learning and memory. We restored trimethylation in the $Kmt2d^{+/-}$ mouse brain to concomitantly improve nociception, learning, and memory, as measured by two independent neurobehavioral assays. Deficit in *Kmt2d* affects cellular metabolism and $Kmt2d^{+/-}$ mice show suppression of stem cells, neural progenitors and newborn young neurons in the SGZ of the hippocampal DG. Enhancement of trimethylation in the brain revealed heterogeneous but overall higher levels of doublecortin (DCX) positive newborn young neurons. Our analyses yield an understanding of the relationship between chromatin structure, metabolism and the induction and progression of neurogenesis from stem cells to mature neurons in the SGZ of the DG of the hippocampus. The studies provide key insights into mechanisms by which epigenetic factors control neurogenesis and develop strategies to stimulate neurogenesis and treat intellectual disability in KS as well as more prevalent neurological conditions.

Disclosures: **M. Alam:** None. **A. MysoreRajashekara:** None. **K. Lee:** None. **V. Sanders:** None. **K. Haldar:** None.

Poster

113. Postnatal Neurogenesis

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 113.17/A17

Topic: A.01. Neurogenesis and Gliogenesis

Support: 2018YFA0108000
31872763

Title: Precise positioning of neural progenitors is essential for neocortical development

Authors: ***Y. XIE**, T. TANG, Y. ZHANG, Y. WANG;
Fudan Univ., Shanghai, China

Abstract: Neural progenitors with distinct potential in generating progeny are associated with a spatially distinct microenvironment. Neocortical intermediate progenitors (IPs) located in the subventricular zone (SVZ) of the developing brain generate neurons for all cortical layers and are essential for cortical expansion. We show that spatial control of neural progenitor positioning is essential for neocortical development. We demonstrate that the positioning of IPs is epigenetically regulated by histone deacetylatases in a developmental stage specific manner. In mutant brains, mispositioned neural progenitors are located at the ventricular surface where they divide and differentiate into neurons, thereby leading to the cortical malformation. Our results demonstrate the importance of the spatial positioning of neural progenitors in cortical development and reveal a mechanism underlying the establishment of the SVZ microenvironment.

Disclosures: **Y. Xie:** None. **T. Tang:** None. **Y. Zhang:** None. **Y. Wang:** None.

Poster

113. Postnatal Neurogenesis

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 113.18/A18

Topic: A.01. Neurogenesis and Gliogenesis

Support: ZPNSF Grant LR17C090001

Title: Lin28a is involved in Wnt-dependent regulation of neurogenesis in the adult hippocampus

Authors: *Z. HU[1,2], Y. GU[1,2];
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Abstract: Adult hippocampal neurogenesis is regulated by Wnt ligands and antagonists within the local microenvironment. It remains obscure how neural stem cells and newborn neurons respond to Wnt signals in the neurogenic niche. Lin28a is a conserved RNA binding protein, playing important roles in regulating cellular metabolism of various types of cells. We found that Lin28a remains existent in neural stem cells and granule neurons in the adult dentate gyrus. Conditional knock-out of Lin28a in the hippocampal neural stem cells impairs neurogenesis and the responsiveness of neural stem cells to Wnt3a, a Wnt ligand in the niche. Over-expression of Lin28a increases proliferation of neural stem cells, and promotes the development of newborn neurons. Moreover, over-expression of Lin28a in neural stem cells in the dentate gyrus enhances hippocampal neurogenesis, resulting in improved pattern separation. Our study suggests Lin28a regulates hippocampal neurogenesis by responding to niche Wnt signals.

Disclosures: Z. Hu: None. **Y. Gu:** None.

Poster

113. Postnatal Neurogenesis

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 113.19/A19

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant DK54733
NIH Grant DK60521

Title: Cellular retinoic acid binding protein 1 modulates stem cell proliferation to affect learning and memory in male mice

Authors: *Y.-L. LIN[1], S. D. PERSAUD[1], J. NHIEU[1], L.-N. WEI[2];
[1]Dept. of Pharmacol., Univ. of Minnesota, Minneapolis, MN; [2]Dept. of Pharmacol., Univ. of Minneapolis, Minneapolis, MN

Abstract: Retinoic acid (RA) is the active ingredient of vitamin A. It exerts its canonical activity by binding to nuclear RA receptors (RARs) to regulate gene expression. Increasingly, RA is also known to elicit nongenomic RAR-independent activities, most widely detected in activating extracellular regulated kinase (ERK)1/2. This study validated the functional role of cellular

retinoic acid-binding protein 1 (Crabp1) in mediating nongenomic activity in RA, specifically activating ERK1/2 to rapidly augment the cell cycle by expanding the growth 1 phase and slowing down embryonic stem cell and neural stem cell (NSC) proliferation. The study further uncovered the physiological activity of Crabp1 in modulating NSC proliferation and animal behavior. In the Crabp1 knockout mouse hippocampus, where Crabp1 is otherwise detected in the subgranular zone, neurogenesis and NSC proliferation increased and hippocampus-dependent brain functions such as learning and memory correspondingly improved. This study established the physiological role of Crabp1 in modulating stem cell proliferation and hippocampus-dependent brain activities such as learning and memory.

Disclosures: **Y. Lin:** None. **S.D. Persaud:** None. **J. Nhieu:** None. **L. Wei:** None.

Poster

113. Postnatal Neurogenesis

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 113.20/A20

Topic: A.01. Neurogenesis and Gliogenesis

Support: NMSS RR-1512-07066
FISM 2015/R/13
1U54HD090257

Title: Sox17 regulates notch during the development and regeneration of oligodendrocyte cells

Authors: *L.-J. CHEW[1], X. MING[1], B. MCELLIN[1], J. L. DUPREE[2], E. HONG[1], M. CATRON[1], M. FAUVEAU[3], B. NAIT-OUMESMAR[3], V. GALLO[1];
[1]Ctr. for Neurosci Res., Children's Natl. Med. Ctr., Washington DC, DC; [2]Virginia Commonwealth Univ., Richmond, VA; [3]Inst. du Cerveau et de la Moelle epiniere, Sorbonne Univ., Paris, France

Abstract: Sox17 is, to date, the only member of the Sox F family of factors to have a demonstrated role in neural cell development. To understand its function in the development and spontaneous repair of oligodendrocytes in the subcortical white matter (WM), we established a novel floxed Sox17 mouse to characterize the effects of CNPCre-(CKO) and PDGFRaCreERT2-(PCKO) targeted Sox17 ablation. Postnatal oligodendrogenesis in subcortical WM of both Sox17-deficient strains was altered in biphasic fashion: first transiently increased at postnatal day 18 (P18), then decreased by P30, with increased cell death and reduced Olig2, CC1 and MAG-expressing OLs. P60 Sox17 CKO showed thin myelin and deficits in motor coordination. Decreased WM densities of proliferating NG2+ progenitors at P18 was followed by recovery at P30. Oligodendroglial response after lysolecithin-induced focal demyelination in P60 adults was also deficient in both Sox17 mutant strains, showing attenuated NG2 cell expansion at 7 days

post lesion (7DPL). The NG2 cell reduction in CKO was accompanied by increased cells expressing TCF7L2 at 7DPL, indicating differentiation. However, TCF7L2 cells were later reduced at 10 DPL. This pattern is also observed in developing WM. Sox17 ablation significantly reduced the numbers of cells expressing Activated Notch1 (ActN1/NICD) and Hes gene targets in developing WM. In cultured oligodendrocyte progenitor cells, Sox17 siRNA decreased ActN1 protein and Notch1 RNA. siRNA silencing of Sox17 and Notch1, and pharmacological gamma-secretase inhibition significantly decreased TCF7L2 expression, indicating shared targets between Sox17 and Notch1 in the control of progenitor cell development. Despite decreasing TCF7L2 RNA levels, gamma-secretase inhibition increased progenitor differentiation into TCF7L2+ and O4+ pre-myelinating oligodendrocytes in culture. Gel-shift assays using either P12 WM lysate or cultured oligodendrocyte progenitor cell nuclear extracts revealed a specific Sox17-containing complex that bound a Notch1 enhancer probe, suggesting possible direct Sox17 regulation of Notch1 expression. Injection of gamma-secretase inhibitor into adult WM of Sox17-overexpressing transgenic mice led to reduction of its enhanced NG2 cell population, indicating intrinsic Notch-dependent progenitor signaling by Sox17. These studies reveal a previously uncharacterized expansion function for Sox17 in CNS progenitors, with subsequent impact on oligodendrocyte differentiation and accumulation.

Disclosures: **L. Chew:** None. **X. Ming:** None. **B. McEllin:** None. **J.L. Dupree:** None. **E. Hong:** None. **M. Catron:** None. **M. Fauveau:** None. **B. Nait-Oumesmar:** None. **V. Gallo:** None.

Poster

113. Postnatal Neurogenesis

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 113.21/A21

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant R01DK108230

Title: Control of proliferation and neural competence in hypothalamic tanycytes

Authors: *S. YOO, S. BLACKSHAW;
Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Tanycytes are specialized glia cells residing in the mediobasal hypothalamus, which have been proposed to function as neural progenitors in adult animals. However, the mechanisms that regulate tanycyte proliferative and neurogenic competence are lacking. Here, we report the effects of conditional ablation of hypothalamic tanycytes using *RaxCreER;Eno2-lsl-DTA* mice. We show that tamoxifen-induced Cre activation leads to a selective and dose-dependent ablation of tanycytes. Short-term expose to tamoxifen selectively ablates beta2 tanycytes of the median

eminence, while prolonged tamoxifen exposure efficiently kills alpha2, beta1 and beta2 tanycytes. Ablation of beta2 tanycytes leads to a rapid, compensatory increase in proliferation of surrounding tanycytes, that results in full regeneration of the ablated tanycytes. In contrast, loss of alpha2 tanycytes prevents tanycyte regeneration. This also leads to a transient increase in the number of tanycyte-derived neurons in hypothalamic parenchyma, although these cells do not label with BrdU. In parallel, we also generated tanycyte-specific deletion of the NFI class transcription factors *Nfia*, *Nfib*, and *Nfix*. This resulted in a rapid induction of proliferation in all classes of tanycytes, and led to high levels of neurogenesis in alpha tanycytes. Tanycyte-derived HuC/D+ immature neuronal precursors migrated into the dorsomedial and ventromedial hypothalamus, eventually maturing and expressing NeuN. These data demonstrate the existence of quorum signaling that acts to maintain correct numbers of tanycytes in adult hypothalamus, and establish NFI factors as key negative regulators of proliferative and neurogenic competence in tanycytes.

Disclosures: S. Yoo: None. **S. Blackshaw:** None.

Poster

113. Postnatal Neurogenesis

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 113.22/A22

Topic: A.01. Neurogenesis and Gliogenesis

Support: F32CA168330
K22NS092767
R01 NS032817
CPRIT RP130464

Title: ASCL1 regulates neurodevelopmental transcription factors and cell cycle genes in glioblastoma

Authors: *T. VUE[1], R. K. KOLLIPARA[2], M. D. BORROMEO[2], T. SMITH[3], T. MASHIMO[4], D. K. BURNS[5], R. M. BACHOO[4], J. E. JOHNSON[3];
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Abstract: Glioblastoma (GBM) are highly aggressive brain tumors with high degree of cellular heterogeneity and genetic mutations that contribute to their poor prognosis. Remarkably, critical transcription factors that normally regulate glial-lineage development are aberrantly co-expressed in GBM, conferring cancer stem-like properties which may be responsible for driving tumor progression and therapeutic resistance. The functional role of individual transcription factors in GBMs *in vivo* remains elusive. Here, using patient-derived-xenograft (PDX) GBMs,

we demonstrate that Achaete-scute homolog 1 (ASCL1), a bHLH transcription factor, binds to a host of transcriptional targets that are central to GBM development and progression, which include neural stem cell and glial transcription factors (NFIA, OLIG2, SOX2, SOX10), oncogenic intracellular signaling molecules, chromatin modifying genes, and cell cycle and mitotic genes. We also show that the loss of ASCL1 significantly reduces the proliferation of GBMs *in vivo* in a murine glioma model, resulting in an extended survival for these animals. RNA-seq analysis of the mouse GBM tumors reveal that the loss of ASCL1 is associated with down-regulation of cell cycle genes, complementing the findings in the PDX-GBMs and illustrating an important role for ASCL1 in controlling the proliferation of GBMs.

Disclosures: T. Vue: None. **R.K. Kollipara:** None. **M.D. Borromeo:** None. **T. Smith:** None. **T. Mashimo:** None. **D.K. Burns:** None. **R.M. Bachoo:** None. **J.E. Johnson:** None.

Poster

113. Postnatal Neurogenesis

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 113.23/A23

Topic: A.01. Neurogenesis and Gliogenesis

Support: NSFC31271123

Title: Pen-2 is essential for the maintenance of neural stem cells in the developing neocortex

Authors: *G. CHEN, S. CHENG, T. LIU;
Nanjing Univ., Nanjing, China

Abstract: Presenilin enhancer 2 (Pen-2) is a key subunit of γ-secretase and has been implicated in neurodevelopmental disease. However, it remains unknown whether Pen-2 is required for the maintenance of neural stem cells (NSCs) and cortical development. To address this question, we generated *Pen-2* conditional knockout (cKO) mice in which Pen-2 is specifically inactivated in neural progenitor cells (NPCs) in the dorsal telencephalon. We find that *Pen-2* cKO cortices display remarkable depletion of radial glial progenitors (RGPs) but transiently increased number of intermediate progenitors (IPs) as compared to controls. We demonstrate that the proliferation rate of RGPs and IPs is not changed in *Pen-2* cKO cortices. Molecular analyses reveal decreased levels of Hes1 and Hes5 in *Pen-2* KO cells. We show that reintroduction of Notch1 intracellular domain (NICD) into *Pen-2* cKO cortices restores the population of RGPs/IPs but not cortical morphology. Taken together, these findings suggest that Pen-2 regulates the maintenance of NSCs in the developing neocortex in a Notch-dependent mechanism.

Disclosures: G. Chen: None. **S. Cheng:** None. **T. Liu:** None.

Poster

113. Postnatal Neurogenesis

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 113.24/A24

Topic: A.01. Neurogenesis and Gliogenesis

Support: Graduate School Of Life Sciences,Wuerzburg PhD fellowship
DFG - BL567/3-2
DFG - TRR58 A10

Title: Intracellular auto-activation of Trk receptors modulates actin dynamics via focal adhesion kinase

Authors: *R. GUPTA[1], V. LUZAK[1], V. WEGAT[1], G. LANGLHOFER[1], B. WACHTER[1], P. LÜNINGSCHRÖR[1], J. KUPER[2], C. MONORANU[3], M. SENDTNER[1], R. BLUM[1];
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Abstract: Receptor tyrosine kinase B (TrkB), the receptor for the neurotrophin brain-derived neurotrophic factor (BDNF), plays an important role in neuronal survival, differentiation and plasticity. Conventionally, TrkB activation is induced by dimerization of receptor monomers on binding of BDNF at extracellular sites. The intracellular activation domain of TrkB consists of a tyrosine kinase core, with three tyrosine (Y) residues at positions 701, 705 and 706, that catalyses the phosphorylation reaction between ATPγ and tyrosine. The release of cis-autoinhibition of the kinase domain and its subsequent auto-phosphorylation then phosphorylates tyrosine residues outside of the catalytic domain. Phosphorylation of Y515 creates the binding site for Shc (Src homologous and collagen-like protein) and phosphorylated Y816 serves as the adaptor site for Phospholipase C (PLCγ). Initially, when investigating the role of the Y705 residue in TrkB transactivation, we observed that while on the one hand, growth factor depletion after overexpression of TrkB was sufficient to reduce TrkB downstream signaling, it also increased TrkB phosphorylation. Therefore, we cloned multiple TrkB phosphorylation mutants and found that a high, local abundance of the receptor is sufficient to activate TrkB in a ligand-independent manner. This autoactivation of TrkB is blocked when either the ATP-binding site or Tyr705 in the core domain is mutated. Confocal analysis of overexpressed TrkB mutants revealed that the vast majority of phosphorylated TrkB, in the absence of a ligand, is found at intracellular locations and was preferentially seen in roundish cells, lacking filopodia. Therefore we performed live cell imaging of actin dynamics and saw that autoactive TrkB changed the cellular morphology by reducing actin filopodia formation. Surprisingly, this process was fully reversible when treated with K252a, a known inhibitor of TrkB kinase activity and was not seen in TrkB Y705F mutants. Signaling cascade analysis confirmed that autoactive TrkB is a

powerful activator of Focal Adhesion Kinase (FAK) and therefore disrupts actin filopodia formation. This signaling axis from Y705 to FAK can be mimicked by expression of the soluble, cytosolic TrkB kinase domain and is not active when the TrkB kinase domain becomes membrane bound by artificial membrane anchors. The biological function of the signaling pathway is not clear yet, but recent research on the protumorigenic function of NTRK2 lets us assume that 'intracellular autoactivation by abundance' might contribute to the protumorigenic and promigratory function of Trk receptors.

Disclosures: **R. Gupta:** None. **V. Luzak:** None. **V. Wegat:** None. **G. Langlhofer:** None. **B. Wachter:** None. **P. Lüningschrör:** None. **J. Kuper:** None. **C. Monoranu:** None. **M. Sendtner:** None. **R. Blum:** None.

Poster

113. Postnatal Neurogenesis

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 113.25/A25

Topic: A.01. Neurogenesis and Gliogenesis

Title: The function of diazepam binding inhibitor during development of the telencephalon

Authors: *I. EVERLIEN[1], J. ALFONSO[2], H. MONYER[3];
[1]Clin. Neurobio., Universitätsklinikum Heidelberg, Heidelberg, Germany; [2]Clin. Neurobio., DKFZ, Heidelberg, Germany; [3]Clin. Neurobio. A230, Med. Fac. of Univ. Heidelberg & DKFZ, Heidelberg, Germany

Abstract: The small molecule diazepam binding inhibitor (DBI) is a versatile regulator of adult brain functions. It is a key housekeeping gene in astrocytes throughout the mature brain where it is involved in fatty acid transportation as well as the production of neurosteroids to fine-tune synaptic transmission. Studies of recent years also identified DBI as a key modulator of GABAergic effects on neural progenitor cell populations of the adult stem cell niches. Gamma-amino-butyric-acid (GABA) is an important modulator of many processes during central nervous system (CNS) development. It acts tonically on progenitor cells in the telencephalic ventricular (VZ) and subventricular zone (SVZ) as well as on immature neurons, regulating various processes including progenitor cell proliferation and neuronal migration even before the formation of the first chemical synapses. Coinsidering the multiple pathways by which DBI exerts its diverse functions in the adult brain, raises the pressing question as to its role during the development of the telencephalon.
Here we investigated the functions of DBI during telencephalic development in vivo. We used in utero genetic manipulations in mice to alter the expression of DBI from the onset of neurogenesis and analysed different features of brain development. We found that DBI

expression shows regional heterogeneity, it is restricted to progenitor cells during development and affects developmental trajectories of the generated progeny.

Disclosures: I. Everlien: None. **H. Monyer:** None. **J. Alfonso:** None.

Poster

113. Postnatal Neurogenesis

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 113.26/A26

Topic: A.03. Stem Cells and Reprogramming

Support: Alana Foundation
Burroughs Wellcome Fund
LuMind Foundation
UNCF/Merck

Title: Altered 3D-Genome Architecture of Neural Progenitor Cells as a Consequence of Down Syndrome

Authors: *H. MEHARENA[1], L.-H. TSAI[2];
[1]MIT, Cambridge, MA; [2]The Picower Inst. for Learning and Memory, MIT, Cambridge, MA

Abstract: The precise epigenomic regulation of the transcriptome plays an integral role in the regulation of brain development and function. Epigenetic modifications of histones and DNA determine the way chromatin is packaged in the nuclei, in-turn the chromatin architecture regulates gene-expression through long-range interactions. We and others have shown that the epigenome plays a pivotal role in the process of memory and learning and its dysregulation has been implicated in numerous human diseases. Moreover, recent technological advances allow the interrogation of the three-dimensional (3D) nuclear architecture (Hi-C) and have revealed that A/B compartments, topologically associating domains (TADs), and long range interactions play an integral role during neurodevelopment and abrogation of the nuclear architecture has been linked to intellectual disability. Furthermore, recent whole-genome transcriptional profiling of various tissue samples from individuals with Down Syndrome (DS) as well as DS-mouse models have revealed that T21 induces genome-wide transcriptional disruption in addition to the dosage dependent up-regulation of the genes on chromosome 21 (HSA21). We observe that these transcriptional alterations are associated with 3D-genome architecture reorganization as a consequence of DS.

Disclosures: H. Meharena: None. **L. Tsai:** None.

Poster

113. Postnatal Neurogenesis

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 113.27/A27

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant P20GM103620
NIH Grant P20GM103548
NSF Grant DGE-1633213

Title: Genetic disruption of cholesterol biosynthesis inhibits astrogenesis and induces neurodevelopmental deficits through perturbation of Wnt signaling

Authors: *B. FREEL[1], N. COUNGERIS[1], R. ANDERSON[1,2], K. FRANCIS[1,2];
[1]Cell. Therapies and Stem Cell Biol., Sanford Res., Sioux Falls, SD; [2]Dept. of Pediatrics, Univ. of South Dakota Sanford Sch. of Med., Vermillion, SD

Abstract: Disorders of cholesterol synthesis constitute a class of eight rare pediatric diseases exhibiting broad clinical phenotypes, including neurological dysfunction. Smith-Lemli-Opitz syndrome (SLOS) is the most common disease in this class and results from mutations in *DHCR7*, inhibiting the final step of cholesterol synthesis. SLOS presents with a wide range of neurological phenotypes including intellectual disability, autistic behaviors, microcephaly, cerebellar malformation, and hippocampal hypoplasia. However, the mechanisms whereby *DHCR7* mutations lead to the neurodevelopmental and functional deficits observed in patients are unclear. To uncover cellular phenotypes and causative signaling deficits resulting from *DHCR7* mutations, we previously developed patient-derived induced pluripotent stem cell (iPSC) models from multiple SLOS subjects. Through analysis at the transcriptional, protein, and cellular level, we have determined that *DHCR7* disruption induces defects at multiple stages of development. At the neural stem cell (NSC) stage, inhibited proliferation and a four-fold decrease in MS-1 and hNestin expression highlights loss of SLOS multipotency. Using 2D and organoid differentiation models, we demonstrated a 3-fold increase in neuronal differentiation of SLOS NSCs corresponding to attenuated astrogenesis, identified by a six-fold decrease in GFAP expression and glial transcript expression in SLOS. Transcriptomic and functional analyses suggested that the shift away from astrogenesis in SLOS is due to intrinsic signaling differences present at the NSC stage, altered signaling mechanisms are only partially correctable by cholesterol supplementation, and dynamic signaling deficits in SLOS models occur throughout differentiation in both 2D and 3D models. Through stabilization of inhibited Wnt signaling, we were able to normalize aberrant differentiation patterns and promote astrogenesis. Conversely, iPSC models of Lathosterolosis, a separate and distinct cholesterol synthesis disorder, do not exhibit Wnt signaling defects and are non-responsive to Wnt stabilization, demonstrating

mechanistic differences between these disorders. These studies detail a basic requirement for cholesterol homeostasis for neurodevelopment, identify critical cellular phenotypes potentially contributing to CNS malformations and neurological dysfunction in SLOS, and highlight the precise regulation of signaling pathways by cholesterol homeostasis during neurodevelopment.

Disclosures: B. Freel: None. **N. Coungeris:** None. **R. Anderson:** None. **K. Francis:** None.

Poster

114. Axon and Dendrite Development

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 114.01/A28

Topic: A.05. Axon and Dendrite Development

Support: JHU Catalyst Award
Brain & Behavior Research Foundation
NIH Bench-to-Bedside Award

Title: *In vivo* epigenetic editing of Sema6A promoter reverses impaired transcallosal connectivity caused by C11orf46/ARL14EP neurodevelopmental risk gene

Authors: *M. NAGPAL[1], A. SAITO[2], Y. HASEGAWA[2], A. KAMIYA[3];
[1]Dept. of Psychiatry and Behavioral Sci., Johns Hopkins Univ., Baltimore, MD; [2]Dept. of Psychiatry and Behavioral Sci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [3]Dept Psychiatry and Behavioral Sci., Johns Hopkins Univ. Sch. Med., Baltimore, MD

Abstract: Many neuropsychiatric risk genes contribute to epigenetic regulation of gene expression but very little is known about specific chromatin-associated mechanisms governing the formation and maintenance of neuronal connectivity. Here we show that transcallosal connectivity is critically dependent on C11orf46 (also known asARL14EP), a small nuclear protein encoded in the chromosome 11p13 Wilms Tumor, Aniridia, Genitourinary Abnormalities, intellectual disability (formerly referred to as Mental Retardation) (WAGR) risk locus.C11orf46 haploinsufficiency in WAGR microdeletion cases was associated with severe hypoplasia of the corpus callosum. In utero short hairpin RNA-mediated C11orf46 knockdown disrupted transcallosal projections of cortical pyramidal neurons, a phenotype that was rescued by wild type C11orf46 but not the C11orf46R236Hmutant associated with autosomal recessive intellectual disability. Multiple genes encoding key regulators of axonal growth and differentiation, including Sema6A, were hyperexpressed in C11orf46-knockdown neurons. Importantly, RNA-guided epigenetic editing of neuronal Sema6a gene promoters via a dCas9 protein- conjugated SunTag scaffold with multimeric (10x) C11orf46 binding during early developmental periods, resulted in normalization of expression and rescue of transcallosal dysconnectivity via repressive chromatin remodeling, including up-regulated histone H3K9

methylation by the KAP1-SETDB1 repressor complex. Our study demonstrates that interhemispheric communication is highly sensitive to locus-specific remodeling of neuronal chromatin, revealing the therapeutic potential for shaping the brain's connectome via gene-targeted designer activators and repressor proteins.

Disclosures: **M. Nagpal:** None. **A. Saito:** None. **Y. Hasegawa:** None. **A. Kamiya:** None.

Poster

114. Axon and Dendrite Development

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 114.02/A29

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant R00NS083714
NIH Grant R01NS109197

Title: A novel role for Gpc3 in pre-target topographic axon sorting in the developing visual system

Authors: ***O. SPEAD**, F. E. POULAIN;
Univ. of South Carolina, Columbia, SC

Abstract: Brain connectivity and function depend on the precise formation of neuronal connections during development. In the central nervous system, most axonal projections are organized into topographic maps according to the spatial organization of the neurons they originate from or the type of stimulus they respond to. An important mechanism contributing to topographic map formation is pre-target axon sorting, where axons become pre-ordered en route to their destination. A salient example is the visual system, where retinal axons are topographically sorted along the dorso-ventral axis in the optic tract before reaching the optic tectum. While optic tract sorting contributes to the topographic fidelity of retinotectal connections, little is known about how it is established. Our previous studies in zebrafish have shown that optic tract sorting is achieved through the selective degeneration of missorted dorsal axons that have erroneously misrouted along the dorsal branch of the tract. Heparan sulfate, a type of sugar chains carried by core proteins known as heparan sulfate proteoglycans (HSPGs), acts non-cell-autonomously along ventral axons to regulate the degeneration of these missorted dorsal axons. We have now identified the HSPG Glypican-3 (Gpc3) as specifically expressed in ventral retinal ganglion cells (RGCs) throughout development. Using CRISPR/Cas9 genome editing, we have generated several *gpc3* mutant alleles encoding a truncated, non-functional protein. Analysis of retinal axon sorting in *gpc3* mutants reveals that some dorsal retinal axons are missorted along the dorsal branch of the optic tract, demonstrating a novel function for Gpc3 in axon-axon interactions. We are now using genetic and biochemical approaches to identify the

signaling pathway and molecular mechanism by which Gpc3 regulates pre-target optic tract sorting *in vivo*. Overall, our study unravels a novel function for Gpc3 in trans-axonal signaling and developmental axon degeneration during neural circuit formation.

Disclosures: O. Spead: None. **F.E. Poulain:** None.

Poster

114. Axon and Dendrite Development

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 114.03/A30

Topic: A.05. Axon and Dendrite Development

Support: Grant-in Aid from The Ministry of Education, Culture, Sports, Science and Technology of Japan
the Yokohama Foundation for Advancement of Medical Science

Title: The soluble form of lotus suppresses Nogo receptor signaling by inhibiting Nogo receptor-P75NTR interaction

Authors: *Y. KAWAKAMI[1], Y. KURIHARA[1], K. TAKEI[2];
[1]Med. Life Sci., Yokohama City Univ. Grad. Sch. of Medica, Yokohama, Japan; [2]Dept. of Medl Life Sci., Yokohama City Univ. Grad Sch. of Med. Life Sci., Yokohama, Japan

Abstract: Nogo receptor type 1 (NgR1) is known to inhibit neuronal regeneration in the CNS. Ligand binding to NgR1 leads to the activation of interaction between NgR1 and its co-receptor p75NTR, activating RhoA, resulting in actin depolymerization. So far, several reports have shown that regulation of NgR1-mediated signaling promotes neuronal regeneration. We identified lateral olfactory tract usher substance (LOTUS) as a membrane-bound protein and found that LOTUS interacts directly with NgR1 and inhibits its function by blocking the binding of its all five ligands: Nogo, myelin-associated glycoprotein (MAG), oligodendrocyte myelin glycoprotein (OMgp), B lymphocyte stimulator (BLyS) and chondroitin sulfate proteoglycans (CSPGs). Therefore, LOTUS is expected to have therapeutic potential for the promotion of neuronal regeneration. On the other hand, soluble form of LOTUS (s-LOTUS) is identified abundantly in human cerebrospinal fluid, and its concentration fluctuates according to the morbidity of neuronal inflammation such as multiple sclerosis and meningitis. This evidence implicates that s-LOTUS also has some physiological functions. However, it remains unknown whether s-LOTUS has an inhibitory action on NgR1 function as a candidate for therapeutic agents. Here, we show that s-LOTUS inhibits NgR1-mediated signaling by inhibiting the molecular interaction between NgR1 and its co-receptor p75 neurotrophin receptor (p75NTR). In contrast to the membrane-bound form of LOTUS, s-LOTUS did not block ligand binding to NgR1. However, we identified p75NTR as a novel LOTUS binding partner. Binding assays and

pull-down assays revealed that s-LOTUS suppressed the interaction between p75NTR and NgR1. s-LOTUS inhibited Nogo or MAG-induced RhoA activation in murine primary cortical neurons. Functional analyses in vitro revealed that s-LOTUS inhibited growth cone collapse and neurite outgrowth inhibition induced by all of NgR1's five ligands in chick DRG neurons. In addition, treatment with s-LOTUS inhibited ligand-induced growth cone collapse in olfactory bulb neurons of *lotus*-knockout mice. Finally, we observed that intravitreous injection of s-LOTUS promoted axonal regeneration in optic nerve crush injury in adult mice of either sex. These findings suggest that s-LOTUS inhibits NgR1-mediated signaling by interfering with the interaction between NgR1 and p75NTR. Thus, s-LOTUS may have potential as a therapeutic agent for neuronal regeneration in the damaged CNS.

Disclosures: Y. Kawakami: None. **Y. Kurihara:** None. **K. Takei:** None.

Poster

114. Axon and Dendrite Development

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 114.04/A31

Topic: A.05. Axon and Dendrite Development

Support: NSERC Discovery Grant 2015-03780
NSERC Discovery Grant 2017-00008

Title: Micrornas and regulation of retinoid acid-induced growth cone turning

Authors: *S. E. WALKER, G. E. SPENCER, R. L. CARLONE;
Biol. Sci., Brock Univ., St Catharines, ON, Canada

Abstract: During development and regeneration, neurons navigate through a changing and highly complex environment to ultimately establish remarkably accurate connections with their target cells. The tips of these growing axons, growth cones, rapidly respond to various environmental cues, including classical guidance proteins such as netrins and semaphorins. Recent studies have led to the identification of other, non-traditional guidance cues, including the Vitamin A metabolite, retinoic acid (RA). RA has been shown to act as a chemoattractant for both vertebrate and invertebrate neurons *in vitro*. Little is known, however, about the nature of the underlying regulatory molecules or biochemical pathways involved in fine-tuning growth cone turning responses to a gradient of RA. MicroRNAs (miRNAs), a class of conserved non-coding RNAs, have recently been proposed to regulate gene expression and local protein synthesis during growth cone guidance in response to traditional protein cues. Our goal is to determine the role that miRNAs play as mediators of axonal guidance in response to a non-traditional guidance cue such as RA. We have previously established that growth cones from neurons of the pond snail, *Lymnaea stagnalis*, exhibit positive turning responses toward RA in a

local protein synthesis-dependent manner. We now have evidence for the compartmentalization of miR-124 in these growth cones and axons, as well as in cell bodies within the brain. We utilize LNA-FISH to precisely localize miR-124 in the growth cone during different phases of a turning response to RA. Moreover, we have also upregulated/inhibited miR-124 to identify its importance to growth cone guidance. To determine the specificity of miR-124 to RA-induced growth cone responses, we have further investigated the localization of this miRNA in response to a different attractive guidance cue, serotonin. These studies will advance our knowledge of the fine-tuning of growth cone dynamics, especially the underlying mechanisms of RA-induced chemoattraction. Further, this research will elucidate the role of miRNAs in local protein synthesis in both development and regeneration of the central nervous system.

Disclosures: S.E. Walker: None. **G.E. Spencer:** None. **R.L. Carlone:** None.

Poster

114. Axon and Dendrite Development

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 114.05/A32

Topic: A.05. Axon and Dendrite Development

Support: Grant from the Lilly Endowment, Inc, Indianapolis, IN to Cary Lai

Title: Effect of neuregulins 1, 2, and 3 on cortical GABAergic interneuron neurite outgrowth *in vitro*

Authors: A. RAHMAN, C. LAI, ***A. L. PRIETO**;
Psychological and Brain Sci., Indiana Univ., Bloomington, IN

Abstract: The neuregulins (Nrgs 1-4) are a family of structurally related signaling molecules that play fundamental roles in the developing and mature nervous system. Nrg1 has been shown to influence neurite outgrowth in PC12 cells and in multiple populations of neurons including those in the retina, hippocampus, cerebral cortex, midbrain, spinal cord, and cerebellum. In comparison, the ability of Nrg2 and Nrg3 to promote neurite outgrowth has remained understudied. The Nrgs have the potential to bind to and activate members of the ErbB family of receptor tyrosine kinases, which are comprised of 4 receptors, ErbB1-4. In rodent cortical neurons, the expression of ErbB4 is limited to GABAergic interneurons, with predominant expression in the parvalbumin-expressing subset of these cells. In this study, we characterized and compared the effects of Nrg1, Nrg2, and Nrg3, on neurite number and neurite elongation in cortical GABAergic interneurons at early stages of their differentiation *in vitro*. For these efforts, we prepared neuronal cultures from embryonic day 17-18 rat cerebral cortices and treated them with either GST-Nrg1, GST-Nrg2, GST-Nrg3 or GST for 2 or 5 days in culture. Following treatment, we assessed the length of all primary neurites, the length of the longest primary

neurite, the average length of the primary neurites (excluding the longest one), and also counted the number of primary neurites per cell. We observed that all three Nrgs enhanced neurite outgrowth in cultured ErbB4(+)/GABA(+) interneurons, which were predominately parvalbumin -positive in our cultures. We did not detect enhanced neurite outgrowth of ErbB4(-)/GABA(+) and other ErbB4 (-) neurons. At 2 days *in vitro* (DIV), all morphometric parameters measured were enhanced by Nrg treatment, while at 5 DIV, only axon length (longest neurite) was significantly enhanced. All 3 Nrgs increased the number of total neurites in ErbB4(+)/GABA(+) neurons at both 2 and 5 DIV. These results broaden our limited understanding of the molecular mechanisms regulating neurite outgrowth and the differentiation of GABAergic interneurons. Additional studies will be required to determine if the ability of the Nrgs to promote neurite outgrowth *in vitro* has functional relevance *in vivo*.

Disclosures: **A. Rahman:** None. **C. Lai:** None. **A.L. Prieto:** None.

Poster

114. Axon and Dendrite Development

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 114.06/A33

Topic: A.05. Axon and Dendrite Development

Support: NIH-NINDS NS094499
NIH-TRA 1R01NS092474

Title: Development of myelinated inhibitory axons in mouse cortex

Authors: *K. D. MICHEVA, M. KIRALY, M. M. PEREZ, D. V. MADISON;
Stanford Univ. Sch. Med., Stanford, CA

Abstract: Many axons in the vertebrate nervous system are surrounded by myelin, an insulating sheath that keeps axonal impulse propagation velocity high and energy consumption low, and thus enables rapid and efficient signal transmission. The myelination of axons is an important parameter determining the function of neuronal circuits and contributing to their plasticity. Recently, we showed that a large fraction of myelin in the mouse and human neocortex ensheathes axons of inhibitory parvalbumin-positive interneurons, and that these axons have distinctive structural and molecular features that contrast with those of the majority of excitatory myelinated axons. It is known that in mammalian neocortex, myelination occurs postnatally over a prolonged period of time. However, there are no data on the developmental course of myelination specifically of inhibitory axons. In the present study we address this question by using immunofluorescent array tomography on mouse neocortical tissue from different ages spanning between 2 weeks postnatally to 2 years. We focus on 2 different areas, visual cortex and piriform cortex.

Our results show that, as expected, in both cortical areas there is a prolonged period of development of myelination. A period of rapid myelination occurs between 2 weeks of age, when only a few myelinated axons are seen in the deep cortical layers 5 and 6, and 1 month of age, when myelinated axons extend all the way to layer 1 and their density reaches a quarter of the mature levels. By 3 months of age the density of myelinated axons is about 60% and it continues to increase until approximately 7 months. Interestingly, inhibitory myelinated axons follow a different developmental pattern in the earlier ages. At 2 weeks of age, practically no inhibitory axons in cortex are myelinated, but by 1 month they are already at 35% of mature levels, compared to only 20% for the excitatory axons. When analyzing individual dye-filled parvalbumin interneurons a similar pattern is observed, with no myelination seen at 2 weeks of age, then highly variable intermittent myelination of the proximal axonal arbor at 1 month, which continues to increase until at least 7 months of age.
The developmental pattern of cortical myelination suggests that neuronal circuits in mouse mature much later than generally appreciated, after 6 months of age.

Disclosures: **K.D. Micheva:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aratome LLC. **M. Kiraly:** None. **M.M. Perez:** None. **D.V. Madison:** None.

Poster

114. Axon and Dendrite Development

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 114.07/A34

Topic: A.05. Axon and Dendrite Development

Title: Control of axon-guidance of human ipsc derived neurons by microstructured gradients

Authors: *S. MEFFERT, **A. OFENHÄUSSER**;
Forschungszentrum Jülich, Jülich, Germany

Abstract: Neural stem cells are promised for cell therapy of neurodegenerative disorders and spinal cord injury. That requires - next to controlled differentiation of the stem cells to neurons - integration in the neural tissue and formation of precisely wired neural circuits. Substrate-bound gradients expressed in numerous spatio-temporal patterns play a crucial role during the development of complex neural networks. Previous study has shown that using a discontinuous substrate-bound gradient the neuronal cell position, the neurite growth and axon directionality of rat cortical neurons could be controlled. In this study we examined if these data could be transduced to human neurons. The gradient pattern was fabricated by microcontact printing using laminin/poly-L-lysine (PLL). The gradients were tested for their impact on axon guidance of neural stem cells-derived human neurons. The axon was evaluated by TAU-1 and the dendrites by MAP-2 immunostaining. We found that the microgradient directed neurons' adhesion and

guided up to 80% of the axons. Our approach proved to be very successful in guiding axons of single human neurons with very high efficiency Thereby, we could see that the data previously gained by using rat cortical neurons could be confirmed for human neurons. These data could be useful to engineer defined functional circuits of human neurons for cell therapy application.

Disclosures: S. Meffert: None. **A. Ofenhäusser:** None.

Poster

114. Axon and Dendrite Development

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 114.08/A35

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant NS090030
NIH Grant NS055272

Title: Crispr/Cas9 interrogation of the murine Pcdhg gene cluster reveals a crucial isoform-specific role for Pcdhgc4 in organismal and neuronal survival

Authors: *A. M. GARRETT[1], P. J. BOSCH[2], L. C. FULLER[2], D. M. STEFFEN[2], A. A. KOCH[1], J. A. WEINER[2], R. W. BURGESS[3];
[1]Pharmacol., Wayne State Univ., Detroit, MI; [2]Biol., Univ. of Iowa, Iowa City, IA; [3]Jackson Lab., Bar Harbor, ME

Abstract: The mammalian *Pcdhg* gene cluster consists of 22 variable exons and 3 constant exons, which encode the gamma-protocadherins (γ-Pcdhs), a family of 22 cell adhesion molecules. The proteins form dimers promiscuously in *cis*, but with distinct homophilic preferences in *trans*, resulting in a zipper-like lattice of dimers between membranes. When many isoforms are shared between opposing membranes, a larger lattice can form, and in cell aggregation studies, subtle differences between cells in isoform ratios greatly affect cell binding. Thus, through isoform combination, the γ-Pcdhs, alone or in complex with the related α- and β-clustered Pcdhs, could generate many thousands of distinct cellular recognition units. The γ-Pcdhs critically regulate multiple neurodevelopmental processes, including neuronal survival, synapse formation, dendrite self-avoidance, and dendrite arborization, and mice null for the *Pcdhg* cluster exhibit perinatal lethality. However, the requirement for isoform variety in these functions is still unclear. To precisely ask whether molecular diversity is essential for normal γ-Pcdh function, and to determine if any particular isoform(s) is critically important, we used CRISPR/Cas9 genome editing to generate an unbiased array of mutations in the variable exons of the *Pcdhg* locus in mice. sgRNAs were designed to target sequences near the start codon of each of the 22 *Pcdhg* variable exons and were injected together as a mixture into fertilized eggs. We established ~30 new lines with between 1 and 21 disrupted variable exons, with disruptions

ranging from small indels to large rearrangements between guide sites. We found that isoform diversity *per se* was not required for neuronal survival or for survival of the animal. Multiple lines with substantially reduced isoform diversity were viable as homozygotes. However, mutations that included disruption of the γC4 variable exon resulted in widespread neuronal cell death in the spinal cord and retina and perinatal lethality. To directly test the crucial nature of this isoform, we analyzed two additional lines – one in which γC4 was the only intact isoform, and one in which γC4 was the only disrupted isoform. We found that γC4 is necessary and sufficient among γ-Pcdh isoforms for organismal survival and for cell survival in many of the neuronal subtypes assayed.

Disclosures: **A.M. Garrett:** None. **P.J. Bosch:** None. **L.C. Fuller:** None. **D.M. Steffen:** None. **A.A. Koch:** None. **J.A. Weiner:** None. **R.W. Burgess:** None.

Poster

114. Axon and Dendrite Development

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 114.09/A36

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant NS090030
NIH Grant NS055272

Title: CRISPR/Cas9 interrogation of the murine Pcdhg gene cluster reveals that both stochastically- and constitutively-expressed isoforms contribute to dendritic arborization

Authors: D. M. STEFFEN[1], C. G. MARCUCCI[1], P. VALIÑO RAMOS[1], A. M. HERBER[1], C. HANES[1], A. M. GARRETT[2], R. W. BURGESS[3], ***J. A. WEINER**[1];
[1]Biol., The Univ. of Iowa, Iowa City, IA; [2]Pharmacol., Wayne State Univ., Detroit, MI; [3]Jackson Lab., Bar Harbor, ME

Abstract: The mammalian *Pcdhg* gene cluster consists of 22 variable exons and 3 constant exons, which together encode the gamma-protocadherins (γ-Pcdhs), a family of 22 cell adhesion molecules. The proteins form dimers promiscuously in *cis*, but with distinct homophilic preferences in *trans*, resulting in a zipper-like lattice of dimers between membranes. When many isoforms are shared between opposing membranes, a larger lattice can form, and in cell aggregation studies, subtle differences between cells in isoform ratios greatly affect cell binding. Thus, through isoform combination, the γ-Pcdhs, alone or in complex with the related α- and β-clustered Pcdhs, could generate many thousands of distinct cellular recognition units. In previous work, we showed that the γ-Pcdhs are required for normal dendritic arbor complexity in cortical neurons, and presented evidence that arborization could be increased or decreased *in vivo* by, respectively, manipulations that promoted or blocked homophilic *trans* interactions. Though

single isoforms may mediate some *Pcdhg* functions when misexpressed, the requirement for isoform diversity during normal development remains unclear. To ask whether molecular diversity is essential for normal γ-Pcdh function, and to determine if any particular isoform(s) is critically important, we used CRISPR/Cas9 genome editing to establish ~30 new lines of mice with between 1 and 21 disrupted *Pcdhg* variable exons, with disruptions ranging from small indels to large rearrangements between guide sites. Analysis of multiple lines retaining distinct functional *Pcdhg* isoform repertoires indicates that molecular diversity indeed contributes to the formation of complex dendritic arbors in the cortex. Arborization is significantly reduced in mice specifically lacking the ubiquitously-expressed γC3 isoform, which regulates dendrite development via Axin1 signaling. Arbor complexity is also adversely affected in lines lacking all or most of the stochastically-expressed γA (12) and γB (7) isoforms but retaining expression of the 3 ubiquitous C-type isoforms (γC3, γC4, and γC5). As mouse lines lacking γC4 uniquely exhibit neonatal lethality (see adjacent linked poster), we are currently breeding *trans*-heterozygotes harboring one cortex-restricted conditional null allele to allow analysis of postnatal arborization.

Disclosures: D.M. Steffen: None. **C.G. Marcucci:** None. **P. Valiño Ramos:** None. **A.M. Herber:** None. **C. Hanes:** None. **A.M. Garrett:** None. **R.W. Burgess:** None. **J.A. Weiner:** None.

Poster

114. Axon and Dendrite Development

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 114.10/A37

Topic: A.05. Axon and Dendrite Development

Support: R01NS096098

Title: Ribosomal protein SA (Rpsa) signaling regulates cortical neuronal morphogenesis

Authors: *S. BLAZEJEWSKI, S. BENNISON, N. HA, X. LIU, T. SMITH, K. J. DOUGHERTY, K. TOYOOKA;
Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Defects in neuronal morphology can lead to functional consequences and are related to neurodevelopmental disorders. Development of proper neuronal morphology depends on early mechanisms including neurite formation and dendritic branching. To better understand cortical development, it is useful to determine which signaling pathways are important for regulating neuronal morphology. Ribosomal protein SA (Rpsa) is known to interact with the extracellular matrix and function in neuroprotection. Here, we identified a novel function for Rpsa in regulating several aspects of neuronal morphogenesis by using *in utero* electroporation to

knockdown (KD) Rpsa *in vivo*. At P3, Rpsa deficient neurons displayed orientation defects in the mean angle at which the apical dendrite extends from the middle of the soma relative to the cortical plate. At P15, defects in neuronal morphogenesis were observed in Rpsa KD neurons, which had significantly fewer and shorter extensions with less branching as compared with control neurons. The Rpsa KD phenotype could be rescued by Rpsa overexpression (OE) at both time-points. To investigate the functional consequences of these morphological defects, the GCaMP6s calcium indicator was used to record spontaneous activity in live brain slices. Rpsa KD neurons had a significantly lower mean difference between maximum and minimum fluorescence intensity peaks, suggesting that the spontaneous activity of Rpsa deficient neurons is lower than that of control neurons. Preliminary whole-cell recordings also suggest a possible functional difference, with decreased spontaneous EPSC amplitudes in Rpsa deficient neurons. To further delineate the signaling mechanism of Rpsa, we investigated its extracellular ligand, pigment epithelium derived factor (PEDF), and Integrin subunit alpha 6 (Itga6), which is known to interact with Rpsa on the plasma membrane. Similar defects in neuronal morphogenesis are observed following Rpsa KD, PEDF KD, and Itga6 KD. Additionally, Rpsa OE rescued morphological defects resulting from PEDF KD *in vivo*, suggesting that PEDF is the ligand responsible for initiating Rpsa signaling involved in regulating neuronal morphogenesis. We hypothesize that Itga6 OE will rescue morphological defects caused by PEDF KD by facilitating its interaction with Rpsa. Further investigation will advance our understanding of neuronal morphogenesis in normal brain development and in the context of neurodevelopmental disorders, as well as increase our knowledge of a relatively unstudied and complex signaling mechanism.

Disclosures: S. Blazejewski: None. **S. Bennison:** None. **N. Ha:** None. **X. Liu:** None. **T. Smith:** None. **K.J. Dougherty:** None. **K. Toyooka:** None.

Poster

114. Axon and Dendrite Development

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 114.11/A38

Topic: A.05. Axon and Dendrite Development

Support: R01NS096098

Title: Activity dependent neuroprotective protein (Adnp) regulates neuronal morphogenesis in the developing cortex

Authors: *S. BENNISON, S. BLAZEJEWSKI, X. LIU, T. SMITH, K. TOYO-OKA; Neurobio. & Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Activity-dependent neuroprotective protein (Adnp) is a master regulator of ~400 genes essential to embryonic and postnatal development. Mutations in Adnp are the most

frequent underlying Autism Spectrum Disorder (ASD) and lead to a distinctive combination of clinical features. Adnp functions in the nucleus as a transcription factor, and in the cytoplasm during neuronal maturation to promote dendritic spine/synapse formation via microtubule (MT) interactions. Adnp's activities in earlier neuronal development remain elusive but are important to understand how Adnp mutations result in pathology. Previous research has suggested that Adnp may play a role in promoting neurite formation, which prompted us to investigate this role in more detail. Our RNA-Sequencing (RNA-Seq) data suggest increased Adnp expression throughout neuritogenesis, and our cortical neuritogenesis analyses implicate a much more complex role for Adnp in neuronal morphogenesis than previously reported. Overexpression (OE) of Adnp in primary cortical neurons leads to premature spine-like formation on all neurites, including the neurite most likely to become the axon, suggesting that Adnp may function in neuronal polarization. Knockdown (KD) of Adnp in primary cortical neurons leads to increased neurite initiation and defective neurite elongation, suggesting that Adnp has distinct roles in each. Neurite elongation defects included increasing the length of the neurite most likely to become the axon, while decreasing the length of the remaining neurites. This suggests that Adnp also has distinct roles in the neurite most likely to become the axon vs. the neurites most likely to become the dendrites. We postulate that this may occur via multiple mechanisms: either Adnp interacts with differentially distributed cytoskeletal proteins such as Tau in the primitive axon and MAP2 in the primitive dendrites for example, or that Adnp itself also plays a role in neuronal polarization. *In vivo* analysis using *in utero* electroporation revealed Adnp KD leads to another morphological deficit, disrupting the angle of the apical dendrite at P3. This suggests altered connectivity and response to guidance cues, which is ultimately based on microtubule stabilization. Adnp is known to promote MT polymerization and we conclude that this defect at P3 as a result of Adnp KD is due to altered MT dynamics. We conclude that Adnp works via multiple distinct mechanisms to establish proper neuronal morphogenesis which is crucial for cortical development.

Disclosures: S. Bennison: None. **S. Blazejewski:** None. **X. Liu:** None. **T. Smith:** None. **K. Toyo-oka:** None.

Poster

115. Behavioral Study and Animal Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 115.01/A39

Topic: A.07. Developmental Disorders

Title: Sexually dimorphic effects of propionic acid in adult rats: Implications for an animal model of autism spectrum disorder

Authors: *K. BENITAH, P. OSSENKOPP, M. KAVALIERS;
Univ. of Western Ontario, London, ON, Canada

Abstract: Autism spectrum disorder (ASD) is a developmental disorder of variable severity characterized by impairments in social interaction and communication as well as restricted and repetitive patterns of movement. Past research suggests that certain gut and dietary factors may transiently worsen symptoms in ASD. Propionic acid (PPA) is a short chain fatty acid and an important intermediate of cellular metabolism. PPA is also a by-product of a subpopulation of human gut enterobacteria. Previous studies have shown that treatment with PPA can create both brain and behavioural responses in rats that are characteristic of ASD in humans. A strong and consistent male bias in ASD prevalence has been observed, and several sex-differential genetic and hormonal factors have been suggested to contribute to this bias. Past studies have reported a neuroprotective effect of the sex hormones prolactin and estrogen, for both hippocampal neurodegeneration and neuroinflammation, which have been proposed as potential etiological mechanisms in autism. Very little research has examined the effects of PPA in females. The present study explored putative sex differences in the effects of PPA on a rodent behavioural ASD phenotype. Male (N = 16) and female (N = 16) rats were systemically treated with PPA (500mg/kg) or PBS control and tested in a light-dark anxiety procedure. PPA-treated females displayed similar patterns of anxiety-like behaviour (i.e. duration of time spent in the light chamber and nosepokes into the light chamber) to PPA-treated males, which differed significantly from PBS treated rats.

Disclosures: K. Benitah: None. **P. Ossenkopp:** None. **M. Kavaliers:** None.

Poster

115. Behavioral Study and Animal Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 115.02/A40

Topic: A.07. Developmental Disorders

Support: NIH Grant R15S088776

Title: Characterizing ultrasonic vocalizations in the NS Pten knockout model: Implications for autism

Authors: *M. S. BINDER[1], S. L. HODGES[2], S. O. NOLAN[3], P. D. WOMBLE[3], D. G. JONES[4], J. N. LUGO, JR[3];
[1]Psychology & Neurosci., [2]Inst. of Biomed. Studies, [3]Psychology and Neurosci., [4]Baylor Univ., Waco, TX

Abstract: A signaling cascade that plays a crucial role in the development of an autistic-like phenotype is the PI3K/AKT/mTOR pathway. A mouse model that illustrates this connection is the neuronal subset specific (NS) *Pten* knockout model which exhibits hyperactivity of mTOR. Despite the importance of communicative deficits in an autistic-like phenotype, few studies have assessed communicative behaviors in NS-*Pten* knockout animals. The present study sought to characterize communicative behaviors in NS-*Pten* wildtype and knockout pup and adult mice. Neonatal vocalizations were elicited from males and females on postnatal days 8 and 11 via the maternal isolation paradigm. Adult vocalizations were elicited from 7-week-old males via the female urine induced vocalization paradigm using a separate cohort of mice. Scent marking, another form of communicative behavior, was also assessed in adult NS-*Pten* knockout and wildtype mice. In pups, we found that NS-*Pten* knockout mice emitted fewer vocalizations for both sexes, (*p* &lt .05). Knockout males had calls of a shorter duration (*p* &lt .001) and lower peak amplitude on day 8 (*p* &lt .001), while emitting calls of a shorter duration (*p* &lt .001), lower peak amplitude (*p* &lt .001), and higher peak frequency (*p* &lt .001), and fundamental frequency (*p* &lt .001), on day 11 relative to wildtype males. Knockout female pups vocalized at a lower peak amplitude (*p* &lt .001), and fundamental frequency (*p* &lt .001), and a higher peak frequency (*p* &lt .001), on day 8, while showing a shorter duration (*p* &lt .001), and higher peak frequency (*p* &lt .001), and fundamental frequency (*p* &lt .001), on day 11 relative to wildtype females. Spectrographic analyses revealed significant differences in call-type utilization for both genotypes (*p* &lt .05). In adulthood, NS-*Pten* knockout mice did not display a significant difference in call quantity or scent marking behavior (*p* &gt .05). NS-*Pten* adult knockout mice exhibited calls of a lower mean peak frequency than wildtype males (*p* &lt .05). Additionally, adult knockout mice utilized different types of calls than wildtype mice (*p* &lt .05). This study indicates that hyperactivity of mTOR results in both quantitative and qualitative changes in communicative behaviors for both pup and adult NS-*Pten* knockout mice.

Disclosures: M.S. Binder: None. **S.L. Hodges:** None. **S.O. Nolan:** None. **P.D. Womble:** None. **D.G. Jones:** None. **J.N. Lugo:** None.

Poster

115. Behavioral Study and Animal Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 115.03/A41

Topic: A.07. Developmental Disorders

Support: 2017-ATESP-0106-FAQC_01_2017

Title: Targeting the PPARα and endocannabinoid systems in the rat valproic acid model of autism spectrum disorder: Focus on gender differences

Authors: S. SCHEGGI[1], M. G. DE MONTIS[1], F. GUZZI[2], M. PARENTI[2], *C. GAMBARANA[1];
[1]Mol. and Developmental Med., Univ. of Siena, Siena, Italy; [2]Med. and Surgery, Univ. of Milano-Bicocca, Monza, Italy

Abstract: The social motivational theory of Autism Spectrum Disorder (ASD) focuses on social anhedonia as the key causal feature of the impaired peer relationships that characterize patients and are a core symptom of ASD. Accordingly, ASD can be regarded as an extreme case of early-onset reduced social motivation. We previously reported that motivational anhedonia induced in rats by exposure to a chronic stress protocol is relieved by repeated treatment with fenofibrate (FBR), an agonist of PPARα and CB_1/CB_2 receptors clinically used to treat hyperlipidemia (1). In this study, we assessed whether a long-term FBR treatment started at early age relieved social motivational anhedonia in a model of ASD. ASD-like symptoms were induced in Sprague-Dawley rats by a single *in utero* exposure to valproic acid (VPA, 500 mg/kg) at gestational day 12.5. ASD has a 4:1 prevalence in boys, yet, increasing evidence suggests that it is often underdiagnosed and undertreated in females. Thus, we aimed to identify an ASD-like behavioral phenotype in male and female VPA-exposed rats and to assess whether FBR administration differentially affected one sex over the other. At weaning (P21) the VPA- or saline-exposed offspring were fed with a FBR-enriched diet (0.2% FBR) or standard diet until P48-53. At this time, rats of the four experimental groups (n = 8-10) were behaviorally tested to evaluate ASD core domains, i.e. social behaviors (3-chamber sociability, 3-ch ST; social transmission of food preference, STFP), and repetitive (RB) and perseverative (marble burying, MB) behaviors. VPA-exposed male rats showed impaired social behaviors (3-ch ST and STFP) and FBR administration relieved these ASD-like deficits. In VPA-exposed female rats only the STFP test demonstrated an impaired social interaction that was relieved by FBR administration. Repetitive and perseverative behaviors were increased in VPA-exposed male rats and MB behavior was normalized upon FBR administration. VPA-exposed female rats only showed increased RB that was not affected by FBR treatment. These results support the hypothesis that motivational mechanisms underpin ASD social impairments. Moreover, they offer a new perspective for ASD therapy since subsets of patients may significantly benefit from a pharmacological treatment that targets the core symptoms of the disorder and FBR is already chronically employed with no overt toxicity. The study also provides preliminary information on the neurobiology of distinct core symptom expression in the two sexes and their responses to specific treatments, rarely addressed issues by experimental studies of ASD.
(1) Scheggi et al. Neuropharmacol. 110:251-259, 2016.

Disclosures: S. Scheggi: None. **M.G. De Montis:** None. **F. Guzzi:** None. **M. Parenti:** None. **C. Gambarana:** None.

Poster

115. Behavioral Study and Animal Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 115.04/A42

Topic: A.07. Developmental Disorders

Title: Shank3 social deficits and repetitive behavior reflect preventable context-induced anxiety

Authors: *S. KRÜTTNER, P. CARONI;
Friedrich Miescher Inst. For Biomed. Res., Basel, Switzerland

Abstract: Autism Spectrum disorders (ASD) is a mental condition with a strong genetic component that affects 1% of the population. Despite the identification of a large variety of genes involved, the underlying mechanisms are still poorly understood. ASD is difficult to diagnose because of the complex spectrum of core symptoms and connected co-morbidities involved. These symptoms include social deficits, anxiety, stereotypic and repetitive behaviors as well as impaired cognitive capabilities. To date, it is unclear whether the variety of co-morbidities and symptoms described arise independently as a consequence of altered developmental processes or whether they involve shared underlying mechanisms. Furthermore, the onset and development of deficits can vary even among patients harboring the same genetic defect, suggesting that genetic predisposition alone might not be sufficient to trigger the full extent of ASD symptoms. Using Shank3-/- mice as a model for ASD, we searched for experience-dependent mechanisms which might acutely control manifestation of ASD symptoms. We show that experience of a novel context induces long-lasting and context specific sensitization, which accounts for several characteristic ASD phenotypes including anxiety, repetitive locomotion and failure to engage with novelty including social cues. We identify elevated Parvalbumin (PV) expression as a biomarker for ASD, and correlate sensitization to the induction of aberrant plasticity in ventral hippocampus PV-interneurons of *Shank3-/-* mice. Based on these findings, we developed behavioral, pharmacological and pharmacogenetic interventions schemes that are effective to prevent circuit and behavioral alterations in Shank3-/- mice, and might serve as a basis to be develop rational therapies to prevent and treat core symptoms of ASD.

Disclosures: S. Krüttner: None. **P. Caroni:** None.

Poster

115. Behavioral Study and Animal Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 115.05/A43

Topic: A.07. Developmental Disorders

Support: T32 (MH065215)
Vanderbilt University

Title: Similarities and distinctions in behavioral phenotypes of three CAMK2A mutant mouse lines

Authors: *J. R. THOMAS[1,2], K. SPIESS[1], R. J. COLBRAN[1,2,3];
[1]Mol. Physiol. and Biophysics, Vanderbilt Univ. Med. Ctr., Nashville, TN; [2]Vanderbilt Brain Inst., Nashville, TN; [3]Vanderbilt-Kennedy Ctr. for Res. on Human Develop., Nashville, TN

Abstract: It is well established thatCa^{2+}/calmodulin dependent protein kinase II alpha(CaMKII alpha) is important for synaptic plasticity, learning and memory. Mutations in the *CAMK2A* gene, which encodes CaMKII alpha,have been linked to Autism Spectrum Disorder(ASD)and intellectual disability. Previous characterization of mice harboring an ASD-associated Glu183 to Val knock-in mutation in CaMKII alpha (E183V-KI), which reduces CaMKII alpha expression and activity, revealed an impairment in social motivation, increased repetitive behaviors, as well as hyperactivity. To better understand behavioral roles of CaMKII alpha, we compared behavioral phenotypes of mice with various CaMKII alpha genotypes (homozygous): E183V-KI; CaMKII alpha-null (KO); Thr286 to Ala knock-in (T286A-KI) mutation (prevents Thr286 autophosphorylation). Compared to wild-type (WT) mice, all three genotypes displayed similar hyperactivity in a novel open field arena and spent less time in the center of the arena. Although open field behavior is consistent with increased anxiety, testing on an elevated plus or zero maze revealed that all three genotypes spent more time in the open domains compared to the closed relative to their WT controls, indicating decreased anxiety. Moreover, testing in a light-dark box revealed that none of the mouse lines displayed an anxiety phenotype. We interpret these data to indicate that these CaMKII alpha mutations do not affect anxiety *per se*, and that phenotypes in the open field arena and elevated mazes are driven by a heightened “escape” behavior, consistent with robust increases in jumping and rearing along the walls of the open field arena, as well as the overall hyperactivity. Despite these strong phenotypic similarities, testing in a 3-chamber arena revealed no deficits in social exploration in CaMKII alpha-KO mice, in contrast to E183V-KI mice (analyses of T286A-KI mice are ongoing). We also explored the impact of these CaMKII alpha mutations on tactile sensitivity. All three CaMKII alpha mutant mouse lines displayed increased threshold to respond in the Von-Frey filament test, compared to WT mice. Moreover, WT mice preferentially explored a textured object relative to a visually very similar

but smooth object, whereas all three CaMKII alpha mutant mice failed to differentiate between these objects. Together these data suggest that tactile sensation is impaired in these CaMKII alphamutant mouse lines. In summary, our data demonstrate that CaMKII alphahas an important role in behavioral phenotypes associated with ASD that involve multiple regions of the brain.

Disclosures: J.R. Thomas: None. **K. Spiess:** None. **R.J. Colbran:** None.

Poster

115. Behavioral Study and Animal Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 115.06/A44

Topic: A.07. Developmental Disorders

Support: NIH Grant P50 NS22343
NIH Grant T32DC7361

Title: Developmental differences in eye contact for school age children with high functioning autism and children with perinatal stroke

Authors: *S. EDWARDS, E. BEAVER, M. MEYER, P. LAI;
Communication Disorders, Univ. of Nebraska, Kearney, Kearney, NE

Abstract: This study included three groups of school age children as they communicated in a semi-natural conversation. The groups of children included individuals with High Functioning Autism (HFA) and children with Perinatal Stroke (PS). This study focused on social communication in individuals with neurodevelopmental disorders of different nature and origin with the goal of better characterizing the social phenotype of each group. Children with HFA and children with PS all display atypical social profiles. Individuals with HFA tend to be withdrawn, while children with PS show a more nuance social profile, dictated by the side of injury. Past research has shown that young children with Right Hemisphere Injury (RHI) but not those with Left Hemisphere Injury (LHI) show affective impairments as early as the first year of life. Few studies have investigated the social phenotypes of these children during school-age as the majority of studies occur before the age of 6. This study included 43 children in total between 7 to 14 years of age; 23 children with HFA, 20 children with PS. The first task was a social dialog between the child and an experimenter. During this task, coding of eye contact of the child was conducted and instances of initiation was coded to observe which participant lead the conversation. Frequency of eye contact to the experimenter measures and reflected the degree of sociability for each participant. For the second dataset, four questionnaires were distributed to parent/caregivers to complete. Results showed that there was a significant correlation between age of the child and eye contact of the child ($p=0.011$). As the child developed, changes in communicative behaviors through eye contact increased, reflecting a change in communicative

style in all three groups. For example, children ages 7-11, the average initiation of eye contact was 43.64 instances. On the other hand, children ages 12-14 averaged 83.52 instances of initiated eye contact. Initiation of conversation by the experimenter was not significant, suggesting the experimenter was consistent during the task (p=.85). Insights from this study adds unique knowledge to our understanding of social development as well as contribute to better-informed treatment methods in the future.

Disclosures: S. Edwards: None. **E. Beaver:** None. **M. Meyer:** None. **P. Lai:** None.

Poster

115. Behavioral Study and Animal Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 115.07/A45

Topic: A.07. Developmental Disorders

Support: NIH Grant R01MH106553

Title: Examining the impact of a two-hit model of neuroinflammation on social behavior in male and female juvenile rats

Authors: *A. TURANO, E. M. MCAULEY, M. C. MUENCH, N. A. HAAS, J. M. SCHWARZ;
Univ. of Delaware, Newark, DE

Abstract: Many neuropsychiatric disorders are associated with deficits in social behavior. While it is common to try to examine these disorders by developing an animal model specific to each disorder, it is becoming increasingly popular to consider mental health disorders in terms of functional dimensions rather than by diagnostic labels (Yuhas, 2017). This way, we can move away from these diagnostic labels, which have proven to be heterogeneous (NIMH Strategic Plan, 2015). Thus, we are aiming to model the complex social behavioral deficits observed across a number of neurodevelopmental disorders, including autism and early onset schizophrenia, as a means of answering important questions relevant to both of these disorders. In addition to genetic factors, epidemiological data indicate that environmental factors also contribute to the risk of early onset neurodevelopmental disorders. Neonatal exposure to infectious pathogens is one of these environmental factors, suggesting that activation of the neonatal immune system may contribute to disease pathology. Microglia, the resident immune cells of the brain, perform functions crucial for normal brain development and behavior. According to a “two-hit model of neuroinflammation,” neonatal neuroimmune activation causes persistent deficits in microglial functioning, resulting in an exaggerated immune response and significant behavioral deficits following subsequent immune activation later in life. Importantly, males are more likely than females to be diagnosed with disorders such as autism and early onset

schizophrenia. During early development, males and females exhibit different microglial phenotypes, possibly leaving males more susceptible to the negative outcomes associated with early-life neuroinflammation. Our goal was to better understand the impact of the two-hit model of neuroinflammation on the development and expression of social behaviors in male and female rats. We piloted behavioral paradigms to characterize the development of social behavior in juvenile rats and applied the two-hit model of neuroinflammation to determine how immune activation may affect the expression of these social behaviors. We concurrently measured cytokine expression in the male and female juvenile brain. Our preliminary analysis indicate that the timing (neonatal or juvenile) and the nature (E.coli or lipopolysaccharide) of the neuroimmune activation both play an important role its impact on the expression of specific social behaviors.

Disclosures: A. Turano: None. **E.M. McAuley:** None. **M.C. Muench:** None. **N.A. Haas:** None. **J.M. Schwarz:** None.

Poster

115. Behavioral Study and Animal Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 115.08/A46

Topic: A.07. Developmental Disorders

Title: Objective measures of articulatory complexity in autism spectrum disorder

Authors: *T. F. QUATIERI[1], L. NOWINSKI[2], J. WILLIAMSON[1], D. HANNON[1], A. LAMMERT[1], H. RAO[1], S. YUDITSKAYA[1], D. STURIM[1], K. CLAYPOOL[1], H. SARO[2], C. STAMM[2], M. MODY[2], J. PALMER[1], C. MCDOUGLE[2];
[1]MIT Lincoln Lab., Lexington, MA; [2]Massachusetts Gen. Hosp. Lurie Ctr. for Autism, Lexington, MA

Abstract: Autism spectrum disorder (ASD) is a prevalent neurodevelopmental disorder with core deficits in social communication, including impairment of speech and language processes. We hypothesize that with such impairment there is a loss in coordination within and across the speech subsystems: the vocal tract (articulators), source (vocal folds), and prosody (pitch, energy, and timing), reflecting possibly loss in neural coordination. Here, we present our initial exploratory findings in support of this hypothesis indicating reduced complexity in coordination of articulators. We designed a non-intrusive platform to collect speech acoustics from audio as part of a larger multimodal data collection involving in addition facial muscle intensities from video and hand pressure and displacement from a custom iPad writing/drawing app. The protocol includes a phonetically-balanced (age-appropriate) short paragraph and a diadochokinetic sequence ‘pa-ta-ka,’ as well as involving facial expression while speaking and hand dexterity over a range of tasks. MIT/MGH IRB approvals were obtained prior to an initial data collection

of 5 controls (4 males) and 5 ASD subjects (males). Subjects are 6-12 years of age, verbal, and able to read the paragraph except for one control subject. We measured articulatory coordination by an approach introduced and validated for other neurological conditions (Quatieri et al, 2017): first, we computed temporal correlations across pairs of the first three vocal tract resonances, sampled at multiple different relative time delays. We computed eigenvalues from the resulting correlation matrices, which were constructed at multiple different time-delay scales. Based on preliminary analysis we found a strong Cohen's d effect size for the average eigenvalues of the ASD class relative to controls for both the paragraph and diadochokinetic sequence. The general pattern of effect sizes, positive and close to +1 for large eigenvalues, and negative and close to -1 for small eigenvalues (with p values under 0.05) indicates *lower complexity of articulatory coordination*, i.e., fewer independent degrees of freedom in controlling muscle-motor movements of the tongue, lips, jaw, and velum. While further validation of both statistical significance and interpretation is needed due to small sample size, our exploratory results suggest the speech markers hold potential in monitoring the efficacy of interventions. Complexity of coordination of facial muscle movements (obtained from video) while speaking, as well as coordination of quantified hand movements while drawing, show similar effect-size patterns.

Disclosures: T.F. Quatieri: None. **L. Nowinski:** None. **J. Williamson:** None. **D. Hannon:** None. **A. Lammert:** None. **H. Rao:** None. **S. Yuditskaya:** None. **D. Sturim:** None. **K. Claypool:** None. **H. Saro:** None. **C. Stamm:** None. **M. Mody:** None. **J. Palmer:** None. **C. McDougle:** None.

Poster

115. Behavioral Study and Animal Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 115.09/A47

Topic: A.07. Developmental Disorders

Support: SC EPSCoR Stimulus Research Grant

Title: Evaluating nicotinamide riboside supplementation in a mouse model of autism

Authors: O. LARNER[1], M. REESE[3], S. M. LAMBERT[2], J. SAXENA[1], T. RAY[1], *L. R. FREEMAN[1];
[1]Neurosci., [2]Furman Univ., Greenville, SC; [3]Claflin Univ., Orangeburg, SC

Abstract: Autism spectrum disorder (ASD) is a developmental disorder with various symptoms such as difficulties with: social interactions, verbal and nonverbal communication, anxiety, and restricted/repetitive behaviors. ASD is common, with a reported incidence of 1 in 63 individuals and a male bias. Previous work has shown that ASD may be characterized by altered mitochondrial respiration through defective tryptophan utilization. The aim of our study is to test

the possibility that a mouse ASD phenotype can be rescued by targeting tryptophan metabolic pathways. Specifically, we are evaluating the role of the kynurenine pathway in ASD, which metabolizes tryptophan and produces nicotinamide adenine dinucleotide (NAD+). We are using a well-characterized in utero exposure to valproic acid (VPA) as our mouse model. Post-natal treatment with the nutraceutical nicotinamide riboside (NR) was used to determine if increasing cellular NAD+ levels would reverse or improve ASD-linked behaviors. We have evaluated an initial cohort of animals on a behavioral battery including: the open field test, three-chamber sociability test, marble burying, and the elevated plus maze. Preliminary data reveal NR supplementation partially rescues sociability deficits, anxiety-related symptoms, but increases hyperactivity. Preliminary data also reveal a greater response to NR supplementation in female mice.

Disclosures: O. Larner: None. **M. Reese:** None. **S.M. Lambert:** None. **J. Saxena:** None. **T. Ray:** None. **L.R. Freeman:** None.

Poster

115. Behavioral Study and Animal Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 115.10/A48

Topic: A.07. Developmental Disorders

Support: NIMH R01 MH100173
NIMH K23 MH086785
Simons Foundation 94924
NIMH R21 MH091309
NARSAD Atherton Young Investigator Award
CTSA Grant Number UL1 RR024139

Title: Pivotal response treatment increases neural efficiency of social perception in autism spectrum disorder

Authors: *S. KALA[1], M. ROLISON[1], A. NAPLES[1], P. VENTOLA[1], J. MCPARTLAND[2]; [1]Yale Univ., New Haven, CT; [2]Yale Child Study Ctr., New Haven, CT

Abstract: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by primary difficulties in social function. Individuals with ASD display slowed neural processing of social visual information, such as human faces, as indexed by the N170, a face-sensitive event-related potential (ERP). Pivotal Response Treatment (PRT), a behavioral intervention, uses naturalistic play-based interactions to improve social communication skills in children with ASD. Prior research has demonstrated the effectiveness of PRT in improving social behavior in autism and provided suggestive evidence of hemodynamic changes in brain function associated

with treatment response. The current study sought to examine the feasibility of a scalable method, extracranial EEG, to characterize neural correlates of behavioral improvement in the context of PRT; specifically, we examined changes in N170 latency in response to treatment. Preschool-aged children with ASD (n=7, mean age = 5.6 years) received a 16-week course of PRT. EEG was recorded while participants viewed computer-generated faces with neutral and fearful affect before and after treatment. Additionally, a subset (n=3) served as a waitlist control group, receiving a second EEG at a 16 week delay but prior to treatment onset.
Analyses revealed a main effect of treatment [$F(1,6)=11.34$, $p=.015$], with reduction in N170 latency for both neutral ($p = .027$) and fearful ($p = .029$) faces. There was no significant change in N170 latency during the waitlist control period [$F(1,2)=2.45$, $p=.26$], suggesting that reductions in N170 latency following PRT were due to treatment effects rather than passage of time. Additionally, participants' N170 latency prior to PRT significantly correlated with treatment-associated change in latency for neutral faces ($r=-.873$, $p=.010$); participants who initially showed more atypical neural response to faces showed the most dramatic normalization during treatment.
This study provides initial evidence of the effectiveness of the face-sensitive N170 as a potential index of treatment response in ASD. Results also suggest that N170 latency may denote a strata within ASD (i.e., individuals displaying the most delayed latencies) more likely to respond to behavioral interventions targeting social function.

Disclosures: S. Kala: None. **M. Rolison:** None. **A. Naples:** None. **P. Ventola:** None. **J. McPartland:** None.

Poster

115. Behavioral Study and Animal Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 115.11/A49

Topic: A.07. Developmental Disorders

Support: NIH Grant 1 R15 NS101608-01A1

Title: Investigating the effects of nicotinic acetylcholine receptor activation on larval motor function in *drosophila* kismet and cell adhesion molecule mutants

Authors: *E. L. HENDRICKS, F. L. W. LIEBL;
Southern Illinois Univ. Edwardsville, Edwardsville, IL

Abstract: The chromodomain helicase DNA-binding (CHD) family of proteins are chromatin remodelers that play a key role in regulating developmental processes. In humans, mutations in CHD7 and CHD8 are implicated in CHARGE syndrome and autism spectrum disorders, respectively. Mutations in *kismet* (*kis*), the *Drosophila* ortholog to CHD7 and CHD8, lead to

defects in synaptic morphology at the neuromuscular junction as well as increased expression of postsynaptic cell adhesion molecules, including the Neuroligins (Nlgs). Behavioral analysis of mutants overexpressing Nlg1 (n=30), Nlg3 (n=30), and Nlg4 (n=30) reveals a significant decline in motor function compared to controls (n=30). *Kis* mutants (n=30) also display this decrease in locomotor activity. The central nervous system of *Drosophila* is primarily cholinergic, with cholinergic neurons synapsing upon the glutamatergic neurons at the neuromuscular junction. To explore the possibility of any downstream effects on glutamatergic synapse organization, we examined the effects of acetylcholine receptor (AChR) activation by nicotine on larval motor function. Control larvae exposed to minimal levels of nicotine (n=40) demonstrate increased crawling behavior when compared to nicotine-deprived larvae (n=40). We are currently investigating if AChR activation by nicotine modifies the downstream expression of cell adhesion molecules, which are responsible for maintaining the structural integrity of the synapse. We will also examine if AChR activation restores the impaired motor function of *kismet* and cell adhesion molecule mutants. Together, these experiments will promote further understanding of mechanisms that lead to the aberrant synaptic organization implicated in neurodevelopmental disorders, like CHARGE syndrome and autism spectrum disorders.

Disclosures: E.L. Hendricks: None. **F.L.W. Liebl:** None.

Poster

115. Behavioral Study and Animal Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 115.12/A50

Topic: A.07. Developmental Disorders

Support: Fondation par la Recherche Médicale
Région Nouvelle-Aquitaine
FEDER

Title: Sex dependent behavioral deficits and neuropathology in environmental mouse models of autism

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Abstract: Autism spectrum disorders (ASD) is a psychiatric disease, difficult to diagnose and with no curative treatment. A wide range of symptoms have been identified in ASD patients such as social interaction deficits, restricted interests and repetitive movements. While motor and gait disorders have been constantly reported, they are not included within the diagnosis criteria.

Determination of behavioral and cellular disturbances associated with motor dysfunction may lead to better diagnosis and help develop new therapeutic approaches. In our study, we aimed at (i) investigating whether ASD mouse models manifest motor and gait impairments and thrived to determine the neuronal network involved in these deficits and (ii) identifying differences between males and females as the sex ratio in ASD is 3 boys for 1 girl. We used environmental mouse models to characterize different aspects of their behavior and determined the neurohistological readouts underlying deficits. For this, pregnant females were exposed at E 12.5 to either valproic acid, an anticonvulsant drug, or poly I:C, a double stranded RNA that provokes a maternal immune activation. Our results demonstrate sociability deficits, fine motor and gait disorders and neuronal loss in ASD mice models. These deficits were present in a sex and model-specific manner underlying the spectrum of the disease. Additionally, a correlation analysis revealed relationships among motor disorders, social interactions and number of neurons in specific sublobules of the cerebellum. Our results indicate that motor disorders in ASD could be used as a marker of the disease severity and that the cerebellum could be targeted for therapeutic strategies.

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Poster

115. Behavioral Study and Animal Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 115.13/A51

Topic: A.07. Developmental Disorders

Support: CIHR, IDRC, ISF and Azrieli Foundation Grant 2425/15
ISF Grant 1650/17

Title: Impaired sensorimotor synchronization in autism reveals slow updating of internal priors

Authors: *G. VISHNE[1], N. JACOBY[2], M. AHISSAR[1];
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Abstract: Autism is characterized by deficits in social communication and repetitive patterns of behavior, interests and activities. In recent years there is growing understanding that atypical sensory processing and motor deficits are core to the disorder. Recent findings show that individuals with autism underuse previous environmental information in making perceptual decisions relative to neurotypical individuals (Karaminis et al., 2016). This finding has been interpreted within a Bayesian framework, but in different ways: According to one hypothesis

individuals with autism over-estimate the volatility of the environment (Lawson et al., 2017), therefore they are expected to adequately track fast environmental changes, and underuse knowledge acquired over a long time period. By contrast, according to the slow updating account (Lieder et al., 2019) they are expected to track and update changes slowly but adequately accumulate information over long durations. We tested these conflicting predictions by measuring sensorimotor synchronization (finger tapping) to an external metronome with both fixed and alternating tempos (control n=47, autism n=30). Performance in finger tapping is characterized by: (a) phase - the temporal interval between the metronome beat and the participant's response; (b) period - participant's inter-response-interval. Participants with autism showed substantially larger phase variability ($p<0.005$, Mann-Whitney U test in all group comparisons) and higher correlation between consecutive phases ($p<0.01$), implying reduced correction of phase errors. Using an autoregressive model of the phase responses we show that this reduction is specific to the most recent phase, so the deficit has a fixed time-course, hampering only the use of very recent stimuli. Using a computational model for tapping (Jacoby et al., 2015) we find that individuals with autism have noisier phase correction, but their period retention and motor noise are in the neurotypical range (both $p>0.24$). In the alternating tempo conditions, individuals with autism failed to adapt their phase to the changes, and we show that this impairment stems from a reduction in the period compensation response directly following the change ($p<0.002$). Lastly, we show these two responses are correlated in both groups ($r_{con}=0.34$, $r_{asd}=0.8$, both $p<0.02$), pointing to a shared mechanism of slow integration of the external metronome stimuli as a dynamic temporal trigger for sensorimotor synchronization. These results support the slow updating account of autism and are in line with behavioral reports of the difficulties individuals with autism face when the environment changes abruptly.

Disclosures: G. Vishne: None. **N. Jacoby:** None. **M. Ahissar:** None.

Poster

115. Behavioral Study and Animal Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 115.14/A52

Topic: A.07. Developmental Disorders

Support: NIH Grant 029028-00001
Brain and Cognitive Sciences, MIT 154500

Title: Autistic traits are associated with reading difficulty and reduced neural suppression to print

Authors: *I. R. FROSCH, A. M. D'MELLO, J. D. GABRIELI;
McGovern Inst. for Brain Res., MIT, Cambridge, MA

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impairments in social communication and the presence of repetitive behaviors. Some of the earliest diagnostic markers for ASD are impairments in the language domain. Crucially, individuals with language impairments are at a high risk for literacy failure. In adults, language and reading impairments can result in reduced workplace preparedness, fewer educational opportunities to enhance social and workforce skills, and low income over the lifetime. Despite this, there are few studies examining the relationship between ASD and reading, and an even greater paucity of studies of reading in adults with ASD.

We explored the relationship between neural activation, reading ability, and severity of autism traits in adults with (n = 12) and without (n = 18) ASD diagnoses. Participants (N = 30, age = 28.7, *SD* = 6.4) completed standardized assessments of ASD traits (Autism Quotient, AQ) and symptom severity (Social Responsiveness Scale, SRS), general cognitive ability, and reading and comprehension skills. Participants also completed a functional magnetic resonance imaging (fMRI) session, during which we probed print-selective regions of the brain using a repetition suppression design.

Across all individuals, the left fusiform gyrus (visual word form area) exhibited significantly reduced activation for repeated print, and increased repetition to print was associated with better reading (r = .41 p = .032). Regardless of diagnosis, individual differences in ASD traits and severity of social cognition impairments were associated reading skills (AQ, r = -.37 p = .051; SRS cognition r = -.49 p = .008). Higher ASD traits were also associated with reduced repetition suppression to text (r = -.39, p = .03).

Together these findings suggest that ASD trait and symptom severity are associated with reading ability and neural response to print across individuals with and without a formal ASD diagnosis. Social-communication impairments are a core diagnostic feature of ASD. However, language impairments may extend beyond social communication into reading. Reading impairments in ASD have lasting consequences for success in the workplace, transition to independent living, and global functioning. Efforts to improve global functioning in adults with ASD should consider and seek to improve reading ability and comprehension.

Disclosures: **I.R. Frosch:** None. **A.M. D'Mello:** None. **J.D. Gabrieli:** None.

Poster

115. Behavioral Study and Animal Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 115.15/A53

Topic: A.07. Developmental Disorders

Support: SFARI Explorer Award #350225
Autism Science Foundation

Title: Eye blink and pupillometry as peripheral indicators of reward responsivity in autism spectrum disorder

Authors: *A. S. DICRISCIO, V. TROIANI;
Geisinger, Lewisburg, PA

Abstract: Altered motivational drives may contribute to clinical phenotypes characterized by aberrant reward response and social impairments, such as those observed in autism spectrum disorders (ASD). Spontaneous eye blink and measures of pupil response have been highlighted as peripheral indicators of noradrenergic and dopaminergic activity that influence homeostatic drive states. The overall objective of the current research was to assess the relationship between reward responsivity and ASD features in children (N=76) with and without a clinical diagnosis of ASD. We assessed the relationship between reward responsivity and ASD by assessing whether quantitative features of reward responsivity (measured using eye blink rate, pupil response, and the Sensitivity to Punishment and Sensitivity to Reward Questionnaire for Children; SPSRQ-C) are linearly related to ASD features (measured using the Social Responsiveness Scale; SRS) and/or a clinical diagnosis of ASD. Resting pupil diameter and blink rate were measured during a passive eye tracking task that displayed alternating dark and light stimuli. We report significant relationships between resting pupil diameter and blink rate, quantitative measures of reward responsivity (SPSRQ-C), and ASD features (SRS). Given these relationships, we assessed whether measures of eye blink and pupil diameter could be used as a neurobiological correlate of reward response and predictor of clinically significant ASD features. In a binary logistic regression, we find that resting pupil measurements can be used as a significant predictor of ASD diagnosis. This research highlights individual differences across objective and behavioral measures of reward response that scale with the presence of ASD features. Finally, this work emphasizes that eye tracking technology can capture peripheral measures that correspond to underlying neurobiology in patients with below and above average cognitive ability, indicating promise for future use in clinical trials of heterogeneous populations.

Disclosures: A.S. Dicriscio: None. **V. Troiani:** None.

Poster

115. Behavioral Study and Animal Models for Autism Spectrum Disorders

Location: Hall A

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Program #/Poster #: 115.16/A54

Topic: A.07. Developmental Disorders

Support: NIH NINDS K08 NS094643
The University of Texas Rising STARs Faculty Award
Child Neurology Society Philip R. Dodge Young Investigator Award
Pediatric Epilepsy Research Foundation (PERF) Scientific Research Grant

Dell Medical School startup funds

Title: Dissection of prefrontal corticothalamic circuitry in the regulation of social behaviors

Authors: *F. MENG[1], A. ALARIO[1], A. C. BRUMBACK[2];
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Abstract: The medial prefrontal cortex (mPFC) plays a key role in regulating social behaviors. Previously work has shown that optogenetic activation of dopamine receptor 2 positive (*Drd2+*) neurons in mPFC disrupted social interaction in wildtype mice and in the prenatal valproic acid exposure (VPA) mouse model of autism. In addition, previously published work showed that in VPA mice, inhibition of *Drd2+* neurons in mPFC increased social exploration behavior. However, because mPFC *Drd2+* neurons project to multiple subcortical locations, the precise subcortical target responsible for these behaviors is unclear. The medial dorsal thalamus (MD) is one of main postsynaptic targets of the mPFC. Here, we tested the hypothesis that the mPFC→MD circuit is required for mPFC-dependent social behaviors. To do this, in wildtype mice we optogenetically activated the terminals of mPFC→MD projection neurons during exploration of novel mice and of novel objects. Then, to test the hypothesis that the abnormal social behavior of VPA mice could be modulated by selective inhibition of the mPFC→MD circuit, we optogenetically inhibited the terminals of mPFC→MD projection neurons in MD in VPA mice and saline controls. To find circuit-level convergence across autism models, we characterized social behavior in the fragile X mouse model of autism. In *Fmr1* knockout (KO) mice, we found abnormalities in social exploration. These differences in social exploration were not associated with general hyperactivity, anxiety, increased interest in novelty, or changes in olfaction. To test the hypothesis that targeting the mPFC→MD circuit would reverse social exploration differences in fragile X mice, we performed optogenetic manipulations of this circuit during social behavior assays in *Fmr1* wildtype and knockout mice. Our ongoing experiments are dissecting the cell types and subregions of the MD thalamus that mediate this social exploration behaviors and their links to abnormal social behavior in autism in both VPA and fragile X mouse models.

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Poster

115. Behavioral Study and Animal Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 115.17/A55

Topic: A.07. Developmental Disorders

Support: NIH R01 MH107515

Title: Gait development in genetic mouse models of autism and other neurodevelopmental disorders

Authors: *C. T. WEICHSELBAUM[1], K. B. MCCULLOUGH[2], S. E. MALONEY[3], J. D. DOUGHERTY[1];
[1]Genet. and Psychiatry, [2]Genet., [3]Psychiatry, Washington Univ. Sch. of Med., St. Louis, MO

Abstract: Motor abnormalities, including gait disruptions, are pervasive in neurodevelopmental disorders such as autism spectrum disorder (ASD) and Williams syndrome (WS). Motor impairments may emerge in the first year of life, often preceding the hallmark social-communicative deficits of ASD (Mosconi and Sweeney 2015), and include abnormalities in locomotor gait such as shorter stride length and greater stance width (Kindregan et al. 2015). As many as 80% of ASD-associated genetic syndromes are also associated with motor impairments (Mosconi and Sweeney 2015), but there have been few studies exploring how these various genetic forms of ASD may differ in their disruptions of gait. Related neurodevelopmental disorders such as WS also feature gait abnormalities (Hocking et al. 2009; Hocking et al. 2013) but have not been directly compared to those observed in ASD. As motor phenotypes lend themselves well to translational research, we propose that mouse models present a promising opportunity to examine gait development across multiple neurodevelopmental disorders in a high-throughput, genetically informative manner. Here we employ the DigiGait treadmill system (Mouse Specifics, Inc.) to quantify changes in spatial, temporal, and postural gait parameters from weaning to adulthood in three genetic mouse models: a SHANK3B deletion model of Phelan-McDermid syndrome and other non-syndromic ASD; an NF1 mutant model of neurofibromatosis associated with ASD; and the “complete deletion” model of WS, which is lacking the entire Williams syndrome critical region. Across these models, we observed changes in gait variability and delayed maturation of distinct gait parameters compared to wildtype littermate controls. We are further investigating how changes in body size may be mediating gait development. This work provides an important step toward linking gait with specific genetic disruptions in neurodevelopmental disorders.

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Poster

115. Behavioral Study and Animal Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 115.18/A56

Topic: A.07. Developmental Disorders

Support: U01-MH106882

Title: LINE1 regulates animal behavior impacting on schizophrenia associated phenotypes

Authors: *A. SARKAR[1], I. GALLINA[2], M. WANG[3], A. C. PAQUOLA[4], J. ERWIN[2], F. H. GAGE[3];
[1]Salk Inst., La Jolla, CA; [2]Salk Inst., San Diego, CA; [3]LOG-G, Salk Inst., La Jolla, CA; [4]Lieber Inst. For Brain Develop., Baltimore, MD

Abstract: DNA derived from mobile elements comprises nearly half of the human genome. Endogenously encoded Long Interspersed Element-1 (LINE-1 or L1) is a mobile element that causes somatic mosaicism in the human hippocampus and other regions. While it has been hypothesized that aberrant somatic L1 activity could be a response to environmental challenges and contribute to neurological disorders, direct evidence of L1 activation and the functional consequences of L1 mediated somatic mosaicism has remained elusive. Herein, we investigate the functional role of inflammation-driven L1 activity in contributing to neurological disorders. Maternal immune activation (MIA) during embryonic neurogenesis increases the risk of developing schizophrenia and autism and correlates with increased L1 copy number in mouse and macaque brain.
We established an *in vivo* mouse model to manipulate levels of L1 during MIA. Mice exposed to fetal MIA demonstrate impaired sensorimotor gating measured by prepulse inhibition. This parallels the sensorimotor-gating deficits observed in humans at high risk of schizophrenia. We demonstrate that attenuating L1 activity during fetal MIA specifically ameliorates the sensorimotor gating function without altering the acute pro-inflammatory immune response. These results show that excessive L1 activity specifically causes sensorimotor abnormalities as a result of a known environmental risk factor for schizophrenia and autism.

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Poster

115. Behavioral Study and Animal Models for Autism Spectrum Disorders

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Program #/Poster #: 115.19/A57

Topic: A.07. Developmental Disorders

Support: "Ricerca finalizzata e Giovani ricercatori" del Ministero della Salute

Title: Creatine transporter disorder: New insights into epileptic phenotype and diagnostic biomarkers

Authors: *F. CACCIANTE[1], L. BARONCELLI[2], G. SAGONA[2], M. GENNARO[2], L. LUPORI[1], R. MAZZIOTTI[3], E. PUTIGNANO[2], T. PIZZORUSSO[2];
[1]Scuola Normale Superiore, Pisa, Italy; [2]Neurosci. Institute, CNR, Pisa, Italy; [3]Univ. of Florence, Firenze, Italy

Abstract: Creatine (Cr) transporter (CrT) deficiency is an orphan disorder (CTD, OMIM #300352) characterized by intellectual disability, epilepsy and autistic-like behavior. Epilepsy is one of the symptoms with the greatest impact on everyday life of patients and families. Animal models are crucial tools to analyze disease mechanisms and to develop new therapeutic strategies. Four murine models of CTD are available so far. However, they have been analyzed only at the behavioral, neurochemical and anatomical level. To expand our knowledge about the face validity of the murine model, we monitored brain excitability and seizure susceptibility in the CrT knockout mice using video-EEG recording sessions. Our data show that CrT loss-of-function results in higher susceptibility to kainic acid (KA)-induced seizures, as assessed both at behavioral and electrophysiological level. Accordingly, we detected a prominent reduction of parvalbuminergic synapses in the cerebral cortex. This activity allowed us to fill a substantial gap in the current literature and to provide a more comprehensive set of normative data for the evaluation of potential therapeutic approaches. In addition, since CTD patients show an epileptic phenotype, we analyzed the EEG pattern of animals during spontaneous behaviours and found some specific band alteration that can be prognostic of seizures predisposition.

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Poster

115. Behavioral Study and Animal Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 115.20/A58

Topic: A.07. Developmental Disorders

Title: Gene replacement ameliorates deficits in mouse and hiPSC models of CDKL5 disorder

Authors: Y. GAO[1], E. E. IRVINE[1], I. ELEFTHERIADOU[1], C. JIMÉNEZ NARANJO[1], F. HEARN-YEATES[1], L. BOSCH[1], J. A. GLEGOLA[1], L. MURDOCH[1], A. CZERNIAK[1], I. MELONI[2], A. RENIERI[2], M. KINALI[3], *N. D. MAZARAKIS[1];
[1]Imperial Col. London, London, United Kingdom; [2]Univ. of Siena, Siena, Italy; [3]The Portland Hosp., London, United Kingdom

Abstract: CDKL5 disorder is a severe neurodevelopmental disorder caused by mutations in the X-linked cyclin-dependent kinase-like 5 (*CDKL5*) gene. It predominantly affects females that typically present with severe early epileptic encephalopathy, global developmental delay, motor

dysfunction, autistic features and sleep disturbances. To develop a gene therapy, we initially characterised the human *CDKL5* (*hCDKL5*) transcript isoforms expressed in the brain, neuronal cell lines, primary astrocytes and embryonic stem cell (ESC)-derived cortical interneurons, in which *hCDKL5_1* and to a lesser extent *hCDKL5_2* isoforms were found ubiquitously expressed. These isoforms were subsequently cloned into recombinant adeno-associated viral (AAV) vector genome and high-titre AAV vectors were produced. Intrajugular delivery of AAV-PHP.B-GFP vector in adult wild-type male mice transduced neurons and astrocytes throughout the brain more efficiently than AAV9 (1×10^{12} vg per animal, n = 3 per group). *Cdkl5* knockout (KO) male mice treated with AAV-PHP.B-*hCDKL5_1* vector via intrajugular injection at age 28-30 days exhibited significant behavioural improvements compared to GFP-treated controls (1×10^{12} vg per animal, n = 10 per group). AAV-DJ vectors efficiently transduced induced pluripotent stem cell (iPSC)-derived neural progenitors, which were subsequently differentiated into neurons. When treating *CDKL5*-mutant neurons, hCDKL5_1 expression led to an increased density of synaptic puncta on secondary dendrites, whilst hCDKL5_2 ameliorated the calcium signalling defect, implying distinct functions of these isoforms in neurons. This study provides the first evidence that AAV-mediated gene therapy can be utilised for treatment of CDKL5 disorder.

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Poster

115. Behavioral Study and Animal Models for Autism Spectrum Disorders

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Program #/Poster #: 115.21/A59

Topic: A.07. Developmental Disorders

Support: NIH INTRAMURAL GRANT
CAPPES
CNPQ

Title: Structural and functional connectomes reveal of brain rewiring in callosal dysgenesis

Authors: *D. SZCZUPAK[1], L. GEMAL[2], R. LENT[3], A. C. SILVA[4], F. TOVAR-MOLL[5]; [1]Natl. Inst. of Hlth., Bethesda, MD; [2]Inst. D'or for Res. and Educ., Rio de Janeiro, Brazil; [3]Inst. de Ciencias Biomedicas UFRJ, Rio de Janeiro, Brazil; [4]Dept. of Neurobio., Univ. of Pittsburgh, Pittsburgh, PA; [5]D Or Inst. For Res. and Educ. / UFRJ, Rio de Janeiro, Brazil

Abstract: Callosal dysgenesis (CD) is a neurodevelopmental syndrome that causes a malformation of the Corpus Callosum (CC). Although its ontogenetic mechanisms are still unknown, the anatomical changes in these patients are well documented. There are several different phenotypes in the morphology of the CC and different abnormal bundles that derive from the axonal misguidance, such as the Probst, sigmoid and inter-cortical bundles. We employed a connectomic approach to map the circuits of the whole brain and how they are rewired in this congenital malformation. For the structural connectome, we used the Human Connectome Project control data to compare them with 7 CD patients (5 complete agenesis and 2 hypoplasic). For the functional connectome we excluded one patient (complete agenesis) that could not acquire rsFMRI data. This approach allowed us to reveal a complete, whole brain rewiring in these patients. We showed a structural shift from interhemispheric to intrahemispheric connections that is not seen in the functional map. This suggests that somehow the patients' brains found a way to reconnect regions through alternative multisynaptic routs. Our findings strongly imply that CD is a disorder that does affect the corpus callosum only, but also affects the whole cerebral cortex, creating a different, rewired brain along development.

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Poster

115. Behavioral Study and Animal Models for Autism Spectrum Disorders

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Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 115.22/A60

Topic: A.07. Developmental Disorders

Support: Donors to TGen's C4RCD

Title: A *de novo* mutation in the ATP6V1A gene causing developmental encephalopathy with epilepsy is associated with mitochondrial dysfunction

Authors: *R. PANDEY[1,2], L. LLACI[1,2], A. SOGGE[1], C. BILAGODY[1,2], R. GUPTA[1], K. CHAIN[1], G. MILLS[1], V. NARAYANAN[1,2], S. RANGASAMY[1,2];
[1]Neurogenomics Div., Translational Genomics Res. Inst. (tgen), Phoenix, AZ; [2]Ctr. For Rare Childhood Disorders, Phoenix, AZ

Abstract: V-type proton (H+) ATPase (V-ATPase) is ATP-dependent proton pump involved in the active transport of hydrogen ions and plays ubiquitous role in pH homeostasis of endosomes, lysosomes, and other intracellular organelles. V-ATPase is a multi-subunit enzyme, which is composed of a cytosolic V1 domain that hydrolyses ATP (*ATP6V1A*) and a transmembrane V0 domain that translocate protons by a rotary mechanism. Recently, whole exome sequencing (WES) has identified *de novo* heterozygous mutation (p. Pro27Arg, p.Asp00Tyr, p.Asp349Asn,

p.Asp371Gly) in the ATP6V1A gene encoding the V1A subunit of the V-ATPase in patients with developmental encephalopathy with epilepsy. The heterozygous variant p. Pro27Arg was identified at Center for Rare Childhood Disorders (C4RCD). To understand the biology of the ATP6V1A mutation, we established patient-derived dermal fibroblast cultures. Patient-derived ATP6V1A-mutant fibroblasts demonstrated increased vacuolar pH levels indicating that p. Pro27Arg mutations may cause reduced function of V-ATPase . The patient fibroblast also showed decreased Lysosomal protein (LAMP-1) without any alterations in the EEA1 levels compared to controls. Interestingly, patient cell also displayed markedly reduced ATP synthesis machinery molecules such as complex I, II, IV and ATP synthase or Complex V. This was accompanied by significantly less mitochondrial ATP generation, which was measured by ATP-monitoring luminescence assay in patient derived fibroblasts. Furthermore, mutated fibroblasts significantly down-regulated TOM20 protein levels as compared to control cells. Collectively, our study provides evidence that p. Pro27Arg de novo heterozygous mutation in ATP6V1A is associated with mitochondrial dysfunctions

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Poster

115. Behavioral Study and Animal Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 115.23/A61

Topic: A.07. Developmental Disorders

Title: Decreased parvalbumin expression is correlated with tactile hypersensitivity in Shank3$^{-/-}$ and Cntnap2$^{-/-}$ mice models of autism

Authors: *T. DEEMYAD[1], S. PUIG[1], H. QI[3], A. E. PAPALE[2], E. LANOCE[3], N. N. URBAN[2]; [2]Dept. of Neurobio., [1]Univ. of Pittsburgh, Pittsburgh, PA; [3]Univ. of Pittsburgh, Dept of Neurobio., Pittsburgh, PA

Abstract: Hypersensitivity to light touch and tactile defensiveness are common symptoms in patients with autism spectrum disorders (ASD). However, the underlying neuronal changes leading to these symptoms are not known. An alteration in the balance between excitation and inhibition (E/I balance) is thought to be commonly altered in ASD and the activity of parvalbumin (PV) positive interneurons is critical for maintaining the E/I balance. Recent studies suggest that the number of cortical PV cells is decreased in ASD patients as well as in several mouse models of ASD. To investigate whether changes in interneurons underlies the alteration in light touch sensitivity in ASD, we compared expression of PV, Somatostatin (SST), Calbindin (CB) and Calretinin (CL) in the somatosensory cortex of two genetic mouse models of ASD, (contactin associated protein-like 2 knockout (Cntnap2$^{-/-}$) and SH3/ankyrin domain gene 3

knockout (Shank3$^{-/-}$). Consistent with previous studies, we found that the expression level of PV was decreased in interneurons in the somatosensory cortex of Cntnap2$^{-/-}$ and Shank3$^{-/-}$ animals with no change in SST, CB or CL or the overall number of interneurons. Notably, we found that the reduction in PV expression was stronger in one hemisphere. To investigate whether such lateralization could correlate with hypersensitivity to tactile stimulation (i.e., similar to that observed in ASD patients), we evaluated bilateral hind paw sensitivity to mechanical and thermal stimulation using Von-Frey and Hargreaves tests respectively. Left and right hind paw sensitivities were compared and a potential correlation with lateralized changes in PV cells in the somatosensory cortex were investigated. There was no difference in thermal sensitivity between left vs. right hind paws of WT and ASD mice. In addition, while there was no difference in mechanical sensitivity between the two sides in WT mice (left/ right ratio: 1.13 ± 0.12), the ratio of the low intensity mechanical stimulation on the two sides in ASD models almost doubled (Shank3$^{-/-}$: 2.34 ± 0.7, Cntnap2$^{-/-}$: 2.10 ± 0.52, two-way ANOVA, p < 0.001 re WT). This effect was due to an increase in sensitivity on one side, which correlated with a reduced expression of PV in the contralateral somatosensory cortex in the same animals. These results suggest that lateralized changes in PV expression in the somatosensory cortex may underlie the tactile hypersensitivity and defensiveness in ASD patients.

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Poster

116. Autism: Synaptic and Cellular Mechanisms II

Location: Hall A

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Program #/Poster #: 116.01/A62

Topic: A.07. Developmental Disorders

Support: The research was supported by the Cognitive and Neurobiological Approaches to Plasticity (CNAP) Center of Biomedical Research Excellence (COBRE) of the National Institutes of Health under grant number P20GM113109.

Title: A custom movement tracking software for behavioral analyses of an adult zebrafish shank3-mutant model of autism

Authors: C. TUDOR[1], L. WANER[1], J. STONEBREAKER[2], J. NEWELL[1], J. E. DALLMAN[3], P. PRAKASH[2], ***T. MUELLER**[1];
[1]Div. of Biol., [2]Electrical and Computer Engin., Kansas State Univ., Manhattan, KS; [3]Dept. of Biol., Univ. of Miami, Coral Gables, FL

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder that affects more than 1% of the world population. ASD is characterized by social and cognitive deficits, aberrant

fear responses and altered sensory processing. The pathogenesis of ASD is not well understood because it involves complex interaction between genetic, neurobiological and environmental factors. Zebrafish due to its genetic amenability and overall conserved vertebrate specific brain organization represents an increasingly important model organism to study neural circuits of behavior in health and disease. Using zebrafish as a model, our overall objective aims to dissect the neural circuitry underlying autistic characteristics with a focus on odor-driven behaviors and sensory processing. Towards this aim, we have generated a software tool for movement tracking behavior applied to a recently established zebrafish *shank3a/b*double mutant model of autism. Specifically, we compare normal wt versus mutant models in behavioral paradigms of fear conditioning and social-approach behavior. The results of this project will form the foundation for detailed neural circuit analyses focusing on aberrant behavioral output as it occurs in patients with autism.

Disclosures: C. Tudor: None. **L. Waner:** None. **J. Stonebreaker:** None. **J. Newell:** None. **J.E. Dallman:** None. **P. Prakash:** None. **T. Mueller:** None.

Poster

116. Autism: Synaptic and Cellular Mechanisms II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 116.02/A63

Topic: A.07. Developmental Disorders

Support: NIH Grant P20GM10340

Title: Assessment of Shank3 expression at excitatory synapses in the cerebellar cortex

Authors: *R. J. HYDE[1], B. D. RICHARDSON[2,3];
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Abstract: Multidomain proteins Shank1, Shank2, and Shank3 are core components of postsynaptic densities at many excitatory synapses. Mutations of Shank genes, Shank3 in particular, are associated with ASD and like disorders (e.g. Phelan-McDermid Syndrome). Similarly, the absence of specific Shank3 isoforms in mice result in ASD-like behavioral phenotypes often used to understand the role of Shank3 in brain regions like striatum, hippocampus, and cortex. However, little is known about the role of Shank3 in cerebellar function, despite high Shank3 (isoforms c and d) expression in cerebellar granule cells, our budding understanding of the cerebellum's role in both motor and non-motor circuits, and implications for cerebellar involvement in ASD. To understand how Shank3 may be involved in glutamatergic transmission in the cerebellum, adult male and female wildtype C57bl/6 mice (n=5) were euthanized and cerebelli extracted and sectioned. Shank3, pre-, and postsynaptic protein expression patterns in the cerebellar cortex were determined using

immunohistochemistry and imaged using a Nikon Spinning Disk Confocal microscope, then qualitatively analyzed for co-expression patterns. Shank3 is expressed in areas surrounding nearly all VGLUT1/2-expressing presynaptic mossy fiber terminals, composing classical rosette structures. Likewise, Shank3 co-localizes with specific glutamatergic receptor subtypes/subunits in the granule cell layer, many of which are specifically expressed in cerebellar granule cells and include NR2C, GluA2, GluA4, and GluK5. While NR2C, GluA2, and GluA4 colocalized with a higher percentage of Shank3 protein (nearly 1:1), GluK5 and Shank 3 were localized, but not exclusively. These data indicate the nature of excitatory synaptic transmission in the cerebellar cortex may depend upon signaling by Shank3, a known ASD-related gene/protein. As such, work to understand the precise functional role of Shank3 in mossy fiber-granule cell synaptic transmission may be key in understanding how a known ASD-related gene/protein could perturb signal integration in the cerebellum. These data highlighting the importance of and support broadening the research focus to include a deeper understanding of the cerebellum's role in ASD.

Disclosures: R.J. Hyde: None. **B.D. Richardson:** None.

Poster

116. Autism: Synaptic and Cellular Mechanisms II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 116.03/A64

Topic: A.07. Developmental Disorders

Support: Autism Research Trust
Commonwealth scholarship
EU-AIMS

Title: Investigating the role of autism related presynaptic NRXN1 and postsynaptic SHANK3 in synaptic mechanisms using human stem cell derived cortical neurons

Authors: *A. PAUL[1], A. MASSRALI[1], L. D. POLIT[2], N. GATFORD[2], D. ADHYA[2], D. P. SRIVASTAVA[2], M. R. KOTTER[3], S. BARON-COHEN[1];
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Abstract: Background: NRXN1 and SHANK3 are presynaptic and postsynaptic cell adhesion molecules respectively and are known to be highly penetrant for syndromic and idiopathic autism. However, the contribution of NRXN1 and SHANK3 towards autism pathophysiology through the regulation of presynaptic neurotransmitter release and activity dependent

homeostatic plasticity is not well characterised in human neurons.**Objectives:**The aim is to study NRXN1 and SHANK3 deletion-mediated differences in the structure and function of synapses and to investigate whether and how presynaptic NRXN1 affects presynaptic neurotransmitter release and postsynaptic SHANK3 mediate homeostatic control of activity dependent plasticity.**Methods:** In this study we are using iPSCs from individuals with deletions in SHANK3 (n=2, 1 male, 1 female), NRXN1 (n=2, 1 male, 1 female) and healthy controls (n=2). NGN2-GFP was introduced in the iPSCs using lentiviral vectors to generate excitatory cortical neurons for assays. The neurons were tested for expression of MAP2 and synaptic markers such as Synaptophysin and Homer1. The expression of immediate early genes were measured with qRT PCR. Neurite formation was assayed using live imaging of GFP-tagged NGN2 neurons. Presynaptic release of synaptic vesicles was measured using FM4-64X dye under stimulating conditions. The spontaneous and activity dependent firing was measured with calcium imaging under non-stimulating and stimulating conditions. The network activity of neurons was further measured using extracellular (multielectrode arrays). **Results:**There was no significant change in presynaptic and postsynaptic puncta number in NRXN1 and SHANK3 lines but calcium imaging revealed impairment in synaptic connectivity induced by channel blockers. Moreover, the neurite outgrowth assay on patient lines demonstrate impaired neuronal migration due to changes in the ratio of filamentous to globular actin. There was a significant change in rate of spontaneous firing (mean firing rate) ($P<0.005$) in all autism lines. Currently we are generating isogenic hiPSC lines by introduction of deletions of NRXN1 and/or SHANK3 gene in wildtype hiPSC lines with CRISPR-Cas9 gene editing technique to validate the results.**Conclusions:** Loss of function of autism related synaptic cell adhesion molecules such as presynaptic NRXN1 and postsynaptic SHANK3 may not have any immediate effect on synaptic puncta number but affects synaptic mechanisms through distinct molecular mechanisms. NRXN1 affects presynaptic release of synaptic vesicles whereas SHANK3 is essential for homeostatic control of activity dependent synaptic plasticity.

Disclosures: **A. Paul:** None. **A. Massrali:** None. **L.D. Polit:** None. **N. Gatford:** None. **D. Adhya:** None. **D.P. Srivastava:** None. **M.R. Kotter:** None. **S. Baron-Cohen:** None.

Poster

116. Autism: Synaptic and Cellular Mechanisms II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 116.04/A65

Topic: A.07. Developmental Disorders

Support: HIAS#15007
HIAS#18005
HIAS#15003
HIAS#18004

Title: Altered expression of cadherin-8 and cadherin-11 suggests their potential roles in excitatory synapse development and autism

Authors: *J. A. FREI, R. F. NIESCIER, J. E. NESTOR, M. W. NESTOR, G. J. BLATT, Y.-C. LIN;
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Abstract: Autism is a neurodevelopmental condition, characterized by both phenotypic and genetic heterogeneity. The condition currently affects 1 in 59 individuals in the United States and is diagnosed by core symptoms in social interaction, communication and repetitive patterns of behavior. The genetic complexity is reflected by the fact that no single gene identified contributes to more than 1% of autism cases. To date, the cause(s) of autism is not known, however association studies have identified a multitude of risk genes that may contribute to its etiology. Many of these risk genes converge into common pathways. The cadherin superfamily, one of the largest families of cell adhesion molecules, may represent such a vulnerable pathway. The cadherin family comprises more than one hundred proteins, which are further grouped into subfamilies including classical type I and II cadherins, clustered and non-clustered protocadherins and atypical FAT cadherins. Although the functions of the majority of the cadherins are not fully understood, genome-wide association implicates cadherins across all subfamilies as candidate risk genes.
The overall goal of this study is to understand the involvement of two autism-associated type II classical cadherins, cadherin-8 (CDH8) and cadherin-11 (CDH11), in excitatory synapse development and autism. We revealed that the temporal expression of both, CDH8 and CDH11, coincides in time with the formation of synapses, a process that has been found to be affected in autism. The distribution of CDH8 and CDH11 within the cell further demonstrated enrichment of these proteins in the synapses. Induced pluripotent stem cell (iPSC)-derived neurons from individuals with autism showed decreased levels of CDH11 and elevated levels of CDH8. Using CDH11 knockout mice, we found similarly increased levels of CDH8 protein in forebrain lysates. The excitatory postsynaptic cell adhesion molecule Neuroligin-1, which we identified as a binding partner of CDH8, but not CDH11, as well as PSD95 also showed increased expression in CDH11 knockout mouse brains. Together, these results provide evidence that CDH8 and CDH11 are involved in regulating the development of excitatory synapses. Alterations in the balance between CDH8 and CDH11, as observed in autism-derived neurons, may result in an increase in the number of dendritic spines. Increased dendritic spine densities have been observed in brains of some autism individuals, indicating a mechanism by which CDH8 and CDH11 may be involved in the etiology of autism.

Disclosures: J.A. Frei: None. **J.E. Nestor:** None. **M.W. Nestor:** None. **G.J. Blatt:** None. **Y. Lin:** None. **R.F. Niescier:** None.

Poster

116. Autism: Synaptic and Cellular Mechanisms II

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Program #/Poster #: 116.05/A66

Topic: A.07. Developmental Disorders

Support: Intramural Research Grant for Neurological and Psychiatric Disorders of NCNP

Title: Abnormal postnatal development of synaptic structure and function in valproate-induced autism model marmosets

Authors: *S. WATANABE, T. KUROTANI, T. OGA, K. NAKAGAKI, J. NOGUCHI, N. ICHINOHE;
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Abstract: Autism spectrum disorder (ASD) is characterized by impaired social interaction and communication, restricted interests, and repetitive behaviors. Although various rodent models have been used for studying synaptic pathology in ASD, synaptic development is highly different between rodents and primates, making the results in rodents difficult to translate to human ASD study. Recently, we have developed an ASD model of a primate (the common marmoset) with exposure to valproic acid (VPA) in utero. The VPA-exposed marmosets show abnormalities in social behavior as seen in human ASD (Yasue et al. Behav. Brain Res. (2015) 292: 323). To explore synaptic abnormalities in the brain region related to social behavior in the ASD model marmoset, we prepared acute slices from the dorsomedial prefrontal cortex (area 8b/9) of VPA-exposed and unexposed (UE) marmosets at the ages of neonates, 3 months as late toddlerhood, and 6 months as early adolescence, and then performed electrophysiological and structural analyses in layer 3 pyramidal neurons.
We revealed that in neonates, the density of dendritic spines was lower in VPA animals than in UE animals, but the spine density was normal or slightly higher in VPA animals at 3 and 6 months compared to UE animals. In contrast, the spine volume was normal in neonates and at 6 months, but was lower in VPA animals at 3 months, suggesting a reduced synaptic efficacy at this age. Electrophysiological analyses revealed that in neonates, the frequencies of both excitatory and inhibitory miniature postsynaptic currents (mEPSCs and mIPSCs) were lower in VPA animals. At 3 months, the mEPSC frequency was also lower in VPA animals, but the mIPSC frequency was similar for UE and VPA animals. At 6 months, both the mEPSC and mIPSC frequencies were similar for UE and VPA animals. As expected from these data, the excitatory/inhibitory (E/I) ratio of evoked synaptic currents was lower in VPA animals at 3 months, while the E/I ratio was similar for UE and VPA neonates and at 6 months. These results suggest that both excitatory and inhibitory synapses are maldeveloped in the ASD model marmoset at the early stage of postnatal development, and that they become normalized in

different time courses. The transient reduction in the E/I ratio around 3 months may be crucial for the development of ASD.

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Poster

116. Autism: Synaptic and Cellular Mechanisms II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 116.06/A67

Topic: A.07. Developmental Disorders

Support: FAPESP #2017/06100-8 to SC
FAPESP #2010/16469-0 to CJ
CNPq to LRGB
CAPES to EMGS

Title: Synaptic disturbances in the retina of a valproic acid mouse model of autism spectrum disorder

Authors: *S. CHIAVEGATTO[1,2], C. JOSELEVITCH[3], L. G. BRITTO[1], E. M. GUIMARÃES-SOUZA[1];
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Abstract: Autism spectrum disorder (ASD) is a developmental disorder that affects communication and behavior. Symptoms generally appear in the first two years of life, when children have difficulty to maintain eye contact and to develop speech. ASD patients are also hypersensitive to sensory stimuli. In this regard,the retina is the primary sensory detector for vision and is the most accessible part of the CNS to study brain wiring and function. We investigated the retinal function and some proteins' expression in adolescent male mice prenatally exposed to the valproic acid (VPA), as a useful model for ASD. **Methods:** Female C57BL/6 mice received VPA (600 mg/kg, *ip*) or saline at the gestational day 11. Their male adolescent pups (P29-35) were tested for anxiety and social interaction. Their retinal function was assessed by full-field scotopic electroretinograms (ERGs), and some retinal proteins related to synaptic function by immunoassays. **Results:** Adolescents prenatally exposed to VPA are smaller and showed increased anxiety-like behaviors and impaired social interest. The a-wave amplitudes of retinas from VPA-exposed mice were smaller than those of CTR animals, whereas no differences were found in b-wave or oscillatory potentials. The glutamatergic receptor mGluR5 was more expressed in the retinas of VPA mice, while GABA, GAD, synapsin-1 and FMRP proteins were reduced when compared to controls. **Conclusions:** Adolescent male mice

prenatally exposed to VPA (at the E11) display behaviors compatible to ASD. They have alterations in retinal function, suggesting photoreceptor dysfunctions, with cones probably more affected than rods, and altered expression of synaptic, glutamatergic and GABAergic markers in both synaptic layers of the retina. These results support the view that synaptic disturbances with excitatory/inhibitory imbalance early in life are implicated in autism phenotypes and highlight the importance to study the retina as a reliable earlier marker in ASD.

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Poster

116. Autism: Synaptic and Cellular Mechanisms II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 116.07/A68

Topic: A.07. Developmental Disorders

Title: Exclusive antagonists of extrasynaptic NMDA receptors for autism

Authors: *A. SAVTCHENKO[1], S. MOORE[2], E. MOLOKANOVA[3], A. R. MUOTRI[2]; [1]Nanotools Biosci., Encinitas, CA; [2]Pediatrics, Univ. of California, San Diego, CA; [3]NeurANO Biosci., Encinitas, CA

Abstract: Autism spectrum disorders (ASDs) are neurodevelopmental conditions that manifest in aberrant social communications and repetitive behaviors. Altered function of NMDA receptors (NMDARs) has been reported in several animal ASD models, but specific roles of synaptic and extrasynaptic NMDARs have not been elucidated. We hypothesized that targeting of eNMDARs may be beneficial in ASDs, because a) extracellular glutamate levels were reported to be elevated in ASD brains, suggesting that eNMDAR overactivation can play a role in the excitability of neuronal networks in ASDs, and b) ASD-prevalent mutations in synaptic anchoring proteins might lead to the increase in eNMDARs due to their lateral diffusion as observed in Huntington's disease.
Memantine, an FDA-approved drug with preferential activity toward eNMDARs, demonstrated promising results in animals, but did not perform well in clinical trials, partly because its concentration had to be kept low (below IC_{50}) to avoid side effects related to inhibition of synaptic NMDARs.
Recognizing the need for exclusive rather than preferential modulators of eNMDARs, we employed a rational drug design strategy (Savchenko et al., 2016) to engineer an eNMDAR antagonist that due to its steric properties is incapable of reaching sNMDARs inside the synaptic cleft while still modulating eNMDARs. Our exclusive eNMDAR antagonist, dubbed AuM, has ~50 memantine molecules conjugated to a 13-nm Au nanoparticle, and exhibits a hydrodynamic diameter of ~35 nm.

We injected 3-day old wild-type (WT) and *Sedt5*+/-mice (Moore et al., 2019) with 2 µl AuM or saline into each ventricle. The 3-month survival rate in all groups was 100%. Animals were monitored for signs of abnormal behavior (locomotion, eating, sleeping), and according to veterinarian's evaluation, appeared to be normal. Three months after injection, animals were subjected to several behavioral tests. We found no statistically significant differences between AuM- and saline-injected WT animals on any of these tests, while AuM improved the performance of *Sedt5*+/- mice.
Based on these findings, we can conclude that targeting of eNMDARs is a valid therapeutic approach for ASDs.

Disclosures: A. Savtchenko: None. **S. Moore:** None. **E. Molokanova:** None. **A.R. Muotri:** None.

Poster

116. Autism: Synaptic and Cellular Mechanisms II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 116.08/A69

Topic: A.07. Developmental Disorders

Support: DST-SERB (SB/YS/LS-215/2013; 2014-2017

Title: Chloride co-transporters: Major contributors in pathophysiology of SYNGAP1$^{-/+}$ mutation

Authors: *V. VERMA[1], T. BEHNISCH[2], V. VAIDYA[3], R. S. MUDDASHETTY[4], J. P. CLEMENT[1];
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Abstract: In the past few decades, hundreds of genetic loci are reported to contribute or cause Intellectual Disability (ID) and Autism Spectrum Disorder (ASD), which affects 1-3% of world population. Autosomal-dominant *de novo* heterozygous mutation in *SYNGAP1* has been reported as a cause of ID. *SYNGAP1* encodes synaptic RAS-GAP, SYNGAP1, which is downstream of NMDA receptors, and negatively regulates RAS, and AMPA receptor trafficking to post-synapse. Using *Syngap1*$^{+/-}$ mouse model, studies have shown that this mutation leads to accelerated maturation of dendritic spine synapses, along-with premature spine pruning, and altered critical period of plasticity during early stages of development. This leads to abnormal excitatory connections that may alter excitatory to inhibitory (E/I) balance. Studies have shown that GABA is the major inhibitory neurotransmitter in central nervous system, which is excitatory in early stages of development and inhibitory in later stages of development. Two Cl^- co-transporters in brain, NKCC1 and KCC2, mediate the dual function of GABA. NKCC1 expressed during early stages of development allows Cl^- to enter the cell, which makes GABA

excitatory. Towards the end of neuronal maturation, i.e. around 1-2 weeks in hippocampus, KCC2 becomes functional that allows extrusion of Cl^- ions and, thus, makes GABA inhibitory. Moreover, KCC2 is shown to gate AMPA receptor trafficking to post-synapse via cofilin phosphorylation through RAC1 pathway, which is shown to be altered in ID models like Rett and Fragile X syndrome. In this study, we are trying to understand the impact of *Syngap1*$^{+/-}$ in maturation and function of KCC2 and NKCC1 in early stages of development. Studying this might enable the possibility of rewiring of the neuronal connections in the adult brain by targeting KCC2 with commercially available drugs, which eventually would help to rescue the physiological and behavioural phenotypes in *Syngap1*$^{+/-}$ mice.

Disclosures: V. Verma: None. **T. Behnisch:** None. **V. Vaidya:** None. **R.S. Muddashetty:** None. **J.P. Clement:** None.

Poster

116. Autism: Synaptic and Cellular Mechanisms II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 116.09/A70

Topic: A.07. Developmental Disorders

Support: GGP16131

Title: Abnormal behaviors and neuronal functions in mice lacking Shank3 in parvalbumin-expressing interneurons

Authors: *J. PAGANO[1,2], L. PONZONI[2], M. SALA[1], F. TEMPIA[3], T. M. BÖCKERS[4], C. SALA[1,2], C. VERPELLI[1,2];
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Abstract: Shank1, Shank2 and Shank3 are scaffold proteins located in the postsynaptic density of excitatory glutamatergic synapses, essential for synaptic function and development. Mutations in *SHANK* genes are strongly associated with Autism Spectrum Disorders (ASDs); in particular, *SHANK3* haploinsufficiency is considered the major cause of the neurological symptoms of Phelan McDermid Syndrome (PMS), a neurodevelopmental disorder characterized by expressive language delay, intellectual disability, hypotonia, autistic-like behaviour, and epilepsy. Since an imbalance between excitatory and inhibitory systems may lead to autism symptoms, we hypothesized that some neuropathological features described in PMS patients might be due to alterations of the inhibitory system. Indeed the function of Shank3 in excitatory synapses on inhibitory neurons has been not fully explored. We thus generated *PV-cre*$^{+/-}$ *Shank3* $^{flox/wt}$ mice, in which *Shank3* is deleted selectively in parvalbumin positive (PV+) interneurons. We

performed behavior tests in adult mice and discovered an impairment in grooming, learning, and memory tasks. Furthermore, we demonstrated that treatment with Ganaxolone, a positive modulator of $GABA_A$ receptors, improves the altered phenotypes of mice. The results of our study suggest that GABAergic system might represent a pharmacological target to develop new treatment for patients with PMS.

Disclosures: **J. Pagano:** None. **L. Ponzoni:** None. **M. Sala:** None. **F. Tempia:** None. **T.M. Böckers:** None. **C. Sala:** None. **C. Verpelli:** None.

Poster

116. Autism: Synaptic and Cellular Mechanisms II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 116.10/A71

Topic: A.07. Developmental Disorders

Support: GGP16131

Title: A novel positive allosteric modulator of mGlu5 receptor rescues behavioral and synaptic defects in Shank3 knock-out mice

Authors: *F. GIONA[1,2], L. PONZONI[2], S. LANDI[3], A. TOZZI[4], M. SALA[1], G. RATTO[3], C. W. LINDSLEY[5], C. K. JONES[5], T. M. BÖCKERS[6], C. SALA[1,2], C. VERPELLI[1,2];
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Abstract: Shank proteins are large scaffold proteins located at post-synaptic density (PSD) of excitatory synapses which have a crucial role in formation, maturation and function of synapses. It is known that haploinsufficiency of SHANK3 is the major cause of neurological symptoms associated with Phelan-McDermid Syndrome (PMS), which include hypotonia, speech delay and autistic behaviour. Indeed, we recently demonstrated that SHANK3 is essential to mediate mGlu5 receptor signalling by recruiting Homer1b/c, another scaffold protein, to the PSD (Vicidomini et al. 2017). In order to better clarify if positive allosteric modulators (PAMs) of mGlu5 might rescue the synaptic and behavioral alterations of Shank3 KO mice we tested the ability of VU0409551, a potent and selective positive allosteric modulator of mGlu5 receptor, to rescue behavioural and synaptic dysfunction in *Shank3Δ11* $^{-/-}$ mice. We found that the acute treatment with VU0409551 rescues repetitive and stereotyped behaviours, social impairments and intellectual inflexibility observed in *Shank3Δ11* $^{-/-}$ mice. Moreover, we found a specific reduction of protein translation, measured by SUnSET methodology, in cortex and striatum of *Shank3Δ11* $^{-/-}$ mice that can be rescued by chronic treatment with VU0409551. In summary our

results suggest that mGlu5 signalling is impaired in *Shank3Δ11* $^{-/-}$ mice and that mGlu5 PAMs may represent a new pharmacologic approach for ameliorating symptoms of patients affected by PMS.
Vicidomini C, Ponzoni L, Lim L, Schmeisser M, Reim D, Morello N, Orelanna D, Tozzi A, Durante V, Scalmani P, Mantegazza M, Genazzani AA, Giustetto M, Sala M, Calabresi P, Boeckers TM, Sala C, Verpelli C (2017) Pharmacological enhancement of mGlu5 receptors rescues behavioral deficits in SHANK3 knock-out mice. Mol Psychiatry 22:689-702.

Disclosures: F. Giona: None. **L. Ponzoni:** None. **S. Landi:** None. **A. Tozzi:** None. **M. Sala:** None. **G. Ratto:** None. **C.W. Lindsley:** None. **C.K. Jones:** None. **T.M. Böckers:** None. **C. Sala:** None. **C. Verpelli:** None.

Poster

116. Autism: Synaptic and Cellular Mechanisms II

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Program #/Poster #: 116.11/A72

Topic: A.07. Developmental Disorders

Support: CONICYT PAI 77180077
CONICYT Fondecyt 11180540
NIH-R01-EY014074
HHMI

Title: Role of the autism spectrum disorder associated gene VPS50 in synaptic function

Authors: *F. J. BUSTOS[1], H. HAENSGEN[1], H. HORVITZ[2], M. CONSTANTINE-PATON[2]; [1]Univ. Andrés Bello, Santiago, Chile; [2]MIT, Cambridge, MA

Abstract: Introduction: Autism Spectrum Disorders (ASD) are incurable and debilitating psychiatric syndromes that have a prevalence of 1 in every 50 children every year. Many studies have tried to identify a single gene that could account for the synaptic defects and phenotype observed in subjects with ASD. However, to date there are more than 400 genes that have been associated with high risk for causing ASD. Many of them have not been characterized on their specific role controlling synaptic transmission. Here we show the functional characterization of VPS50, an ASD associated gene, that controls synaptic transmission by reducing synaptic vesicle acidification.
Material and Methods: To infect neuronal cultures, adeno associated viruses (AAV) coding for CRISPR/Cas9 were designed to specifically knock out VPS50. 7 days after infection deletions in the genomic sequence were determined, and also changes in expression of VPS50 by Western blot and RT-qPCR. The genetically encoded probe SyPhy was used to determine synaptic vesicle acidification. Electrophysiology recordings and calcium imaging were used to determine

synaptic defects of infected neurons.
Results: Our results show that neurons infected with CRISPR/Cas9 targeting VPS50 have indels in their genomic sequence. These indels produce the KO of VPS50 expression measured by mRNA and proteins levels. Using SyPhy we determined that VPS50 KO neurons show decreased acidification of synaptic vesicles. Electrophysiology recordings and calcium imaging demonstrates that neurons KO for VPS50 display deficient synaptic function.
Discussion: Our findings provide insights into the mechanisms by which an associated ASD gene produces synaptic defects. We show that disrupting vesicle acidification by VPS50 KO have severe deficits on synaptic function. We propose that artificially acidifying synaptic vesicles could recover these deficits.

Disclosures: F.J. Bustos: None. **H. Haensgen:** None. **H. Horvitz:** None. **M. Constantine-Paton:** None.

Poster

116. Autism: Synaptic and Cellular Mechanisms II

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Program #/Poster #: 116.12/A73

Topic: A.07. Developmental Disorders

Support: Spectrum Health-MSU Alliance Corporation
UCSF CTSI 1111111 (DV)

Title: Depletion of parvalbumin-positive cortical interneurons and persistence of immature mge-derived oligodendrocytes following conditional biallelic deletion of Nf1 from the medial ganglionic eminence

Authors: *K. P. ANGARA[1], L.-L. PAI[3], A. M. STAFFORD[1], J. NGUYEN[2], J. L. RUBENSTEIN[4], D. VOGT[1];
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Abstract: Neurofibromatosis 1 (NF1), a neuro-cardio-facial-cutaneous (NCFC) syndrome, is a monogenic disorder caused by mutations in the *Nf1* gene, whose protein product, Neurofibromin, inhibits Ras GTPase activity thereby altering RAS/MAPK signaling. Importantly, RAS/MAPK signaling is perturbed in multiple conditions associated with autism spectrum disorder (ASD). NF1 is characterized by café au lait spots, two or more neurofibromas, freckling in the axillary and inguinal regions, optic gliomas and lisch nodules. NF1 often presents clinically with a co-morbid diagnosis of intellectual disability (ID) and/or ASD, with the underlying brain abnormalities and associated cognitive symptoms poorly understood, thus prompting the need for

new assessments. We conditionally deleted (using *Nkx2.1-Cre*) *Nf1* from the medial ganglionic eminence (MGE), an embryonic forebrain progenitor domain that gives rise to a multitude of cells, including early born oligodendrocytes (OGs), cholinergic striatal interneurons and cortical GABAergic interneurons (CINs). Deletion of *Nf1* resulted in a neurobiological phenotype with surprising alterations in both OGs and CINs. Our *Nf1* model induced persistence of immature MGE-lineage OGs in the neocortex that prevented later born OGs from occupying the same regions. Moreover, there was a specific reduction in parvalbumin (PV)-expressing CINs without any changes in other MGE-lineage interneurons. Notably, post-natal deletion of *Nf1* in PV+ CINs did not affect their numbers in the cortex, suggesting important roles for *Nf1* during development. These findings report novel insights into the role of *Nf1*, and potentially RAS/MAPK signaling, which may underlie associated cognitive symptoms in NF1. Moreover, these findings may elucidate potential mechanisms and offer interventional therapeutic targets in ASDs and other RASopathies.

Disclosures: K.P. Angara: None. **L. Pai:** None. **A.M. Stafford:** None. **J. Nguyen:** None. **J.L. Rubenstein:** None. **D. Vogt:** None.

Poster

116. Autism: Synaptic and Cellular Mechanisms II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 116.13/A74

Topic: A.07. Developmental Disorders

Title: Elucidation of molecular mechanism that induce abnormal excitatory synapse formation in valproic acid-induced rodent model of autism spectrum disorders

Authors: *F. YOSHIDA[1], R. NAGATOMO[1], M. KIMURA[1], G. ITO[2], S. TAKATORI[1], T. TOMITA[3];
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Abstract: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by social communication deficits and restricted behaviors or interests. Several genetic and environmental factors have been implicated in the pathogenesis of ASD, and genetic variants identified in ASD patients are identified on genes encoding synaptic proteins. These variations cause aberrant synaptogenesis and synaptic plasticity, supporting the notion that abnormal excitatory and inhibitory (E/I) balance is involved in the pathological process of ASD. Maternal valproic acid (VPA) use during pregnancy has been associated with a higher probability of ASD in the offspring. Previous studies showed that the VPA treatment caused the abnormal E/I balance in *in vivo* and *in vitro* rodent models, although the pathological mechanism of VPA on the synaptogenesis remains largely unknown. To clarify the molecules involved in the VPA-

induced abnormal E/I balance, we analyzed the primary neuronal culture obtained from maternal VPA treated pups. We found an excessive formation of excitatory, but not inhibitory, presynapses in this culture system. Moreover, the conditioned medium of maternal VPA-treated neurons contained the aberrant excitatory presynapse organizer activity. To identify the proteins responsible to this activity, we applied the Secretome Proteins Enrichment with Click Sugars (SPECs) method (Kuhn et al., EMBO J 2012). In combination with the metabolic glycan labelling and the click chemistry-mediated biotinylation, SPECS enables us to identify the secreted proteins at proteome-wide level without contamination of cellular- and serum-derived proteins. We identified 145 proteins, and then quantitatively compared the secretome profile of the conditioned medium from maternal VPA-treated primary neurons with that from control primary neurons. We found that the levels of three proteins were significantly changed in the medium from the maternal VPA-treated neurons. Currently we are verifying the effects of these proteins on the excitatory presynapse formation, and the results will be presented

Disclosures: **F. Yoshida:** None. **R. Nagatomo:** None. **M. Kimura:** None. **G. Ito:** None. **S. Takatori:** None. **T. Tomita:** None.

Poster

116. Autism: Synaptic and Cellular Mechanisms II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 116.14/A75

Topic: A.07. Developmental Disorders

Support: NIH U54HD083211
NIH R01DA038058
NIH R01DA35263
NIH F30MH115535
NIH F31MH114316
NIH R01MH106563
NIH T32GM007347

Title: Autism-linked dopamine transporter mutation alters striatal dopamine neurotransmission and dopamine-dependent behaviors

Authors: *G. E. DICARLO[1], J. AGUILAR[4], H. MATTHIES[5], F. E. HARRISON[7], K. E. BUNDSCHUH[2], A. WEST[8], P. HASHEMI[8], F. HERBORG[9], M. RICKHAG[9], H. CHEN[10], U. GETHER[9], M. T. WALLACE[3], A. GALLI[6];
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of South Carolina, Columbia, SC; [9]Univ. of Copenhagen, Copenhagen, Denmark; [10]DRI Biosci. Corp., Frederick, MD

Abstract: The dopamine transporter (DAT), a presynaptic membrane protein, regulates the temporal and spatial availability of the neurotransmitter dopamine (DA) by rapidly clearing released DA from the synapse. The precise regulation of synaptic DA by the DAT fine-tunes the phasic nature of the DA signal, which is required for reward processing and behavioral learning. Dysregulation of the DA system has been implicated in various neuropsychiatric disorders, including Autism Spectrum Disorder (ASD). A de novo mutation in the *SLC6A3* gene resulting in a threonine to methionine substitution at site 356 (DAT T356M) was recently identified in an individual with ASD. Our group sought to determine the impact of this variant from the level of transporter structure and function to the level of mammalian behavior. Structural modeling demonstrated that a corresponding mutation in a DAT-homologous transporter promotes an outward-facing transporter conformation upon substrate binding, a conformation possibly underlying anomalous DA efflux. We found that this variant supported persistent reverse transport of DA (i.e. anomalous DA efflux) in transfected cells and hyperlocomotion in *Drosophila melanogaster*. Mice homozygous for this mutation (DAT T356M$^{+/+}$ mice) displayed significant impairments in the uptake of released DA and reduced total tissue content of DA. DAT T356M$^{+/+}$ mice also exhibited a number of behavioral changes corresponding with the behavioral characteristics of ASD. These behavioral changes included reduced social preference, repetitive behaviors, and profound increases in spontaneous locomotor activity. Antagonism of the DAT reduced the observed hyperlocomotion in DAT T356M$^{+/+}$ animals, suggesting that DAT-mediated leak of DA may underlie hyperactivity in these animals. Taken together, this research provides new evidence for a role of DAT dysfunction (specifically anomalous DA efflux) in the behavioral changes typically associated with ASD and ADHD. Our model suggests that blockade of the DAT may improve behavior via reduction of DAT-mediated DA efflux. This work presents the exciting possibility of a potential mechanistic underpinning for the behavioral changes seen in ASD and ADHD. Future work should seek to determine to what degree DA dysfunction exists across patients with these neuropsychiatric disorders. Importantly, this work provides a rigorous model for studying the impact of genetic variants on both transporter physiology and behavior from the structural to the mammalian level, a task that is becoming increasingly important with the now-common use of whole exome sequencing in the clinic and the drive to practice precision medicine.

Disclosures: G.E. DiCarlo: None. **J. Aguilar:** None. **H. Matthies:** None. **F.E. Harrison:** None. **K.E. Bundschuh:** None. **A. West:** None. **P. Hashemi:** None. **F. Herborg:** None. **M. Rickhag:** None. **H. Chen:** None. **U. Gether:** None. **M.T. Wallace:** None. **A. Galli:** None.

Poster

116. Autism: Synaptic and Cellular Mechanisms II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 116.15/A76

Topic: A.07. Developmental Disorders

Title: Alterations in dendritic spines and intrinsic excitability of pyramidal neurons of somatosensory cortex in the valproic acid model of autism

Authors: *B. A. GREGORY, M. J. RAILING, J. A. BEATTY, C. L. COX;
Physiol., Michigan State Univ., East Lansing, MI

Abstract: Autism spectrum disorders (ASDs) are characterized by a range of behavioral and neurological deficits. *In utero* exposure to the antiepileptic drug valproic acid (VPA) has been associated with several teratogenic effects including developmental delay and ASD. A mouse model of prenatal VPA exposure exhibits ASD-like behaviors such as impaired social interactions, repetitive behaviors and hypersensitivity. In this study we have investigated anatomical and physiological alterations in layer 5 pyramidal neurons in primary somatosensory cortex (S1) in this mouse model. Using a combined approach of two-photon laser imaging and patch-clamp electrophysiology, we investigated whether dendritic spine phenotype, intrinsic neuronal excitability, and basic synaptic physiology were altered in the VPA-exposure model. We tested for changes at two distinct time points: adolescence (P30) and adult (>P90). We found a decrease in dendritic spine density and spine length at both time points in the VPA model compared to WT animals. Preliminary findings also indicate dampened intrinsic excitability associated with a decreased frequency of spontaneous excitatory synaptic events in the VPA model. Taken together, these data suggest a mechanism of altered morphology and intrinsic excitability in pyramidal neurons of VPA-treated mice that may contribute to the ASD behavioral phenotype that persists in adulthood.

Disclosures: B.A. Gregory: None. **C.L. Cox:** None.

Poster

116. Autism: Synaptic and Cellular Mechanisms II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 116.16/A77

Topic: A.07. Developmental Disorders

Support: SFARI (399853
NIMH (R56 MH117961)
Weill Institute for Neurosciences

Title: Impaired prefrontal cortex-ventral hippocampal synchrony is linked to abnormal anxiety behavior in PogZ mutant mice

Authors: *M. CUNNIFF, E. MARKENSCOFF-PAPADIMITRIOU, J. L. RUBENSTEIN, V. S. SOHAL;
Dept. of Psychiatry, Univ. of California, San Francisco, San Francisco, CA

Abstract: PogZ has been identified as a risk factor for autism and intellectual disability – over 25 *de novo* loss of function mutations have been found in whole-exome sequencing studies. PogZ is one of many chromatin modifiers that has have been linked to autism susceptibility, but it remains unclear how mutations in non-specific genetic modifiers result in a consistent phenotype. We sought to characterize a PogZ mutant mouse in order to identify physiological alterations in physiology to better understandthat might contribute to aspects of PogZ's role in disease etiology. PogZ mice have grossly normal social and cognitive behavior but show a reduction in specific anxiety-related behavioral measures compared to wildtype littermates, spending more time exploring open arms in an elevated plus maze. Based on previous work in our lab and others suggesting a role for communication between the ventral hippocampus (vHPC) and prefrontal cortex (PFC) in anxiety-related behaviorslooking at the link between ventral hippocampal-prefrontal cortex synchrony and anxiety,, we recorded local field potentials from the PFC and vHPC in freely moving mice. PogZ heterozygotes have normal synchrony between brain regions at baseline but have dampened responses in high anxiety specific environments which normally elicit anxiety-related avoidance. This suggests that abnormal communication between the vHPC and PFC may contribute to behavioral phenotypes in these mice. We are currently To further investigating potential underlying mechanisms using characterize this connection, we are using brain slice electrophysiology to characterize the properties of prefrontal neurons as well as vHPC-mPFC synapses in PogZ mutant mice.record from PFC pyramidal cells and interneurons in order to characterize their intrinsic properties and response to ventral hippocampal stimulation.

Disclosures: M. Cunniff: None. **E. Markenscoff-Papadimitriou:** None. **J.L. Rubenstein:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurona Therapeutics. F. Consulting Fees (e.g., advisory boards); Neurona Therapeutics. **V.S. Sohal:** F. Consulting Fees (e.g., advisory boards); Emphatic Therapeutics.

Poster

116. Autism: Synaptic and Cellular Mechanisms II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 116.17/A78

Topic: A.07. Developmental Disorders

Support: NIH/NIMH R01 MH111464
NIH/NIH/NCATS TL1 TR001451 & UL1 TR001450
NARSAD Young Investigator Award

Title: Neuronal and neuroimmune dysfunction underlies behavioral and synaptic phenotypes in a mouse model of MEF2C haploinsufficiency syndrome

Authors: *C. M. BRIDGES[1], A. J. HARRINGTON[1], K. BLANKENSHIP[1], A. ASSALI[1], H. WARREN[2], Y. J. CHO[1], E. TSVETKOV[1], S. A. SKINNER[2], C. W. COWAN[1];
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Abstract: Microdeletions and mutations in the *MEF2C* gene cause a neurodevelopmental disorder termed *MEF2C* Haploinsufficiency Syndrome (MCHS). Symptoms of MCHS include core autism symptoms, such as deficits in speech and social reciprocity, and repetitive motor behaviors. In addition, individuals with MCHS often present with epilepsy, intellectual disability, sleep disturbances, hypotonia and high pain tolerance. Through mouse models, we aim to uncover the mechanisms underlying MCHS. Here, we show that MCHS-associated MEF2C missense mutations cluster in the conserved DNA binding domain, and generation of several of the patient missense mutations produced in a dramatic reduction in MEF2C DNA binding affinity. DNA binding-deficient global *Mef2c* heterozygous mice (*Mef2c*-het) display numerous MCHS-related behaviors as well as input-specific reductions in cortical excitatory synaptic transmission. As a regulator of neuronal synapse development, MEF2C hypofunction in neurons is presumed to underlie most MCHS symptoms, but MEF2C is also expressed in neuroimmune cells, specifically microglia. Our ongoing studies suggest critical roles for MEF2C in both neuronal and neuroimmune populations for behavioral and synaptic phenotypes in a genetic mouse model of MCHS.

Disclosures: C.M. Bridges: None. **A.J. Harrington:** None. **K. Blankenship:** None. **A. Assali:** None. **H. Warren:** None. **Y.J. Cho:** None. **E. Tsvetkov:** None. **S.A. Skinner:** None. **C.W. Cowan:** None.

Poster

116. Autism: Synaptic and Cellular Mechanisms II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 116.18/A79

Topic: A.07. Developmental Disorders

Support: NIH U01# MH106882

Title: Human neural progenitor cells harbor DNA double-strand breaks in long neural genes linked to autism

Authors: *M. WANG[1], C. LIM[1], P.-C. WEI[3], I. GALLINA[1], S. MARSHALL[1], C. MARCHETTO[2], F. ALT[3], F. H. GAGE[2];
[2]LOG-G, [1]Salk Inst., La Jolla, CA; [3]Boston Children's Hospital, Harvard Med. Sch., Boston, MA

Abstract: An association between macrocephaly and autism spectrum disorder (ASD) has long been established. The brain overgrowth prior to most clinical manifestations of ASD suggests that the mechanisms involved in excessive growth also contribute to the pathogenesis of the disorder. Neural progenitor cells (NPCs) derived from induced pluripotent stem cells of ASD individuals with early developmental brain enlargement are inherently more proliferative than control NPCs. Here, we showed that NPCs derived from ASD individuals with macrocephaly displayed an altered DNA replication program and increased DNA damage. High throughput genome-wide translocation sequencing (HTGTS) identified genomic fragile regions in genes in control NPCs upon replication stress. Intriguingly, ASD-derived NPCs harbored elevated DNA double-strand breaks in replication stress-susceptible genes compared to control NPCs. Our results demonstrate that replication-associated genomic instability may cause neurological dysfunction by disrupting long neural genes linked to neurodevelopmental diseases.

Disclosures: M. Wang: None. **C. Lim:** None. **P. Wei:** None. **I. Gallina:** None. **S. Marshall:** None. **C. Marchetto:** None. **F. Alt:** None. **F.H. Gage:** None.

Poster

117. Comparative Brain Anatomy

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 117.01/A80

Topic: A.10. Development and Evolution

Title: Inter-individual and inter-hemispheric variations of the cortical sulcal patterns in human brain: Each of us contains a unique cortical sulcal signature

Authors: *A. KUMAR[1], R. K. NARAYAN[1], V. PAREEK[3], C. KUMARI[4], V. R. DESHMUKH[2], S. K. SANJIB[1], M. A. FAIQ[5];
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Abstract: Introduction Inter-individual variations in specific cortical sulci were often reported but comprehensive sulcal patterns were never studied sufficiently. Further, any study which has described sulcal variations between the two hemispheres of the same individuals, are scarce in literature. A variation in the sulcal pattern of brain will create a cortical signature unique to the individuals which may have important neurophysiological and forensic implications. We have studied here the inter-individual and inter-hemispheric variations of the cortical sulcal patterns in human brain. **Materials and Methods** Fifteen complete human brain specimens along with the meninges were taken out from the formalin fixed (10%) cadavers following standard dissection protocol. Dural coverings were sectioned and removed, and arachnoid matter was gently peeled out from the cortical surfaces for each brain. Further, the specimens were photographed from superior, posterior and lateral aspects under standardized dimensional settings. Individual sulci were identified with the help of a standard text book and a neuroanatomy atlas. An image analyzer software (Image J, from NIH) was used to delineate sulcal patterns in individual images and to study angular relationship of the specific sulci. The angle between the each of the sulci (central, pre-central, post-central, and parieto-occipital, intra-parietal, and lateral frontal sulcus) and the longitudinal fissure, and the superior temporal sulcus and a vertical line passing through the lateral fissure were measured. For each sulcus mean, range, standard deviation (SD), and coefficient of variance (CoV= SD/Mean x 100) were calculated from the measurement values (in degrees). **Results** Inter-individual and inter-hemispheric variation of the sulcal angles ranged from SD: 2.32-9.78, CoV: 18.61-77.09% and SD: 2.9-11.17, CoV: 62.44% -106. 9% respectively. **Discussion** Our results showed that pronounced variations in the sulcal patterns existed not only between the individuals but also between the two hemispheres of the same individuals. The unique cortical sulcal patterns arising of the individual specific variations may explain their uniqueness in terms of the personality and behaviour. An individual specific sulcal pattern constructed from the neuroimaging data can be used as a signature for the forensic identifications. The inter-hemispheric variations in sulcal patterns which indirectly represent varying white matter arrangement in each hemisphere may explain the distinctive information processing between two cerebral hemispheres.

Disclosures: A. Kumar: None. **R.K. Narayan:** None. **V. Pareek:** None. **C. Kumari:** None. **V.R. Deshmukh:** None. **S.K. Sanjib:** None. **M.A. Faiq:** None.

Poster

117. Comparative Brain Anatomy

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 117.02/A81

Topic: I.06. Computation/ Modeling/ and Simulation

Support: MNI-Cambridge Clinical Neurosciences grant (RG90792 RRZD/026)
National Institute of Mental Health Division of Intramural Research Programs

Title: Assessing variability in macaque brain morphology using a fully automated cortical surface extraction pipeline

Authors: *B. JUNG[1], P. L. PERKINS[1], J. SEIDLITZ[3,2], K. WAGSTYL[3,4], C. LEPAGE[4], L. G. UNGERLEIDER[1], A. MESSINGER[1];
[1]Lab. of Brain and Cognition, [2]Developmental Neurogenomics Unit, Natl. Inst. of Mental Hlth., Bethesda, MD; [3]Dept. of Psychiatry, Univ. of Cambridge, Cambridge, United Kingdom; [4]Montreal Neurolog. Inst., Montreal, QC, Canada

Abstract: We previously created an anatomical monkey brain template, called the National Institute of Mental Health Macaque Template - or NMT (Seidlitz et al. 2017). The NMT is a nonlinear average of T1-weighted in vivo MRI scans from 31 adult rhesus macaques. As such, it is highly representative of the species. Using scripts we provide that register a monkey's anatomical scan to the NMT, data from individual monkeys can be presented or analyzed in the NMT space or projected onto its various cortical surfaces. Because CIVET (Kim et al. 2005), FreeSurfer (Fischl 2012), and other automated cortical surface extraction pipelines were designed to work on human anatomical scans, making these original NMT surfaces required extensive manual editing and they were not suited for assessing morphometric properties such as cortical thickness.
We have now extended the CIVET pipeline so that it can create surface maps of individual macaque brains (CIVET-Macaque; Lepage et al. 2018) using only a T1-weighted anatomical MRI. CIVET-Macaque uses an updated, symmetric version of the NMT for registration and generates high resolution white matter, grey matter and mid-cortical surfaces, with corresponding nodes and spatially uniform tiling, through a fully automated and open access pipeline. CIVET also computes various morphological features such as cortical thickness and curvature. These properties can be evaluated at each node or averaged across nodes belonging to the same anatomical structure. Each node is anatomically labeled based on our Cortical Hierarchical Atlas of the Rhesus Macaque (CHARM). Corresponding nodes are directly comparable between monkeys because, while nodes are positioned according to an individual's specific morphology, their anatomical location and numbering remains constant. CIVET-Macaque, in conjunction with the NMT, thus allows macaque neuroimaging data to be evaluated

at the group level or individually both in the volume and on the surface.
We report typical morphological values computed for the surfaces generated by applying CIVET-Macaque to the symmetric NMT volume itself. Additionally, by generating surfaces for over 100 macaques, we assess how the metrics of cortical thickness and curvature vary across subjects and whether they exhibit hemispheric asymmetry. Our quantification of morphological variance in a large population of macaques makes it possible to identify anatomical abnormalities in vivo, whether they be naturally occurring or result from a behavioral, pharmacological, genetic, or other experimental manipulation. These data will also be useful for studies of macaque brain development and comparisons across primate species.

Disclosures: B. Jung: None. **P.L. Perkins:** None. **J. Seidlitz:** None. **K. Wagstyl:** None. **C. Lepage:** None. **L.G. Ungerleider:** None. **A. Messinger:** None.

Poster

117. Comparative Brain Anatomy

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 117.03/A82

Topic: I.06. Computation/ Modeling/ and Simulation

Title: Cortical folding with a mechanical perspective

Authors: *A. YUCESOY[1], R. MEJIA-ALVAREZ[1], T. J. PENCE[1], A. M. WILLIS[2];
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Abstract: The process of development of the human brain can be categorized into three distinct stages including neuronal proliferation, neuronal migration, and cortical folding. The last stage, cortical folding, starts around the 22nd week of gestation and continues until 2 years of age. By the end of this stage, the human brain has developed a very complex and convoluted outer surface. The cortical folding can be portrayed as a biological optimization process aiming to maximize the cognitive abilities while minimizing the cost of the neuronal network within the limited skull volume. The driven mechanism of development of the brain is highly dependent upon the parental genetic background and physical factors that can be assumed to be interrelated. However, this morphological evolution and the key factors shaping the brain still remain to be explained. Furthermore, to explore the folding mechanism would also help in the understanding of abnormal cortical patterns associated with some mental disorders including developmental delay, autism, and schizophrenia. In this computational study, a finite element analysis is implemented to predict the emerging morphological patterns of a developing brain as a result of cortical folding. A two-dimensional, initially smooth, bilayer Finite Element model has been established to simulate folding patterns based on the differential growth hypothesis. From a mechanical point of view, the role of mechanical factors on cortical folding, such as the initial

cortex thickness, growth ratio and stiffness ratio between gray and white matter, is investigated via simulations of volumetric tissue expansion. The results indicate that some mechanical and geometrical regulators play a role in the ultimate morphological configuration. A few instances are: smooth surface patterns akin to Lissencephaly would prevail if the subcortex has lower or equal stiffness than the cortex. Different degrees of folding would depend on initial cortex thickness and on differences in tissue growth ratios (e.g. the degree of convolution decreases when the subcortex has a lower growth rate than the cortex).

Disclosures: A. Yucesoy: None. **R. Mejia-Alvarez:** None. **T.J. Pence:** None. **A.M. Willis:** None.

Poster

117. Comparative Brain Anatomy

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 117.04/A83

Topic: A.10. Development and Evolution

Support: NIH Grant MH113257

Title: MacBrainResource: On-site and web-based access to non-human primate brain slides and EM blocks

Authors: *L. D. SELEMON, A. DUQUE;
Dept. Neurosci., Yale Univ. Sch. Med., New Haven, CT

Abstract: MacBrainResource is comprised of five collections of histologically processed brain slides and EM blocks generated over decades of primate research in the laboratories of Drs. Pasko Rakic and Patricia Goldman-Rakic. These brain materials are now freely available to researchers worldwide for use in their own research initiatives either by visiting the Department of Neuroscience at Yale or via web access (for more information, see macbrainresource.org or https://medicine.yale.edu/neuroscience/macbrain/). To enable researchers to access and analyze slides from their home institutes, slides are digitized on an “as needed” basis by scanning them on an Aperio CS2 scanner and uploading these images to the MacBrainResource database. The requested slides are then made available remotely via free download of the Aperio software packages, eSlideManager and ImageScope. The image files (svs) can be imported into commercially available software programs, such as Stereo Investigator (SI, MicroBrightfield, Williston, VT), that are designed for morphometric and stereologic analyses. For example, Collection 1 slides features cases in which tritiated thymidine was injected systemically at ages spanning embryonic day 25 (E25) to adult. Slides from these cases have been processed autoradiographically to identify newly generated neurons throughout the brain. These slides can be examined at high resolution using the meander scan feature of Stereo Investigator to map the

distribution of labeled neurons in different brain regions. Collection 4 includes cases of non-human primates that were prenatally exposed to x-irradiation, as well as sham-irradiated and non-irradiated control brains. All these brains were processed in a manner compatible for stereologic analyses; therefore area, volume, cell size and population estimates can be made using the Cavalieri, nucleator, optical fractionator and optical disector functions of the SI software. Remote access to MacBrainResource slides should further enhance the value of this resource to foster non-human primate research without the cost or need to sacrifice additional animals.

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

Poster

117. Comparative Brain Anatomy

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 117.05/A84

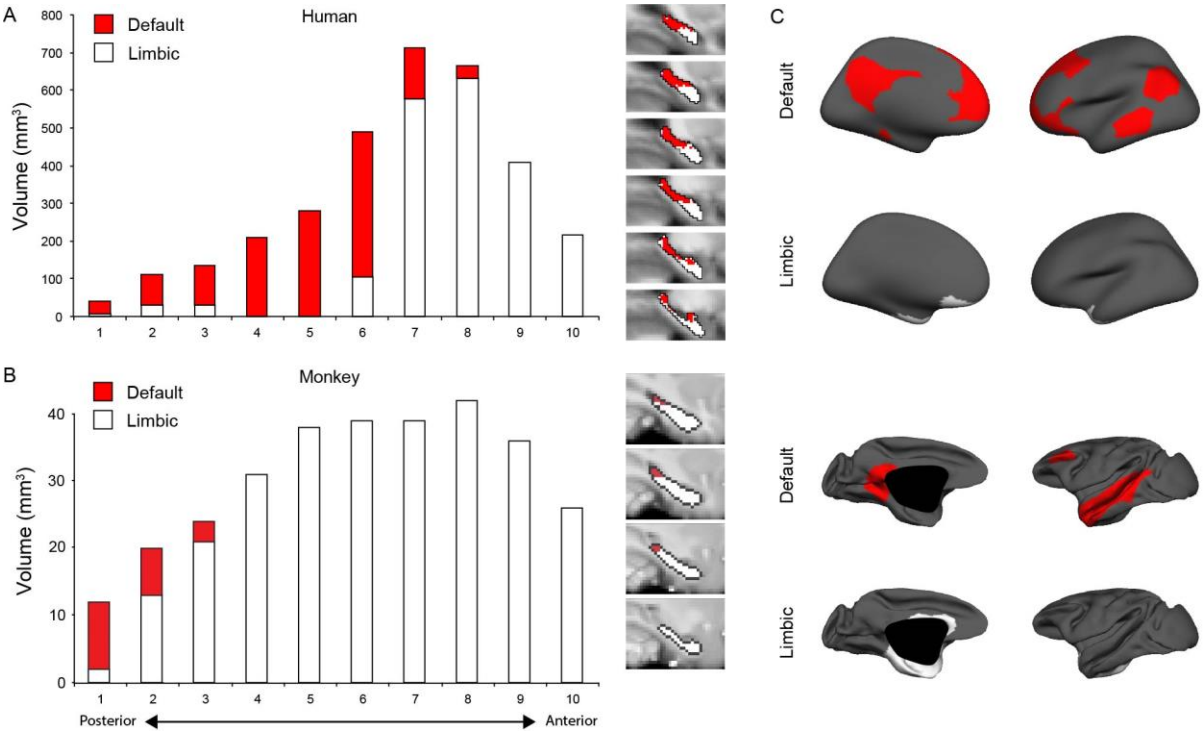
Topic: A.10. Development and Evolution

Title: Comparative analysis of cortical-subcortical resting-state functional connectivity of human and macaque brains

Authors: *G. ZUR, E. BERGMANN, I. KAHN;
Technion, Haifa, Israel

Abstract: While sub-cortical structures have been relatively conserved from non-human to human primates, the cerebral cortex underwent radical changes including massive expansion and potentially the incorporation of new areas. Previous cross-species comparisons showed that this expansion involved mainly association systems whereas motor and sensory areas exhibit only modest differences. Therefore, characterizing the changes in cortical organization and their impact on sub-cortical structures is crucial for our understanding of mammalian brain evolution. To characterize cortical organization, we compared resting-state functional connectivity MRI data of humans and macaque monkeys. First, human and macaque cortices were parcellated using von Mises-Fisher clustering algorithm (Yeo et al., 2011). Similar to humans, cortical surface parcellation of macaque cortex shows a stable division to seven networks. We found similar organization of Visual, Somatomotor, and Limbic cortical networks. However, association networks diverged, demonstrating limited distal connectivity in macaques. After characterizing cortical organization, we sought to parcellate the striatum and hippocampus based on the cortical networks such that each voxel in these regions was assigned to a cortical network. Parcellation of the hippocampus and striatum demonstrated a differential representation of association networks within these areas. In humans a larger proportional volume of the hippocampus and striatum is associated with the Default network relative to the homologue regions in macaques. Our results demonstrate that the expansion of high-order cortical regions in

the human brain is accompanied by changes in cortico-subcortical connectivity with implications to the contributions of these regions. We are now modifying the von Mises-Fisher clustering algorithm to the mouse brain with the aim to evaluate arealization classification changes across the mammalian class extending it to rodents, a species extensively studied both in the context of basic research and models of disease.



Disclosures: **G. Zur:** None. **E. Bergmann:** None. **I. Kahn:** None.

Poster

117. Comparative Brain Anatomy

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 117.06/A85

Topic: A.10. Development and Evolution

Support: NHMRC APP1068140 (DHR)

Title: Cortical connections of the marmoset claustrum complex

Authors: *D. H. RESER[1], M. A. HAGAN[2], E. ZAVITZ[3], Y. WONG[4], D. K. WRIGHT[5]; [1]Monash Rural Health-Churchill, Monash Univ., Churchill, Australia; [2]Dept. of Physiol., [4]Biomedicine Discovery Institute, Dept. of Physiol., [3]Monash Univ., Clayton, Australia; [5]Dept. of Neuroscience, Central Clin. Sch., Monash Univ., Melbourne, Australia

Abstract: The claustrum complex, which in primates includes the claustrum and dorsal endopiriform nucleus, is among the most widely connected forebrain structures. However, its function(s) remain undetermined, and even its structural organization is poorly understood. We have recently identified subdivisions of the marmoset claustrum complex based on myeloarchitectonic and cytoarchitectonic characteristics, and here we describe the patterns of cortical connectivity derived from post-mortem diffusion weighted tractography obtained from high field strength (9.4T) magnetic resonance imaging and tract tracing studies with respect to that compartmentalization. Whole fixed brains from 3 adult marmosets (*Callithrix jacchus*) were obtained after the animals were used for unrelated, MARP Animal Ethics Committee-approved electrophysiological studies. Perfused brains were immersed in proton-free oil and stably mounted for imaging in a 9.4T/20cm Bruker MRI. Multi-gradient echo images were acquired using a 40 mm volume resonator and the following imaging parameters: repetition time = 200 ms; echo times = 6, 12, .., 60 ms; field of view = 35.84 x 24.5 x 19.2 mm^3; matrix size = 512 x 350 x 274; and resolution = 70 x 70 x70 um^3. Total imaging time was approximately 16 hours/brain. Results of the imaging studies were compared to previously published connections from *in vivo* tract tracing studies performed in our lab using neuroanatomical tracers. Comparisons were based on seed regions of interest generated from histological reconstructions of the claustrum complex and cortical injection sites. Differential distribution of claustro-cortical connections was observed with respect to the subdivisions of the claustrum complex and 'rich club' cortical areas known to be components of major resting state functional networks, including the default mode and dorsal attention networks. Our findings provide structural connectivity data which are consistent with recent hypotheses of claustrum-complex involvement in behavioural process which exhibit modulation of functional networks during behavioural and attention-dependent tasks.

Disclosures: D.H. Reser: None. **M.A. Hagan:** None. **E. Zavitz:** None. **Y. Wong:** None. **D.K. Wright:** None.

Poster

117. Comparative Brain Anatomy

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 117.07/A86

Topic: A.10. Development and Evolution

Support: NIH Grant R15 NS090296

Title: Characteristics of myelin development in the anterior corpus callosum of capuchin monkeys (Cebus apella)

Authors: *C. M. WATSON[1], K. A. PHILLIPS[2,3];
[1]Neurosci. Program, [2]Psychology, Trinity Univ., San Antonio, TX; [3]Southwest Natl. Primate Res. Ctr., Texas Biomed. Res. Inst., San Antonio, TX

Abstract: The midsagittal area of the corpus callosum (CC) is frequently used as a marker of brain development, connectivity, and function. Previous work in our laboratory has shown, via MRI quantification, age-related changes in subdivisions of the corpus callosum in capuchin monkeys, with the genu and splenium displaying continued growth over the lifespan (Phillips & Sherwood, 2012). Here we quantify myelin characteristics from electron micrographs to more fully understand differential patterns of white matter development in the CC. Measurements of myelin thickness, density, and myelin fraction were obtained in a systematic random fashion from electron microscopy digital photographs of the anterior regions of the CC from infant (n = 2; male n = 2) and adult capuchin monkeys (*Cebus apella*; n = 2; male n = 1). We hypothesized that infants would have reduced myelin thickness, myelin density, and myelin fraction of axonal fibers compared to adults. Measurements were conducted by CMW who was blind to the age and sex of subjects; validation checks were performed by KAP. In the genu of the corpus callosum, infants had thinner myelin (M = 0.167 μm) than the adult subjects (M = 0.240 μm). Similarly, the subjects had a lower density of myelinated axons (infants: M = 0.376 axons/μm^2 image; adults: M = 0.534 axons/μm^2 image) and lower myelin fraction (infants: M = 0.056 μm^2 myelin/μm^2 image; adults: M = 0.160 μm^2 myelin/μm^2 image). However, these differences were not consistent in the rostral midbody of the CC. These results confirm differences in the rate of development of myelin in the anterior region of the CC.

Disclosures: C.M. Watson: None. **K.A. Phillips:** None.

Poster

117. Comparative Brain Anatomy

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 117.08/B1

Topic: A.10. Development and Evolution

Support: NSF 1653080
Rackham Graduate Student Research Grant

Title: Quantitative spatial models of neural distribution in human lumbar dorsal root ganglia

Authors: *Z. J. SPERRY[1,2], R. D. GRAHAM[1,2], N. PECK-DIMIT[1,2], S. F. LEMPKA[1,2,3], T. M. BRUNS[1,2];
[1]Biomed. Engin., [2]Biointerfaces Inst., [3]Anesthesiol., Univ. of Michigan, Ann Arbor, MI

Abstract: Dorsal root ganglia (DRG) are components of spinal nerves which contain sensory neuronal somata and are located bilaterally in the intervertebral foramina of the spinal column. Recent work has demonstrated the DRG as a potentially advantageous site for neural interface devices. Clinical interfaces with DRG include electrical stimulation to drive reflex organ function or reduce pain, and animal models suggest that neural recordings at the DRG can be used as feedback for medical devices. Any interface with DRG is highly dependent on the unique anatomy of this neural structure and could benefit from a quantitative description of the location of their neurons. In this study, we analyzed the anatomy of L4 and L5 DRG collected from deceased donors through the National Disease Research Interchange (Philadelphia, PA). We screened and excluded donors for disorders that could affect DRG morphology (e.g. neuropathies, spinal stenosis, herniations, spinal injuries etc.). After gross tissue measurement and trimming, DRG were stained (Histowiz Inc., Long Island, NY) for brightfield imaging with a rabbit monoclonal to neurofilament heavy antibody (Abcam ab40796; for identifying neurons) and hematoxylin (for co-location of nuclei). Images were processed with a custom MATLAB graphic user interface (GUI), using validated parameters for thresholding and morphological transformation. The GUI was used to identify the shape and size of each DRG and nerve fascicle, as well as the locations and sizes of their constituent axons and neuronal somata. These spatially-defined elements were normalized to a circular profile for cross-sample density comparisons based on neuron size. GUI output was also used to produce 3D models of DRG profiles. We analyzed images from a total of 31 DRG (13 L4, 18 L5), 1-4 each from 10 donors (7 male, 3 female; 7 Caucasian, 2 Hispanic, 1 Black; age 25-59 [47±10]; BMI 24-38 [30±5]). Preliminary qualitative results indicate that human DRG somata preferentially congregate around the dorsal outer edge of the ganglion, similar to previous results in feline models. Our ongoing quantitative analysis will rigorously characterize the spatial organization of cell location and size as well as the relationship between the two. This is the first time that the microanatomy of human DRG has been described quantitatively. These results provide a basis for computational modeling and design of future clinical neural interfaces with DRG.

Disclosures: Z.J. Sperry: None. **R.D. Graham:** None. **N. Peck-Dimit:** None. **S.F. Lempka:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Presidio Medical, Inc.. F. Consulting Fees (e.g., advisory boards); Presidio Medical, Inc.. **T.M. Bruns:** None.

Poster

117. Comparative Brain Anatomy

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 117.09/B2

Topic: A.10. Development and Evolution

Support: JSPS KAKENHI Grant Number JP17K07943

Title: Histological analysis of the cerebellum of Polypterus senegalus

Authors: *T. IKENAGA, R. SHIMOMAI, K. MATSUMOTO, S. KIMURA;
Dept. of Chem. and Biosci., Kagoshima Univ., Kagoshima, Japan

Abstract: Primitive actinopterygian fish has cerebellum as seen in other gnathostoms including teleost fish but details of their structure including efferent systems are not well understood. Here we investigated morphology, cellular organization, and efferent systems of the cerebellum of *Polypterus senegalus*, most ancestral actinopterygian fish. The *Polypterus* cerebellum is subdivided into the valvula, corpus, and auricula cerebelli, and Giemsa stained sections showed that all of them have typical molecular and granule cell layers. Three-dimensional distribution of these layers was revealed by higher resolution X-ray micro-CT analysis. Immunohistochemistry with anti-IP_3 receptor 1 antibody, marker of the Purkinje cells, revealed that cell bodies of the Purkinje cells were not distributed as layered manner and clustered in specific regions especially in the valvula and corpus. Immunolabeling with some antibodies which were known markers of the cerebellar neurons were performed for further characterization of cells in the *Polypterus* cerebellum. The climbing fibers labeled by anti-parvalbumin antibody did not reach to the cell bodies and dendrites, and terminated mainly on around initial portion of the axon. Anti-calretinin antibody labeled cell bodies in the posterior-lateral area of the auricula cerebelli. Retrograde labelings of cerebellar efferent neurons were also performed by application of carbocyanine dye in outside of the cerebellum. Labeled cell bodies were observed around the ventro-lateral region of auricular cerebelli and made the nuclear-like structure. These results indicate that the *Polypterus* cerebellum has cerebellar nucleus-like structure as cerebellar efferent system as seen in the elasmobranch and other tetrapods.

Disclosures: T. Ikenaga: None. **R. Shimomai:** None. **K. Matsumoto:** None. **S. Kimura:** None.

Poster

117. Comparative Brain Anatomy

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 117.10/B3

Topic: A.10. Development and Evolution

Support: MRC grant G0901525/1

Title: Ectodermal interactions in the development of the pineal organ

Authors: N. STAUDT[1], T. FIELDING[2], J. HUTT[2], I. FOUCHER[3], V. SNOWDEN[2], ***C. KIECKER**[2], C. HOUART[2];

[1]Wellcome Trust Sanger Inst., Cambridge, United Kingdom; [2]Dept. for Developmental Neurobio., King's Col., London, United Kingdom; [3]Inst. Pasteur, Paris, France

Abstract: The pineal organ is one of the vertebrate circumventricular organs. It is a neuroendocrine gland situated in the roof of the 3rd ventricle that regulates circadian and circannual rhythms through the cyclical release of melatonin. Pineal organ development has been studied as a paradigm for asymmetrical neurogenesis in zebrafish embryos and for photoreceptor evolution; however, little is known about the earliest steps of pineal cell fate specification. Classical fate mapping studies in amphibian and avian embryos suggested that pineal progenitors originate from the anterolateral border of the neural plate. Using gene expression and cell lineage mapping in zebrafish we show here that a subset of pineal progenitors originates in the non-neural ectoderm that borders the neural plate. Gene expression in chick embryos also suggests a non-neural origin of pineal progenitor cells. Using gain and loss-of-function experiments targeting the *dlx* gene family (encoding homeodomain transcription factors that specify the neural plate border region and that are required for placode formation), we demonstrate that the non-neural contribution to the pineal organ is placodal. We also show that Fibroblast Growth Factor signalling from the anterior neural border and midbrain-hindbrain boundary restricts the pineal progenitor domain to its specific position. These data suggest an underlying similarity of the pineal organ with the eye and pituitary gland, both of which form through interactions between neural and non-neural tissues.

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Poster

117. Comparative Brain Anatomy

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 117.11/B4

Topic: A.10. Development and Evolution

Support: Kahenhi
Sumitomo

Title: Bairds beaked whale brain in numbers

Authors: *I. M. FERNANDEZ ARTILES[1], A. WATANABE[1], K. K. THA[1], M. KOBAYASHI[2], K. WADA[1], T. MATSUSHIMA[1], B. MOTA[3], N. PATZKE[1];
[1]Hokkaido Univ., Sapporo, Japan; [2]Tokyo Agr. Univ., Abashiri, Japan; [3]Univ. Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Abstract: The evolutionary process of adaptation to an obligatory aquatic lifestyle made crucial modifications in cetacean brain structure and function. One of the most important adaptations is related to their brain size, which is larger than expected for their body size in toothed whales. This finding led to many comparisons with humans, as it is commonly accepted that cetaceans evolved these large brains to support complex cognitive capabilities. Nevertheless, the neuroanatomy of only few toothed whales' species has been examined to date. For this purpose, we analyzed the brain morphology and volumetric of Baird's beaked whale (*Berardius bairdii*), one of the largest and rarest toothed whales found in the North Pacific Ocean deep waters. Two paraformaldehyde-fixed brains (ca. 5000g) were scanned with a 3.0T Siemens system (MAGNETOM Prisma) scanner in the axial, horizontal and sagittal. The volume of the whole brain, amygdala, hippocampus, cerebellum, cortex, superior and inferior colliculi, ventricular system, corpus callosum (volume and mid-sagittal cross-sectional area) and the cortical perimeter were measured. The results obtained for the external and internal anatomical organization revealed typical characteristics previously observed in other toothed whales: (1) brain wider than long; (2) considerable encephalization; (3) the typical odontocete neocortical gyrification pattern; (4) lack of olfactory bulb and tract; (5) thin cortical grey matter; (6) thin corpus callosum; (7) large size of many components of the auditory system, e.g. large inferior colliculus and lateral lemniscus, presumably necessary for echolocation. In addition, the cortical volume and exposed cortical surface reveal the same scaling relationship as that of a multi-species data set previous reported. This finding indicates that overall external shape of Baird's beaked whale cortex is typical mammalian, despite its unusual detailed shape, large size and high gyrification level. Nevertheless, further histological and immunohistochemical analysis are needed to provide a full description of their neuroanatomy (such as cell composition, cell type distribution, etc.).

Disclosures: I.M. Fernandez Artiles: None. **A. Watanabe:** None. **K.K. Tha:** None. **M. Kobayashi:** None. **K. Wada:** None. **T. Matsushima:** None. **B. Mota:** None. **N. Patzke:** None.

Poster

117. Comparative Brain Anatomy

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 117.12/B5

Topic: A.10. Development and Evolution

Support: NSF CAREER Award 1149446

Title: Reconstruction of the African naked mole rat vascular map

Authors: *R. OSMANI, V. CASTAGNA, D. M. THEVALINGAM, E. C. JENKINS; CUNY Col. of Staten Island, Staten Island, NY

Abstract: African naked mole-rats are eusocial, fossorial rodents that have adapted a tolerance for hypoxia and hypercapnia which they encounter within their densely populated underground nests. Previous work has demonstrated that this tolerance in the brain is achieved by both systemic changes, such as an improved oxygen carrying capacity in blood, and neuronal changes, such as the preservation of neotenous features and alternative strategies to reduce oxygen demand. Here, we examined whether naked mole-rats also demonstrate changes to the brain vasculature to accommodate their environmental tolerance. Brain clearing methods were used and optimized for India ink perfused brains of naked mole-rats and age matched mice. Cleared brains were imaged on a custom built light sheet microscope and vascular measurements were made on 3D reconstructed stacks. This approach consistently produced superior clearing in the naked mole-rat brain, compared to the mouse brain, confirming it as a suitable model for brain clearing studies. Gross observations demonstrated a vascular network of major arteries more similar to the guinea pig than to the mouse. At the microscopic level, naked mole-rat's cortical vascular diameter is within a similar range to that of the mouse, but the proportion of the brain that accounts for vasculature appear to be larger. This initial vascular map of the naked mole-rat brain may yield important clues to the significant adaptations this species possesses. This work was supported by NSF CAREER Award 1149446 (to DM).

Disclosures: R. Osmani: None. **V. Castagna:** None. **D.M. Thevalingam:** None. **E.C. Jenkins:** None.

Poster

117. Comparative Brain Anatomy

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 117.13/B6

Topic: F.01. Neuroethology

Title: Correcting for phylogeny: Why to do it, how to do it, and what happens when you do it

Authors: S. THOMAS[1], S. GRETA[4], N. SCHOTTLER[2], W. TOMITA[3], A. L. BURRE[5], D. ROSTAMIAN[2], O. PISHCHALENKO[2], *W. E. BABIEC[3], W. E. GRISHAM[2];
[1]Dept. of Biomath., [2]Dept. of Psychology, [3]Undergraduate Interdepartmental Program for Neurosci., UCLA, Los Angeles, CA; [4]Dept. of Chem., Univ. of Chicago, Chicago, IL; [5]W.M. Keck Sci. Dept., Claremont McKenna Col., Claremont, CA

Abstract: We wanted to look for differences among orders in the volume fraction devoted to six different brain structures: hippocampus, striatum, neocortex, schizocortex, thalamus, and cerebellum. We quantified the volume of each structure and of the whole brain for each species. We sought to analyze the differences in the volume fractions across orders with an ANCOVA and use regression to determine any differences among the slopes of lines relating volume fraction to log10 brain size. **PROBLEM: the analyses that we wished to use requires**

independent groups and our data points were phylogenetically related.
Our data comes from the Comparative Mammalian Brain Collection, which has images of coronal sections of 61 different mammals of 5 groups: primates (n = 10), carnivores (n =17), artiodactyla (n = 8), rodents (n = 7) and Others (n = 19). The others group was included because we only had three or fewer representatives for certain orders.
We created a phylogenetic tree by using an online tool called PhyloT, which generates a tree based on data from NCBI using our species. To make the correction, we used the R packages "ape" and "phytools", both of which consist of various functions that allow for data analyses in a phylogenetic framework. The ape package plotted the tree and formatted it according to the regression model described by Grafen (1989), which essentially equalizes tree branch length. Next, we computed the expected variances and covariances within and among the variables using the vcv.phylo function. Using the phyl.resid function from the phytools package, we computed a multiple regression model, using log brain volume as our beta vector and the covariance matrix as our X values, solved for Y, and found the residuals.
With the corrected residuals, we ran ANCOVAs using a covariate of log brain size to remove the effects attributable to brain size. The pattern of results did not vary much between the raw and corrected volume fraction. Revel (2009) notes that these corrections result in fewer Type I errors, and we correspondingly found fewer significant results--especially for the log brain covariate.

Disclosures: **S. Thomas:** None. **S. Greta:** None. **N. Schottler:** None. **W. Tomita:** None. **A.L. Burre:** None. **D. Rostamian:** None. **O. Pishchalenko:** None. **W.E. Babiec:** None. **W.E. Grisham:** None.

Poster

118. Ionotropic Glutamate Receptors: Pharmacology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 118.01/B7

Topic: B.02. Ligand-Gated Ion Channels

Support: NS036654 NINDS to SFT
NS065371 NINDS to SFT
NS097536 NIH to KBH
a research grant from Janssen to Emory (SFT)

Title: Unique pharmacological properties of GluN1/GluN2A/GluN2D triheteromeric receptors

Authors: *S. BHATTACHARYA[1], K. B. HANSEN[2], S. F. TRAYNELIS[3];
[1]Dept. of Pharmacology, Emory Univ. Sch. of Med., Atlanta, GA; [2]Dept Biomed and Pharm Sci., Univ. of Montana, Missoula, MT; [3]Dept Pharmacol, Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Ligand-gated ion-channel *N*-methyl-*D*-aspartate receptors (NMDARs) are major excitatory receptors in the brain and have critical developmental and maintenance roles in the central nervous system. Most studies of recombinant NMDARs have focused on diheteromeric receptors that contain two copies of GluN1 and two identical GluN2 subunits. However, diverse GluN2-subunits are expressed in most neurons, and there is strong evidence suggesting many native NMDARs are triheteromeric assemblies of two GluN1 and two different GluN2 subunits (e.g. GluN1/GluN2A/GluN2D or any other combination). Receptors that contain two different subunits, such as GluN2A and GluN2D, may have unique pharmacological and functional properties that are distinct from the corresponding diheteromeric assemblies. Elucidating triheteromeric NMDAR pharmacology has been impeded by limited methods to express triheteromeric assemblies without diheteromeric receptors in a heterologous system. We have adapted a strategy using the masking of the endoplasmic reticulum retention signal to selectively express triheteromeric GluN1/GluN2A/GluN2D receptors with minimal contribution of diheteromeric assemblies ("escape currents") in *Xenopus laevis* oocytes. We have profiled expression of GluN1/GluN2A/GluN2D receptors and their "escape current" controls over a 5-day period post injection and found that optimal triheteromeric expression happens on the 4th day post injection for this triheteromeric combination. Furthermore, triheteromeric GluN1/GluN2A/GluN2D receptors have distinct pharmacological profile in experiments with selective and non-selective agonists, endogenous ions (zinc, magnesium), and allosteric modulators, demonstrated by distinct EC_{50}/IC_{50} values compared to the diheteromeric GluN1/GluN2A and GluN1/GluN2D receptors. For example, GluN1/GluN2A/GluN2D receptors had intermediate EC_{50} values for glutamate and glycine. Interestingly, as seen for other triheteromeric NMDARs, a single copy of GluN2A was enough for zinc-mediated inhibition in GluN1/GluN2A/GluN2D NMDARs. This study will advance our understanding of triheteromeric NMDAR function and pharmacology, and aid in development of compounds with therapeutic potential.

Disclosures: S. Bhattacharya: None. **K.B. Hansen:** None. **S.F. Traynelis:** A. Employment/Salary (full or part-time):; Emory University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; consultant for Janssen Pharmaceuticals Inc., is PI on a research grants from Janssen and from Allergan, member of the SAB for Sage Therapeutics, is co-founder of NeurOp Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); receives royalties for software, and is a co-inventors on Emory-owned Intellectual Property that includes allosteric modulators of NMDA receptor function..

Poster

118. Ionotropic Glutamate Receptors: Pharmacology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 118.02/B8

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant NS036654
NIH Grant NS065371
NIH Grant NS092989
NIH Grant HD082373

Title: Functional effects of *de novo* GRIN variants in NMDA receptor M2 channel pore-forming loop associated with neurological diseases

Authors: *D. LIU[1,3], J. LI[1], J. ZHANG[1], W. TANG[1], R. K. MIZU[1], H. KUSUMOTO[1], W. XIANGWEI[1], Y. XU[1], S. J. MYERS[1,2], S. A. SWANGER[1], J. R. LEMKE[4], L. P. WOLLMUTH[5], S. PETROVSKI[6], H. YUAN[1,2], S. F. TRAYNELIS[1,2];
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Abstract: N-methyl-D-aspartate receptors (NMDARs) mediate a slow component of excitatory postsynaptic transmission in the central nervous system, thereby exerting a critical role in neuronal development and brain function. Here we summarize the clinical presentations for 18 patients harboring 12 *de novo* missense variants in *GRIN1*, *GRIN2A*, *GRIN2B* that alter residues in the M2 reentrant loop, a region that lines the pore and is intolerant to missense variation. The resulting amino acid exchanges are GluN1 p.Gly618Arg, p.Gly620Arg; GluN2A p.Leu611Glu, p.Leu614Ser, p.Asn615Lys; GluN2B p.Trp607Cyc, p.Gly611Val, p.Asn615Ile, p.Asn615Lys, p.Asn616Lys, p.Val618Gly and p.Val620Met. These *de novo* variants were identified in children with a set of neurological and neuropsychiatric conditions. The pharmacological properties, biophysical characteristics, and receptor cell surface expression were evaluated *in vitro*. Compared to WT receptors, the mutant NMDARs exhibited either no or modest changes in potency (EC_{50} values) for both glutamate and glycine, as well as no or modest changes in proton inhibition (percentage current inhibition at pH 6.8 compared with the pH 7.6 using two-electrode voltage clamp current recordings from *Xenopus laevis* oocytes. Whole cell patch clamp recordings and beta-lactamase assays on transiently transfected HEK293 cells indicated that these variants can have modest changes in synaptic-like response time course and surface expression. However, functional evaluation showed that all the mutant NMDARs showed

significantly reduced Mg^{2+} inhibition, with significantly increased IC_{50} values and decreased degree of Mg^{2+} inhibition of agonist-evoked current response. The voltage-dependence of Mg^{2+} inhibition is significantly reduced in all variants. The NMDARs hosting a single copy of a mutant subunit showed a dominant reduction in Mg^{2+} inhibition for some variants. These variant NMDARs also show reduced single channel conductance, as well as altered open probability. The data suggest that missense variants increase NMDAR charge transfer in addition to varied and complex influences on NMDAR functional properties, which may underlie the patients' phenotypes.

Disclosures: D. Liu: None. **J. Li:** None. **J. Zhang:** None. **W. Tang:** None. **R.K. Mizu:** None. **H. Kusumoto:** None. **W. XiangWei:** None. **Y. Xu:** None. **S.J. Myers:** None. **S.A. Swanger:** None. **J.R. Lemke:** None. **L.P. Wollmuth:** None. **S. Petrovski:** None. **H. Yuan:** None. **S.F. Traynelis:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Is PI on a research grants from Janssen and from Allergan to Emory University School of Medicine.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); is co-founder of NeurOp Inc, receives royalties for software, and is a co-inventors on Emory-owned Intellectual Property that includes allosteric modulators of NMDA receptor function.. F. Consulting Fees (e.g., advisory boards); is a consultant for Janssen Pharmaceuticals Inc., is a member of the SAB for Sage Therapeutics..

Poster

118. Ionotropic Glutamate Receptors: Pharmacology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 118.03/B9

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant NS036654
NIH Grant NS065371
NIH Grant NS092989
NIH Grant HD082373

Title: Functional changes for GRIA variants associated with epilepsy and intellectual disability

Authors: *N. LIU[1,3], W. XIANGWEI[1,3], Y. XU[1,4], S. KIM[1,2], S. J. MYERS[1,2], Y. JIANG[3], H. YUAN[1,2], S. F. TRAYNELIS[1,2];
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Abstract: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPARs) mediate fast excitatory synaptic signals, and thus are of great significance in nervous system development and various pathological conditions. Here, we described 24 rare variants identified by next-generation sequencing in multiple *GRIA* genes encoding AMPAR subunits in children with epilepsy and/or developmental delay/intellectual disabilities. The patients' phenotypes are summarized and functional properties of the AMPA receptors with these variants are evaluated in addition to wild type (WT) human AMPA receptors. Identical variants were introduced into human cDNAs encoding GluA receptors through the QuikChange methods. cRNA were synthesized from cDNA and injected into *Xenopus laevis* oocytes, and two-electrode voltage-clamp recordings were used to evaluate agonist potency. We studied 24 patients with GRIA variants, which included 2 *GRIA1* variants, 2 *GRIA2* variants, 20 *GRIA3* variants. Of these 24 patients, 4% (1/24) present with epilepsy and developmental delay/intellectual disability, 8% (2/24) present with epilepsy without developmental delay/intellectual disability, and 38% (9/24) show only developmental delay/intellectual disability without seizures. Meanwhile, 4% (1/24) of the variants are located in the amino terminal domain (ATD), 33% (8/24) are in the agonist binding domain (ABD), 33% (8/24) are in transmembrane domains (TMD), 29% (7/24) of variants are in linker regions. Functional evaluation by using voltage clamp to record current revealed 7 gain-of-function variants (7/24 or 29%) that enhanced glutamate and kainate potency by 1.5~15-fold, compared to the corresponding WT receptors. We also found 5 loss-of-function variants (5/24 or 21%) that reduced glutamate and kainate potency by 1.6~51-fold, compared to the corresponding WT receptors. 5 of the 7 the gain-of-function variants are located in TMD or the linker regions, and 2 of the 5 loss-of-function variants are located in the linker regions. Noteworthily, similar phenotypes were associated with both gain- and loss-of-function variants in the same gene. Overall, our results suggest that AMPAR variants are highly relevant with epilepsy and developmental delay/intellectual disabilities in children, and the transmembrane domain or the linker regions were associated with functional variation to a large extent.

Disclosures: **N. Liu:** None. **W. Xiangwei:** None. **Y. Xu:** None. **S. Kim:** None. **S.J. Myers:** None. **Y. Jiang:** None. **H. Yuan:** None. **S.F. Traynelis:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Is PI on a research grants from Janssen and from Allergan to Emory University School of Medicine.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); is co-founder of NeurOp Inc, receives royalties for software, and is a co-inventors on Emory-owned Intellectual Property that includes allosteric modulators of NMDA receptor function.. F. Consulting Fees (e.g., advisory boards); is a consultant for Janssen Pharmaceuticals Inc., is a member of the SAB for Sage Therapeutics..

Poster

118. Ionotropic Glutamate Receptors: Pharmacology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 118.04/B10

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant NS036654
NIH Grant NS065371
NIH Grant NS092989
NIH Grant HD082373

Title: Functional features of GRIN2D variants-related developmental and epileptic encephalopathy

Authors: *Y. XU[1,3], W. XIANGWEI[1,4], V. KANNAN[1], S. BHATTACHARYA[1], A. PODURI[5], J. R. LEMKE[6], S. J. MYERS[1,2], Y. JIANG[4], S. F. TRAYNELIS[1,2], H. YUAN[1,2];
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Abstract: N-methyl-D-aspartate receptors (NMDARs) are ligand-gated ionotropic receptors which mediate a slow, calcium-permeable component of excitatory synaptic transmission in the central nervous system. The NMDARs are encoded by the *GRIN* gene family. Genetic variants in *GRIN* genes are associated with multiple nervous system disorders. In this study we report six novel *GRIN2D* variants and one previously-described disease-associated *GRIN2D* variant in two patients with developmental and epileptic encephalopathy (DEE). These variants are Ser573Phe (c.1718C>T, S573F), Val667Ile (c.1999G>A, V667I), Leu670Phe (2008C>T, L670F), Ala675Thr (2023G>A, A675T), Ala678Asp (2033C>A, A678D), Ser1271Leu (3812C>T, S1271L) and Arg1313Trp (3937C>T, R1313W). The amino acids affected by the variants are located in the pre-M1 helix, transmembrane domain M3, and the intracellular carboxyl terminal domain (CTD). DEE is the unifying phenotype across these patients and the seizure types ranged from focal seizures, atypical absence seizures, tonic or atonic seizures, to epileptic spasms. We performed two-electrode voltage clamp current recordings (TEVC) on *Xenopus laevis* oocytes, in addition to whole-cell voltage-clamp current recordings and beta-lactamase assay on HEK293 cells to explore the associated functional changes for these variants. Our results reveal that GluN2D-A675T significantly enhanced agonist potency by 19-fold for glutamate (EC_{50}: 0.02 μM vs 0.39 μM for wild type, WT), 3-fold increase for glycine (EC_{50}: 0.04 μM vs 0.12 μM for WT) and 14-fold increase for D-serine (EC_{50}: 0.01 μM vs 0.14 μM for WT). GluN2D-A678D

produced a 13-fold increase of glutamate potency (EC_{50}: 0.03 μM), a 2-fold increase of glycine potency (EC_{50}: 0.06 μM), and a 3.5-fold increase of D-serine potency (EC_{50}: 0.04 μM). By contrast, the GluN2D-S573F, S1271L and R1313W substitutions only modestly decreased of glycine potency, without changing glutamate or D-serine potency. GluN2D-S573F, GluN2D-L670F, and GluN2D-A675T reduced the sensitivity to endogenous protons. The GluN2D-L670F and GluN2D-A678D mutant receptors have higher calculated open probability compared to WT receptors. The GluN2D-S573F, A675T, and A678D substitutions significantly decrease current amplitude. GluN2D-L670F variant slows current response deactivation time course and increased charge transfer. All six variants decreased receptor surface trafficking. In addition, we evaluated a set of FDA-approved NMDAR channel blockers to rescue functional changes of mutant receptors. This work may shed light on the pathological mechanisms of *GRIN2D*-mediated DEE and provide opportunities for precision medicine.

Disclosures: **Y. Xu:** None. **W. Xiangwei:** None. **V. Kannan:** None. **S. Bhattacharya:** None. **A. Poduri:** None. **J.R. Lemke:** None. **S.J. Myers:** None. **Y. Jiang:** None. **S.F. Traynelis:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Is PI on a research grants from Janssen and from Allergan to Emory University School of Medicine.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); is co-founder of NeurOp Inc, receives royalties for software, and is a co-inventors on Emory-owned Intellectual Property that includes allosteric modulators of NMDA receptor function.. F. Consulting Fees (e.g., advisory boards); is a consultant for Janssen Pharmaceuticals Inc., is a member of the SAB for Sage Therapeutics.. **H. Yuan:** None.

Poster

118. Ionotropic Glutamate Receptors: Pharmacology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 118.05/B11

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant NS036654
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NIH Grant HD082373

Title: Negative allosteric modulation of GluN1/GluN3A N-methyl-D-aspartate receptors

Authors: *H. YUAN[1,2], Z. ZHU[1], M. P. EPPLIN[3], F. YI[4], S. L. SUMMER[3], R. MIZU[1], K. B. HANSEN[4], D. C. LIOTTA[3], S. F. TRAYNELIS[1,2];
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Chem., Emory Univ. Sch. of Med., Atlanta, GA; [4]Dept. of Biomed. and Pharmaceut. Sci., Univ. of Montana, Missoula, MT

Abstract: NMDA receptors (NMDARs) are ligand-gated ion channels that assemble into tetrameric receptor complexes that contain glycine-binding GluN1 and GluN3 subunits (GluN3A-B) and glutamate-binding GluN2 subunits (GluN2A-D). Functional receptors can be assembled from GluN1/GluN2, GluN1/GluN3, and GluN1/GluN2/GluN3 subunits, although the functional properties of these latter tri-heteromeric receptors remain enigmatic. Co-assembly of glycine-binding GluN1 and GluN3 subunits creates glycine-activated receptors that possess a strikingly different pharmacological profile than conventional NMDARs that contain GluN1 and GluN2 and require both glutamate and glycine binding to activate. In addition, the GluN3 subunit has been suggested to influence synaptic development and plasticity. The study of GluN3-containing receptors has been hampered by the lack of pharmacological tools selective for the GluN3 subunit, which would allow dissection of the contribution of this receptor to neuronal function. We have identified a negative allosteric modulator (EU-1180-438) that is highly selective for GluN1/GluN3 receptors over GluN1/GluN2 NMDA, AMPA, and kainate receptors. The concentration-effect relationship using two-electrode voltage clamp current recordings indicated that EU1180-438 inhibited GluN1/3A receptors (co-expressed with GluN1-F484A,T518L) by 72% at saturating concentrations with an IC_{50} value of 2.9 μM. GluN1-F484A,T518L/GluN3A receptors showed a comparable glycine potency in the presence and absence of 30 μM EU1180-438. EU1180-438-induced maximal inhibition of GluN1-F484A,T518L/GluN3A current response was not altered by increasing glycine concentration, consistent with a non-competitive mechanism of action. Analysis of the current-voltage relationship revealed a similar degree of inhibition of the current response by EU1180-438 at all voltages, which we interpret as evidence that the inhibition does not reflect voltage-dependent channel block. These data support the idea that EU1180-438 is a negative allosteric modulator. The compound inhibits neuronal responses mediated by GluN3A-containing NMDA receptors recorded from hippocampal slice preparations in the presence of CGP-78608. These findings demonstrate that structural differences between GluN3 and other glutamate receptor subunits can be exploited to generate subunit-selective ligands, which have utility in exploring the role GluN3 plays in neuronal function.

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Poster

118. Ionotropic Glutamate Receptors: Pharmacology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 118.06/B12

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant NS036654
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NIH Grant HD082373

Title: Molecular mechanism of a GRIN2A variant associated with early-onset epileptic encephalopathy

Authors: *W. HAN[1,2,3], C. R. CAMP[1], C. BOSTICK[4], A. AMADOR[4], D. KRIZAY[4,5], S. J. MYERS[1,2], A. PODURI[6], D. B. GOLDSTEIN[5,4], M. BOLAND[7,4], W. N. FRANKEL[5,4], S. F. TRAYNELIS[1,2], H. YUAN[1,2];
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Abstract: N-methyl-D-aspartate receptors (NMDARs), ionotropic glutamate receptors, are comprised of two GluN1 and two GluN2 subunits. NMDARs can also be formed by tri-heteromeric receptors through combination of GluN1 and two different GluN2 subunits, and the expression of these different complexes is both spatially and temporally defined. Genetic variation in the *GRIN2A* gene encoding the GluN2A subunit has been associated with focal epilepsy and speech disorder with or without cognitive disability. Here, we investigated the functional effects of a *de novo GRIN2A* missense variant (c.1930 A>G; p.Ser644Gly, S644G) identified in a child with epilepsy, developmental delay, absence of speech, and intellectual disability. Our *in vitro* studies using two-electrode voltage clamp recordings on *Xenopus* oocytes revealed that the S644G variant enhanced glutamate and glycine potency by 17-fold (0.18 μM vs 3.0 μM of wild type, WT) and 10-fold (0.08 μM vs 0.86 μM of WT), respectively, indicating the variant receptors can be activated by much low concentrations of agonists. The variant also was virtually insensitive to endogenous negative allosteric modulators, such as extracellular H^+ and zinc. Whole-cell patch clamp recordings of transfected HEK cells suggests that S644G has a significantly prolonged deactivation time course following synaptic-like glutamate application by 44-fold (2,129 ms vs 48 ms of WT), and increased charge transfer by 50-fold. The channel open probability calculated from the degree of potentiation by the sulfhydryl-modifying reagent

methanethiosulfonate ethylammonium (MTSEA) for the mutant receptor was 6.7-fold higher than that of the WT, and indicates that the mutant channel can generate profound neuronal depolarization by spending more time in the open state. NMDARs that contained only a single copy of S644G showed an intermediate, but significantly enhanced agonist potency and synaptic-like response time course. Taken together, these data suggest the S644G is a gain-of-function variant. Homozygous *Grin2a*-p.S644G knockin mice showed severe spontaneous seizures, and died between PND15 and PND17. Heterozygotes mice had altered seizure threshold. Multielectrode array (MEA) analysis from cultured cortical neurons indicated both mutant heterozygous and homozygous networks displayed significantly increased spontaneous firing and altered bursting kinetics compared to the WT controls. The influence of this mutation on sensitivity to FDA-approved NMDAR antagonists was also evaluated, including memantine, dextromethorphan, and amantadine *in vitro* and dextromethorphan *in vivo* to explore potential rescue pharmacology.

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Poster

118. Ionotropic Glutamate Receptors: Pharmacology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 118.07/B13

Topic: B.04. Ion Channels

Support: AHA 16SDG27480023
VCOM REAP 10303

Title: An agonist concentration-dependent allosteric modulator separates triheteromeric (GluN1/2A/2B) from diheteromeric (GluN1/2A) NMDA receptors

Authors: B. N. VACCA[1], T. V. JOHNSTON[1], D. N. BLEDSOE[1], A. K. WAGNER[1], ***B. M. COSTA**[2];

[1]Edward Via Virginia Col. of Osteo. Med., Blacksburg, VA; [2]Ctr. for One Hlth. Research, Virginia Tech., Blacksburg, VA

Abstract: The N-methyl-D-aspartate (NMDA) subtype of glutamate receptor plays a crucial role in brain physiology and pathogenesis of disorders. Functional tetra-heteromeric NMDA receptor ion channels can be formed by two GluN1 subunits and two identical or different non-GluN1 subunits. Recent studies report that GluN1/2A/2B subunit containing triheteromeric NMDA receptors are predominantly expressed in the adult cortex and hippocampus. Further understanding of this complex combination of NMDA receptor subunits is limited by lack of chemical tools to isolate the GluN1/2A/2B from the diheteromeric receptors. In the present study, using two-electrode voltage clamp electrophysiology, we have identified and characterized a compound (CNS004), which selectively potentiates agonist-evoked recombinant GluN1/2A/2B and GluN1/2B receptor currents, but it has no effect on GluN1/2A receptors. In contrast, with a tenfold increase in agonist concentration, CNS004 potentiates GluN1/2A while leaving GluN1/2A/2B and GluN1/2B receptor currents unaltered. A whole-cell patch-clamp electrophysiology assay with rapid solution exchange reveals that co-application of agonist and CNS004 inhibits current amplitude in GluN1/2A, GluN1/2B, and GluN1/2A/2B receptors; however, a prolonged co-application of agonist and CNS004 potentiates the steady state current in GluN1/2A/2B receptors. Decay curve analysis indicates a CNS004-mediated increase in desensitization and deactivation time constants in GluN1/2A/2B receptors. These results reveal an agonist concentration-dependent positive and negative allosteric modulatory effect of CNS004 that is selective to GluN1/2A/2B over GluN1/2A receptors. This novel selectivity pattern will be helpful to separate the native GluN1/2A/2B receptors from GluN1/2A receptors. Therefore, CNS004 or its future analogs will be helpful in developing clinically useful GluN1/2A/2B receptor selective pharmacological agents.

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Poster

118. Ionotropic Glutamate Receptors: Pharmacology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 118.08/B14

Topic: B.04. Ion Channels

Title: Development and validation of NMDA ligand-gated ion channel assays using the Qube 384 automated electrophysiology platform

Authors: A. MARKLEW, J. KAMMONEN, E. RICHARDSON, *V. LAZARI, J. MANN; Charles River, Saffron Walden, United Kingdom

Abstract: Ligand-gated ion channels are of particular interest to the pharmaceutical industry for the treatment of diseases from a variety of therapeutic areas including CNS disorders, respiratory disease and chronic pain. Ligand-gated ion channels have historically been investigated using fluorescence-based and low throughput patch-clamp techniques. However the development of the Qube 384 automated patch-clamp system has allowed rapid exchange of liquid and direct measurement of ion channel currents on a millisecond timescale, making it possible to run HTS campaigns and support SAR with a functional readout. Here, we have used the Qube platform to develop an assay against the NR1/NR2A receptor, which is part of the N-methyl-D-aspartate (NMDA) glutamate receptor family. For this assay we utilized stacked liquid addition which enabled us to assess the open state kinetics of the channel and to investigate the effects of antagonists with multiple modes of actions. We observed stable agonist responses for both NMDA and glycine, with EC_{50} values (2.7 and 42.1 μM, respectively) which compared to literature values. The assay format was stable over multiple agonist applications and wash periods, this meant it was suitable for compound testing and sensitive enough to detect antagonists with multiple modes of action. D-AP-5, a competitive antagonist, inhibited NR1/NR2A receptors with an IC_{50} value of 0.48 μM and the inhibition was stable over six consecutive agonist/antagonist applications. Whereas, the potency of ketamine, a use-dependent inhibitor, increased over the six agonist/antagonist applications, IC_{50} values of 30.2 μM at application one, versus 2.7 μM at application six. By utilizing of stacked liquid additions in a 384 automated electrophysiology platform we have created an assay against NMDA receptors which is suitable for compound testing and sensitive enough to detect different modes of actions.

Disclosures: **A. Marklew:** A. Employment/Salary (full or part-time):; Charles River. **J. Kammonen:** A. Employment/Salary (full or part-time):; Charles River. **E. Richardson:** A. Employment/Salary (full or part-time):; Charles River. **V. Lazari:** A. Employment/Salary (full or part-time):; Charles River Labs. **J. Mann:** A. Employment/Salary (full or part-time):; Charles River.

Poster

118. Ionotropic Glutamate Receptors: Pharmacology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 118.09/B15

Topic: B.04. Ion Channels

Support: NIH R33DA041876

Title: NMDAR phosphorylation and function is regulated by PP1 targeting protein, spinophilin

Authors: *A. B. SALEK[1], M. C. EDLER, JR[3], J. MCBRIDE[2], A. J. BAUCUM II[1]; [1]Biol., [2]Indiana University-Purdue Univ. Indianapolis, Indianapolis, IN; [3]Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Excitotoxicity is a major hallmark of many neurodegeneration cases like cerebral ischemia. Excitotoxicity is caused by accumulation of glutamate in extracellular space leading to hyperactivation of glutamate receptors such as NMDA receptor. Excessive influx of calcium following NMDA receptor activation, will eventually activate apoptotic pathways leading to loss of neurons. Various research shows that regulation of NMDA receptor subunit composition, localization, surface expression, and activity strongly determine the activation of pro-death or pro-survival pathways after a course of an ischemic insult. Strong evidence suggests that differential subunit phosphorylation of NMDAR define receptor activity, downstream signaling pathways, and localization. NMDA receptor subunit phosphorylation and activity is regulated by protein phosphatases such as protein phosphatase 1. PP1 obtains substrate specificity by forming complexes with targeting subunits. Spinophilin, the major PP1 targeting protein in the postsynaptic density, is known to change phosphorylation state of various substrates via its PP1 binding ability. Our data show that spinophilin can function to decrease both PP1γ1 and PP1α binding to the GluN2B subunit of the NMDAR in vitro and in vivo. We also demonstrate that, overexpression of spinophilin attenuated PP1-induced decreases in Ser-1284 phosphorylation on GluN2B. Furthermore, we show that global loss of spinophilin alters GluN2B phosphorylation and interactome in mouse hippocampus. Taken together, our data demonstrate a unique mechanism by which spinophilin attenuates PP1γ1-dependent dephosphorylation of GluN2B at Ser-1284, a site that has altered phosphorylation in ischemia.

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Poster

118. Ionotropic Glutamate Receptors: Pharmacology

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Topic: B.02. Ligand-Gated Ion Channels

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BIOCEV – Biotechnology and Biomedicine Centre of Academy of Sciences and Charles University in Vestec, project supported from European Regional Development Fund.

Title: Pregnane based steroids: A new class of positive allosteric modulators of NMDA receptors

Authors: *T. SMEJKALOVA[1], **B. KYSILOV**[1,2], B. KRAUSOVA[1], V. VYKLICKY[1], A. BALIK[1], M. KORINEK[1], M. LADISLAV[1], P. HUBALKOVA[1], M. HORAK[1], M. NEKARDOVA[3,4], H. CHODOUNSKA[3], E. KUDOVA[3], J. CERNY[1], L. VYKLICKY, Jr.[1]; [1]Inst. of Physiology, CAS, Prague, Czech Republic; [2]Third Fac. of Medicine, Charles Univ. in Prague, Prague, Czech Republic; [3]Inst. of Organic Chem. and Biochemistry, CAS, Prague, Czech Republic; [4]Fac. of Mathematics and Physics, Charles Univ. in Prague, Prague, Czech Republic

Abstract: N-methyl-D-aspartate receptors (NMDARs) are glutamate-activated ion channels which play a key role in excitatory synaptic transmission. Their hypofunction has been shown to be implicated in the etiology of neuropsychiatric disorders like autism, intellectual disability, and schizophrenia. Therefore, positive allosteric modulators of NMDARs, such as some neurosteroids, may have a favorable effect on these disorders. Experimental data from several laboratories indicate that steroids with a flat structure at the A/B ring – like an endogenous steroid pregnenolone sulfate (PE-S) – potentiate NMDAR responses, while steroids with a "bent" structure – like an endogenous steroid pregnanolone sulfate (PA-S) - inhibit them. The aim of this study is to characterize the structure-activity relationship underlying the positive modulatory effect for newly synthetized analogs of PA-S.

We have prepared a set of pregnane analogs in which the ester bond was substituted by a C-C bond (ω5β-pregnan-3α-yl derivatives of carboxylic acids) and assessed their activity at recombinant GluN1/GluN2B receptors expressed in HEK293 cells using the patch-clamp technique. The results of our experiments show that analogs with short C3 residues (e.g. 3α5βPA-Carboxylate (200 μM) and 3α5βPA-Acetate (10 μM)) inhibit NMDAR responses (65±3%; n= 5 and 79±3%; n=6, respectively). However, derivatives with elongated aliphatic chain (3α5βPA-Butyrate (15 μM) and 3α5βPA-Propionate (30 μM)) potentiate the responses (82±10%; n=7 and 37±1%; n=4, respectively), which is unexpected due to their "bent" structure. 15μM 3β5βPA-Butyrate (3β5βPA-But) also showed a strong potentiating effect on NMDAR (190±6%; n=7).

Subsequent analysis of the steroid effect showed that 3β5βPA-But is similar to PE-S in terms of slow on- and off- kinetics, and disuse-dependence of the effect at GluN1/GluN2B receptors. However, further experiments showed additive effects of both steroids, providing evidence for distinct binding sites or mechanisms of action. Alanine scanning mutagenesis of the amino acid residues within outer segments of the transmembrane domains (TMDs) of GluN1 (Q559A-V572A; T809A-V825A) and GluN2B (S555A-I568A; D814-L825A) subunits indicated that residues which affect the degree of 3β5βPA-But potentiation are located at the interface between the TMDs TM1and TM4 of both subunits and are different from those which affect PE-S potentiation.

Our study shows that the newly synthesized pregnane analogs are potent positive modulators of NMDARs whose effect is mediated by the interaction with the TMDs and indicates new possibilities for *in silico* development of new therapeutics.

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Poster

118. Ionotropic Glutamate Receptors: Pharmacology

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Topic: B.02. Ligand-Gated Ion Channels

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Title: *In vitro* activity of structurally diverse steroids on modulation of N-methyl-D-asparate receptors

Authors: *S. ADLA[1], E. SZÁNTI-PINTÉR[1], B. KRAUSOVA[2], B. KYSILOV[2], P. HUBALKOVA[2], L. VYKLICKY JR[2], E. KUDOVA[1];
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Abstract: Introduction: NMDA receptors are glutamatergic ionotropic receptors involved in excitatory neurotransmission, synaptic plasticity and excitotoxic cell death. Positive allosteric modulators that increase the activity of NMDARs may provide a therapeutic aid for patients suffering from neuropsychiatric disorders where NMDARs hypofunction is thought to be involved, such as intellectual disability, autism spectrum disorders, or schizophrenia. Here, we report a structure-activity relationship study defining structural features affording positive modulatory effect. According to the literature, the planar ring structure of the Δ-5 and 5α-steroids favors potentiation of NMDARs. **Experimental approach:** Electrophysiological recordings and live microscopy were performed on heterologous HEK293 cells expressing GluN1/GluN2B receptors. **Key results:** A series of novel steroidal modulators has been prepared targeting structural modifications at C-3, C-5 and C-17. First, dicarboxylic acid hemiesters of various lengths in combination with 5α, Δ-4, and Δ-5 have been prepared. Next, a series of compounds bearing various type of negatively charged C-3 substituents was prepared. Last, isosteric replacement of pregnane 17-acetyl moiety afforded a series of steroidal carbonitriles bearing various geometry. The modulatory effect at GluN1/GluN2B receptors was studied. **Conclusions:** Novel structural requirements affording novel steroidal positive modulators have been identified.

Moreover, our data suggest that the steroid recognition site(s) bear a high degree of structural specificity associated with NMDAR function.

Disclosures: S. Adla: None. **E. Szánti-Pintér:** None. **B. Krausova:** None. **B. Kysilov:** None. **P. Hubalkova:** None. **L. Vyklicky Jr:** None. **E. Kudova:** None.

Poster

118. Ionotropic Glutamate Receptors: Pharmacology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 118.12/B18

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH/NIGMS grant number: R01GM128195

Title: Membrane to channel inhibition of NMDARs by (+)-MK-801

Authors: *A. NIGAM, M. R. WILCOX, J. W. JOHNSON;
Univ. of Pittsburgh, Pittsburgh, PA

Abstract: N-methyl-D-aspartate receptor (NMDAR) antagonism is a research area with broad implications to pathological conditions such as Alzheimer's disease and epilepsy. NMDARs are excitatory glutamate- and glycine-gated ion channels that are formed by pairing two GluN1 subunits with two GluN2 (A-D) and/or GluN3 (A-B) subunits. NMDAR channels exhibit high calcium permeability, allowing them to contribute to development and synaptic plasticity, but also to pathophysiological excitotoxicity. Many NMDAR antagonists, including memantine and MK-801, are channel blockers, which inhibit by blocking ion flow at a site in the ion channel (the "deep site"). Glasgow et.al. (2018, Neuropharm 137, 344) recently showed that memantine can access the deep site via two paths: the traditional channel blocking path from the extracellular solution, or by associating with a "second site" from which memantine can transit to the deep site following NMDAR activation. Unpublished work from our lab indicates that the second site is a reservoir located in the cell membrane; we thus renamed the phenomenon "membrane to channel inhibition" (MCI). Here we investigate MCI by MK-801 using tsA201 cells transfected to express NMDARs. To determine whether MK-801 MCI of NMDARs composed of GluN1 and GluN2A subunits (GluN1/2A receptors) is voltage dependent we applied 1 μM MK-801 at either -65 or +35 mV. To ensure that MK-801 could not block NMDARs from the extracellular solution, we applied MK-801 in the absence of glutamate and presence of 50 μM APV; washed off both antagonists; applied 1 mM glutamate (all solutions contained 100 μM glycine); measured the minimum NMDAR-mediated current relative to control current (I_{MCI}/I_{Con}). We found that I_{MCI}/I_{Con} at -65 mV (0.38 ± 0.04 (n=4)) was significantly different from I_{MCI}/I_{Con} at +35 mV (0.64 ± 0.02 (n=3); p = 0.002). Our results suggest that voltage dependence of MK-801 MCI is weaker than typically observed for channel

block by monovalent blockers. We examined the time course of MCI onset (τ_M) and found that τ_M at -65 mV (0.93 ± 0.02 s (n=4)) was not significantly different from τ_M at +35 mV (0.89 ± 0.1 s; (n=3)). While examining NMDAR subtype dependence of MK-801 MCI we found that at -65 mV, I_{MCI}/I_{Con} of GluN1/2B receptors (0.30 ± 0.02; (n=3)) was not significantly different from I_{MCI}/I_{Con} of GluN1/2A receptors (0.38 ± 0.03 (n=4); p=0.28). The τ_M for GluN1/2B receptors (0.93 ± 0.03 s (n=3)) was significantly different from τ_M for GluN1/2A receptors (1.20 ± 0.05 s; (n=3); p=0.02). Finally, we examined MK-801 MCI in neuronal cultures, I_{MCI}/I_{Con} at -65 mV was 0.48 ± 0.03; (n=3) and τ_M was 1.02 ± 0.03 s; (n=3). Overall, MK-801 MCI has moderate voltage dependence and slow kinetics.

Disclosures: **A. Nigam:** None. **M.R. Wilcox:** None. **J.W. Johnson:** None.

Poster

118. Ionotropic Glutamate Receptors: Pharmacology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 118.13/B19

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH/NIGMS grant number: R01GM128195

Title: Subtype specificity of calcium-dependent NMDA receptor block by memantine

Authors: *M. B. PHILLIPS, J. W. JOHNSON;
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Abstract: N-methyl-D-aspartate receptors (NMDARs) are ionotropic glutamate receptors expressed at nearly all excitatory vertebrate synapses. NMDARs are key mediators of neuronal Ca^{2+} influx and play a central role in synaptogenesis, synaptic plasticity, dendritic integration, and neuronal survival. NMDARs also display impressive functional diversity, with each NMDAR subtype possessing different biophysical and pharmacological properties, expression patterns, and signaling partners. Aberrant NMDAR activity is heavily involved in neurological and psychiatric disorders. Thus, drugs targeting NMDARs are of great clinical interest. The NMDAR channel blocker memantine (Mem) is a well-tolerated Alzheimer's disease medication and shows promise in treatment of other disorders including ischemia and Huntington's disease. Interestingly, Mem displays a strikingly different clinical profile from other NMDAR channel blockers, most notably ketamine (Ket). This discrepancy could partially result from Mem preferentially targeting specific subpopulations of NMDARs distinct from those inhibited by Ket. We recently reported that Mem, but not Ket, enhances desensitization of GluN1/2A receptors (NMDARs composed of GluN1 and GluN2A subunits) in a Ca^{2+}-dependent manner, suggesting that Mem stabilizes a Ca^{2+}-dependent desensitized receptor state. Here we further investigate the relation between Ca^{2+}-dependent desensitization and the mechanism of action of

Mem. Experiments manipulating free [Ca^{2+}] in the recording pipette revealed that inhibition of GluN1/2A receptors by Mem, but not Ket, powerfully depends on intracellular [Ca^{2+}], a phenomenon we term [Ca^{2+}]-dependent block (CDB). Truncation of the C-terminal domain of the GluN1 subunit, a region of the receptor required for Ca^{2+}-dependent desensitization, ablated CDB by Mem. Furthermore, Mem inhibition of GluN1/2B receptors shows no dependence on intracellular [Ca^{2+}]. The subtype specificity and dependence on increased intracellular [Ca^{2+}] of CDB could lead to Mem preferentially inhibiting overactive neuronal subpopulations with enriched GluN2A expression. Our results strongly support the hypothesis that Mem stabilizes a Ca^{2+}-dependent desensitized state of GluN1/2A receptors and describe a previously unexamined form of context-dependent NMDAR antagonism.

Disclosures: **M.B. Phillips:** None. **J.W. Johnson:** None.

Poster

118. Ionotropic Glutamate Receptors: Pharmacology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 118.14/B20

Topic: B.02. Ligand-Gated Ion Channels

Support: Whitehall Foundation #2017-08-30
NIH R21MH116315
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Title: Voltage dependence of synaptic NMDA receptor potentiation by exogenous co-agonist

Authors: *J. M. WONG[1], E. V. BARRAGAN[1], J. A. GRAY[2];
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Abstract: NMDA receptors (NMDARs) are glutamate receptors that also require a co-agonist for channel opening. The identity of the co-agonist, either glycine or D-serine, is developmentally and spatially regulated in the brain, and although the co-agonists regulate synaptic transmission, neuronal function, and synaptic plasticity, their availability at the synapse remains poorly understood. Though initial studies suggested that the NMDAR co-agonist site is saturated, later studies have demonstrated that the co-agonist site is not saturated at synapses and that co-agonist availability may be regulated by activity. Indeed, the co-agonist site has become an attractive target for drug discovery efforts. However, our understanding of the regulation of NMDAR co-agonist site occupancy surprisingly remains quite limited and controversial. Indeed, exogenous application of co-agonists on acute brain slices produces heterogeneous amounts of NMDAR-EPSC potentiation making firm conclusions regarding saturation state difficult. This heterogeneity is especially evident with exogenous glycine application, though this is typically interpreted as being due to synapse-flanking glycine transporters and glycine-dependent

NMDAR endocytosis. However, D-serine application has also produced heterogeneous potentiation indicating that more complex mechanisms are at play. Here we report an additional complexity - exogenous D-serine application on voltage-clamped CA1 pyramidal neurons of P60 mice potentiates NMDAR-EPSCs at negative but not positive holding potentials.

Disclosures: J.M. Wong: None. **E.V. Barragan:** None. **J.A. Gray:** None.

Poster

118. Ionotropic Glutamate Receptors: Pharmacology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 118.15/B21

Topic: B.02. Ligand-Gated Ion Channels

Title: NYX-783, a novel NMDAR modulator, rescues the detrimental effects of encephalitis-causing anti-NMDAR antibodies on GluN2B-NMDAR expression *in vitro*

Authors: *M. E. SCHMIDT[1], S. U. SAHU[2], R. A. KROES[1,2], M. BLAAGJERG[3,4], E. COLECHIO[1], T. BHATTACHARYA[1], J. R. MOSKAL[1,2];
[1]Aptinyx Inc., Evanston, IL; [2]Northwestern Univ., Evanston, IL; [3]Odense Univ. Hosp., Odense, Denmark; [4]Univ. of Southern Denmark, Odense, Denmark

Abstract: N-methyl-D-aspartate receptors (NMDARs) are ionotropic glutamate receptors found in the central nervous system (CNS) and are critical for synaptic plasticity processes underlying numerous cognitive functions, including learning and memory. Loss of NMDAR function is implicated in many psychiatric and CNS disorders, including anti-NMDAR encephalitis (ANRE). Symptoms of ANRE begin with severe psychiatric problems and progress to seizures, movement impairments, and autonomic dysfunction. ANRE is an autoimmune disorder caused by the production of antibodies against the obligatory GluN1 subunit of the NMDAR. Binding of anti-GluN1 antibodies to the NMDAR leads to receptor internalization and thereby a reduction in functional NMDARs. Although ANRE can be treated with various immunotherapies, many patients have persistent cognitive deficits. Here we show that NYX-783, a small molecule NMDAR modulator, enhanced long-term potentiation (LTP) in rat hippocampal and medial prefrontal cortex (mPFC) slices, as well as improved rats' performance in the novel object recognition (NOR) paradigm. Proteomic analysis of rat mPFC found that NYX-783 increased GluN2A- and GluN2B-NMDAR expression in the post-synaptic density. Similarly, in HEK cells expressing recombinant NMDARs, NYX-783 increased GluN2B-NMDAR surface expression. Given the effects of NYX-783 on NMDAR-dependent synaptic plasticity, NOR performance, and NMDAR surface/synaptic expression, we sought to determine whether it could rescue NMDAR internalization in response to anti-GluN1 antibody exposure. In HEK cells expressing recombinant GluN2B-NMDARs, 45min incubation with either ANRE patient IgG or CSF decreased GluN2B-NMDAR surface expression. Without treatment, these expression levels

remained low until 2 hours following antibody removal. In contrast, incubation with NYX-783 after antibody removal resulted in normalized NMDAR expression within 30min of NYX-783 application. In summary, NYX-783 can enhance *ex vivo* LTP, improve learning, increase NMDARs at the synapse *in vivo*, as well as restore lost NMDARs to the surface following ANRE patient antibody exposure. Altogether these data support the further investigation of NYX-783 for the treatment of ANRE.

Disclosures: **M.E. Schmidt:** A. Employment/Salary (full or part-time):; Aptinyx Inc. **S.U. Sahu:** A. Employment/Salary (full or part-time):; Northwestern University. **R.A. Kroes:** A. Employment/Salary (full or part-time):; Aptinyx Inc.. Other; Northwestern University. **M. Blaagjerg:** A. Employment/Salary (full or part-time):; Odense University Hospital. **E. Colechio:** None. **T. Bhattacharya:** A. Employment/Salary (full or part-time):; Aptinyx Inc. **J.R. Moskal:** A. Employment/Salary (full or part-time):; Aptinyx Inc..

Poster

118. Ionotropic Glutamate Receptors: Pharmacology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 118.16/B22

Topic: B.02. Ligand-Gated Ion Channels

Support: FNRS Grant J0148.19
Uliège Grant FSR2018
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Title: Ketamine selectively enhances AMPA neurotransmission onto a subgroup of identified serotoninergic neurons of the rat dorsal raphe

Authors: *C. HMAIED, S. KOULCHITSKY, D. ENGEL, V. SEUTIN;
GIGA - Neurosciences, Univ. of Liège, Lab. of Neurophysiol., Liège, Belgium

Abstract: It is now well established that the non-competitive NMDA antagonist ketamine is effective as a quickly acting antidepressant that can be used in selected clinical situations. However, its mechanism(s) of action is (are) not well understood. One region where an effect of the drug would make great sense is the dorsal raphe. Indirect evidence also points to the possibility of an enhanced excitability of serotoninergic (5HT) neurons in this area. We set out to directly test this hypothesis using whole-cell patch clamp recordings in brainstem slices from juvenile (P21-P30) rats. We used a conventional bicarbonate-based Ringer and a KCl-based intrapipette solution. This allowed us to discriminate presumed 5HT from non-5HT neurons. 5HT neurons were identified as cells generating an outward current > 30 pA in voltage clamp at -60 mV during superfusion of 100 nM of the $5HT_{1A}$ agonist 8-OH-DPAT (calculated E_K was = -93 mV). Voltage clamp experiments were performed to test ketamine's effect on sEPSCs in the

presence of 10µM gabazine and 1 µM CGP55845. We found that 10 µM racemic ketamine markedly enhanced fast AMPA EPSCs (both amplitude and frequency) in about half of 5HT neurons (N total = 18). This effect was very spectacular in some neurons, where « barrages » of EPSCs were observed during ketamine. The 8-OH-DPAT-induced currents had a similar amplitude in both ketamine-sensitive and insensitive neurons. On the other hand, sEPSCs observed in non-5HT neurons were unaffected (N = 6). The effect of ketamine was shared by the racemate of its metabolite 2,6-hydroxynorketamine (HNK). In current clamp recordings, both ketamine and HNK increased the firing of 5HT neurons in whole-cell recordings when AMPA, NMDA, $GABA_A$ and $GABA_B$ receptors were blocked. However, this effect was not observed in extracellularly recorded 5HT neurons from adult rats (using 10 µM phenylephrine to boost their pacemaking). Additional experiments are in progress to resolve this issue. In conclusion, both ketamine and HNK robustly enhance AMPA EPSCs onto a subgroup of pharmacologically identified 5HT neurons. It remains to be determined whether this subgroup projects to specific targets, given that subpopulations of DR 5HT neurons have been shown to have specific and non-overlapping projections (Ren et al., Cell 175, 472-487, 2018).

Disclosures: C. Hmaied: None. **S. Koulchitsky:** None. **D. Engel:** None. **V. Seutin:** None.

Poster

118. Ionotropic Glutamate Receptors: Pharmacology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 118.17/B23

Topic: B.02. Ligand-Gated Ion Channels

Support: MH113189

Title: Sex difference in AMPAR modulation that underlies 17β-estradiol-induced potentiation in the hippocampus

Authors: *A. JAIN, C. S. WOOLLEY;
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Abstract: 17β-estradiol (E2) acutely potentiates hippocampal CA3-CA1 synapses via largely independent pre- and postsynaptic mechanisms. Although E2-induced potentiation is similar in magnitude between males and females, the underlying molecular signaling is strikingly different. We previously reported sex differences in the requirements of estrogen receptor subtypes, calcium sources, and protein kinase A to induce this potentiation. To determine where these distinct signaling mechanisms converge to produce similar potentiation, here we investigated mechanisms underlying the postsynaptic component of E2-potentiation. Using whole-cell voltage clamp recording and glutamate uncaging in acute hippocampal slices from adult rats, we confirmed that E2 acutely potentiates 2-photon evoked excitatory postsynaptic currents

(2pEPSCs) at a subset of synapses in both sexes. In females, E2 potentiated 2pEPSCs by 83±11% in 37 of 78 spines on 18 cells and, in males, by 88±8% in 33 of 54 spines on 12 cells (n.s.).
To investigate whether postsynaptic potentiation is due to increased AMPAR conductance and/or AMPAR number, we performed conventional and peak scaled non-stationary fluctuation analysis (cNSFA and psNSFA) on the decay phase of 2pEPSCs in all spines with >18 events in both baseline and E2 conditions. In females, cNSFA estimated that AMPAR conductance increased in 13 of 17 E2-responsive spines (76%), by 101±24%, whereas in males, cNSFA estimated that conductance increased in only 8 out of 20 (40%) responsive spines, by 117±28% (Fisher's exact test, p=0.04). Subsequent psNSFA on the same spines showed that responsive spines increased in either AMPAR conductance or number, but not both. psNSFA confirmed the increases in conductance observed with cNSFA, and also showed that AMPAR number increased in the other E2-responsive spines where conductance did not increase; psNSFA estimated that AMPAR number increased by 125±35% in females (n=4) and by 127±26 % in males (n=12). In E2 non-responsive spines (females n=12, males n=10), neither cNSFA nor psNSFA indicated changes in AMPAR properties with E2 treatment. Overall, this study demonstrates that: (1) E2 potentiates excitatory synapses through both increased AMPAR conductance and AMPAR number, but that individual synapses show one or the other change and not both; and (2) whereas increased AMPAR conductance and number occur in roughly equal proportions in males, increased AMPAR conductance predominates in females. Thus the distinct molecular signaling that underlies synaptic potentiation in males and females leads to distinct patterns of AMPAR modulation that produce similar magnitude synaptic potentiation.

Disclosures: A. Jain: None. **C.S. Woolley:** None.

Poster

118. Ionotropic Glutamate Receptors: Pharmacology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 118.18/B24

Topic: B.02. Ligand-Gated Ion Channels

Title: AMPA receptor dynamics contribute to tune synaptic plasticity and brain function

Authors: *D. CHOQUET;
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Abstract: The spatio-temporal organization of neurotransmitter receptors in the postsynaptic membrane is a fundamental determinant of synaptic transmission and thus information processing by the brain. Ionotropic AMPA glutamate receptors (AMPAR) mediate fast excitatory synaptic transmission in the central nervous system. Using a combination of high resolution single molecule superresolution imaging and tracking techniques, we have established

that AMPARs are not all stable in the synapse as thought initially, but in large part undergo continuous entry and exit to and from the post-synaptic density through lateral diffusion. The other fraction of AMPAR are highly concentrated inside synapses into a few clusters of around seventy nanometers. These results have opened the new possibility that glutamatergic synaptic transmission is controlled by the regulation at the nanometer scale of the position and composition of these highly concentrated nanodomains. The dynamic exchange of AMPAR within the PSD and between synaptic and extrasynaptic sites is highly regulated by neuronal activity. We have demonstrated that AMPAR conformation strongly impacts their mobility, desensitized receptors being more mobile than naïve ones. This property likely explains how post-synaptic AMPAR receptor mobility can regulate short term synaptic plasticity, a feature previously ascribed to pre-synaptic mechanisms. Recently, using new methods to exogenously control AMPAR surface diffusion, we have demonstrated that AMPAR surface diffusion directly controls the establishment of long term synaptic plasticity. We will now present a series of new results that 1) establish a link between regulation of AMPAR surface diffusion and changes in short term plasticity during Long Term Depression, 2) expand the role of AMPAR surface diffusion to synaptic plasticity *in vivo* and 3) present how controlling AMPAR surface trafficking can provide insight into the implication of synaptic plasticity in various learning paradigms.

Disclosures: D. Choquet: None.

Poster

119. Calcium Channels

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 119.01/B25

Topic: B.04. Ion Channels

Support: AG052934-03

Title: The development and characterization of the new, conditional mouse model over-expressing $Ca_v1.2$

Authors: *R. PARENT[1], L. J. OUILLETTE[1], E. GLASS[1], H. BURNS[1], A. SMARSH[1], G. G. MURPHY[2];
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Abstract: L-type voltage gated Ca^{2+} channels (LVGCC) are integral membrane proteins that modulate the influx of Ca^{2+} into excitable cells in response to membrane depolarization. In the brain, $Ca_v1.2$ is the most abundant LVGCC and accounts for ~89% of LVGCCs. $Ca_v1.2$ has been shown to couple Ca^{2+} currents to transcriptional regulation, playing an important role in dendritic development, synaptic plasticity, neuronal survival and learning and memory. The $Ca_v1.2$ protein

complex is an assemblage of three different subunits, with the main pore forming subunit, α1C, being encoded for by *CACNA1C*. Functional mutations within exon 8 cause Timothy Syndrome, which is characterized by heart defects and autism spectrum symptoms, likely resulting from a gain of function mutation. Additionally, GWAS studies have linked several SNPs within *CACNA1C*, most of which are found in intron 3, to bipolar disorder, schizophrenia and major depressive disorder, which are thought to be a result of alterations in transcriptional regulation. Changes in expression of $Ca_v1.2$ have also been linked to Alzheimer's disease (AD) and its well-supported hypothesis that calcium dysregulation contributes to AD pathology. It has been shown that APP interacts with $Ca_v1.2$, and complete loss of APP results in a substantial increase in $Ca_v1.2$ in GABAergic neurons, and a comparable increase in Ca^{2+} currents.
While it is clear that changes in the *CACNA1C* sequence and $Ca_v1.2$ expression are associated with several disease states, and there has been a significant amount of work demonstrating the loss of $Ca_v1.2$ produces a number of behavioral and affective phenotypes, little effort has been made to understand the impact of *increased* expression. Therefore, we have created a novel line of transgenic mice that over express $Ca_v1.2$, which contains an HA tag allowing us to detect the exogenous protein expression. Expression of the transgene is driven by the CAG promoter, and contains a Lox-Stop-Lox cassette upstream of the cDNA sequence, allowing us to control its expression using cre-recombinase.
In preliminary behavioral studies, mice with pan neuronal expression of the transgene have a decrease in anxiety-like behaviors, as measured by an increase in the amount of time spent in the open arms of the elevated-plus maze and the open sides of the zero maze when compared to controls. Additionally, they have a deficit in contextual associative learning, as assessed by Pavlovian fear conditioning. These mice do not appear to have any deficits in motor coordination or locomotor activity when assessed in the open field and rota-rod tasks. These results are consistent with previous studies associating CACNA1C with affective disease states.

Disclosures: **R. Parent:** None. **L.J. Ouillette:** None. **E. Glass:** None. **H. Burns:** None. **A. Smarsh:** None. **G.G. Murphy:** None.

Poster

119. Calcium Channels

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 119.02/B26

Topic: B.04. Ion Channels

Support: Natural Science and Engineering Council (RWT)
University of Calgary Eyes High PDF (GS)
Alberta Innovates Health Solutions (GS) PDF

Title: Endoplasmic reticular junctophilin proteins maintain a Cav1.3-RyR2-KCa3.1 tri-protein complex at ER-PM junctions in hippocampal pyramidal neurons

Authors: *G. SAHU[1], R.-M. WAZEN[2], P. COLARUSSO[2], G. W. ZAMPONI[1], S. R. W. CHEN[3], R. W. TURNER[1];
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Abstract: Extracellular calcium entry mediated by plasma membrane (PM) L-type voltage gated calcium channels (Cav1 family) and endoplasmic reticular (ER) internal calcium release by ryanodine receptors (RyR) plays a vital role in controlling the firing pattern of hippocampal pyramidal neurons. Our recent work reported the assembly and functional coupling of a tri-protein complex of L-type Cav1.3 channel, intermediate conductance calcium-activated potassium channel (KCa3.1) and ER-localised RyR2 channels in generating a slow afterhyperpolarization (sAHP) in hippocampal pyramidal neurons. However, the molecular details of this multiprotein assembly have not been characterized. With the use of stochastic optical reconstruction super-resolution microscopy (STORM) and FRET to detect protein-protein interactions we found ER junctophilin (JPH) proteins forming close associations with members of the Cav1.3-RyR2-KCa3.1 complex. STORM imaging (30-40 nm precision) of dissociated hippocampal neurons in TIRF mode (100-150 nm) revealed overlapping nano clusters of JPH 3 & 4 proteins with clusters of subunits of the Cav1.3-RyR2-KCa3.1 complex. Further, FRET was observed between all members of the Cav1.3-RyR2-KCa3.1 complex with JPH 3 & 4. As JPH proteins are found at the site of ER-PM junctions we expressed the ER-PM junctional marker MAPPER-GFP in hippocampal neurons with subunits of the Cav1.3-RyR2-KCa3.1 complex. STORM demonstrated close apposition between MAPPER-GFP and members of the Cav1.3-RyR2-KCa3.1 complex, suggesting localization at ER-PM junctions. Treatment of hippocampal neurons with shRNAs against JPH 3 &4 dissociated the multi-protein complex and also abolished the sAHP. Further infusion of CA1 pyramidal neurons in the slice preparation with JPH 3 & 4 antibodies decreased spike accommodation and increased spike frequency in current clamp mode and in voltage clamp mode the infusion of JPH 3 & 4 antibodies resulted in a decrease of sAHP current. These data establish a tight association and functional coupling between JPH proteins and the Cav1.3-RyR2-KCa3.1 complex at ER-PM junctions to trigger activation of the hippocampal sAHP.

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Poster

119. Calcium Channels

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 119.03/B27

Topic: B.04. Ion Channels

Support: Conacyt

Title: The ubiquitin E3 ligase parkin regulates $Ca_v1.3$ channel functional expression

Authors: *L. GRIMALDO[1], A. SANDOVAL[2], P. DURAN[1], R. FELIX[3];
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Abstract: Neuronal L-type calcium channels of the $Ca_V1.3$ subclass are multisubunit complexes composed of the pore-forming $Ca_V\alpha_1$ subunit and auxiliary $Ca_V\alpha_2\delta$ and $Ca_V\beta$ subunits. It is acknowledged that the pacemaker activity in the adult substantia nigra depends on the L-type channel activity. Likewise, experimental evidence suggest that altered function of $Ca_V1.3$ channels may play a role in the progress of the neurodegenerative mechanisms implicated in Parkinson's disease (PD), including a dysregulation of channel expression levels associated with mitochondrial oxidative stress. In addition, $Ca_V1.3$ channel antagonists have been described as potential therapeutic tools for PD. Although $Ca_V1.3$ channel expression is precisely regulated, an increased level of L-type channel expression has been observed in aged animals that could be related with alterations in the synthesis and/or degradation processes. In a previous report, by using a combination of biochemical and functional analyses, we showed that Parkin, an E3 enzyme of the ubiquitin-proteasome system (UPS), may interact with the neuronal $Ca_V2.2$ channels promoting their ubiquitin-mediated degradation. On the other hand, a role for Parkin mutations in the early-onset autosomal recessive and sporadic PD has been also described previously. Therefore, the present report aims to gain insights into the possible degradation mechanisms of the neuronal $Ca_V1.3$ channel protein by the UPS. First, immunoprecipitation assays demonstrated the interaction between Parkin and the $Ca_V1.3$ channels heterologously expressed in HEK-293 cells and in neural tissues. Parkin overexpression led to a reduced level of total and membrane protein, and a decrease in the Ca_V channel current density. Patch clamp recordings performed in the presence of MG132 prevented Parkin effects suggesting enhanced channel proteasomal degradation. In addition, the half-life of the $Ca_V1.3\alpha_1$ protein was significantly reduced by Parkin. Together, these results suggest that Parkin may promote the proteasomal degradation of the $Ca_V1.3$ channel, which might be related, at least in part, to the pathophysiology of PD.

Disclosures: L. Grimaldo: None. **A. Sandoval:** None. **P. Duran:** None. **R. Felix:** None.

Poster

119. Calcium Channels

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 119.04/B28

Topic: B.04. Ion Channels

Support: Austrian Science Fund (FWF) P27809
Austrian Science Fund (FWF) W11010
Austrian Science Fund (FWF) CavX

Title: Disease-causing *de novo* CACNA1D l-type calcium channel missense mutation in a patient with a severe neurodevelopmental disorder of unknown cause

Authors: N. T. HOFER[1], A. PINGGERA[4], P. TULUC[1], Y. NIKONISHYNA[1], E. M. FRITZ[1], M. FERNANDES-QUINTERO[5], K. R. LIEDL[2,3], B. E. FLUCHER[5], G. J. OBERMAIR[5,6], ***J. STRIESSNIG**[1,3];
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Abstract: Gain of Cav1.3 L-type Ca^{2+} channel function has previously been identified as the disease-causing mechanism of *de novo CACNA1D* missense mutations in individuals with a broad neurodevelopmental disease spectrum (autism spectrum disorder with and without neurological and endocrine symptoms). Demonstration of characteristic gain-of-function gating changes for newly identified rare missense mutations in patients with severe neurodevelopmental disorders can therefore strongly support their pathogenic role. We investigated if such *CACNA1D* mutations occur in patients previously sequenced in the Deciphering Developmental Disorders Study (DDD-S).
Among the validated DDD-S *de novo* single nucleotide variants (SNV) we identified the *CACNA1D* SNV S652L (not reported in gnomAD, gnomad.broadinstitute.org) in a patient (#262954) with delayed speech and language development and global developmental delay. S652L has not been classified as disease mutation in DDD-S. C-terminally long (WT_L) and short (WT_S) wild-type and S652L Cav1.3 α1 subunits were co-expressed together with β3 and α2δ-1 subunits in tsA-201 cells and Ca^{2+} or Ba^{2+} currents (15 mM) were measured using the whole-cell patch-clamp technique.
For WT_L and WT_S mutation S652L significantly shifted the voltage-dependence of activation ($V_{0.5,act}$) and steady-state inactivation ($V_{0.5,inact}$) to more negative potentials (13-17 mV), significantly increased window currents at subthreshold voltages, slowed tail currents and increased Ca^{2+} entry during action potential-like stimulations (10 Hz trains). Inclusion of alternative exons 11 or exons 32 caused significantly more negative $V_{0.5,act}$ (3 mV) and $V_{0.5,inact}$ (6 mV) in WT_S without preventing gating changes of the mutation. In contrast, missense variant S652W, which has been identified in three unrelated apparently healthy individuals, shifted $V_{0.5,act}$ and $V_{0.5,inact}$ to more positive voltages.
By demonstrating typical gain-of-function gating changes for S652L we identify its disease-causing role for this patient with a severe neurodevelopmental disorder. Pathogenicity is not observed for S652W, which reduces activation voltage-sensitivity of Cav1.3. Therefore, the prediction of the pathogenicity of *CACNA1D* mutations also has to consider the amino acid change and not only the amino acid position alone. Moreover, we identify exons 11 and 32 as

determinants of the characteristic low-voltage activation of Cav1.3 channels.
Patients carrying the S652L or other gain-of function mutations may benefit from treatment with already available L-type Ca^{2+} channel blockers, such as felodipine.

Disclosures: **N.T. Hofer:** None. **A. Pinggera:** None. **P. Tuluc:** None. **Y. Nikonishyna:** None. **E.M. Fritz:** None. **M. Fernandes-Quintero:** None. **K.R. Liedl:** None. **B.E. Flucher:** None. **G.J. Obermair:** None. **J. Striessnig:** None.

Poster

119. Calcium Channels

Location: Hall A

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Program #/Poster #: 119.05/B29

Topic: B.04. Ion Channels

Support: Project 2015FNWP34 (Italian Miur)
CSTO165284 (Compagnia di San Paolo)
DIACELL project (National Institute of Nuclear Physics))
MiRaDS project (CRT Foundation)

Title: Somatodendritic release of dopamine and role of L-type Ca^{2+} channels in regulating the spontaneous firing of midbrain dopaminergic neurons

Authors: G. TOMAGRA[1], M. BONARDI[1], F. PICOLLO[2,3,4], B. PICCONI[5,6], A. PASQUARELLI[7], P. OLIVERO[2,3,4], P. CALABRESI[8], E. CARBONE[1,3], A. MARCANTONI[1,3], ***V. CARABELLI**[1,3];
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Abstract: Spontaneous firing and exocytosis of midbrain neurons has been recently addressed by means of Micro-Graphitic Multi Electrode Arrays (μG-SCD-MEAs) (Tomagra et al., Front. Neurosci, 2019). When used as potentiometric biosensors, μG-SCD-MEAs can resolve action potential firing of cultured midbrain neurons (14 DIV) in the range of 0.7 to 7 Hz with high signal-to-noise ratio and also assay the different modulatory pathways induced by levodopa (L-DOPA, 20 μM). When used as amperometric biosensors, μG-SCD-MEAs can reveal multisite dopamine release from different dopaminergic (DA) neurons that occurs at a basal mean frequency of 0.11 Hz and increases 4-fold when cells are depolarized by 30 mM KCl.
As the complexity of responses revealed by μG-SCD-MEAs may arise from the interaction of different neuronal populations, we undertook a detailed electrophysiological study on isolated

DA neurons, identified by their TH-GFP positive staining, with the aim of investigating the ionic basis of cell firing and their coupling to dopamine release.
Here we report that L-type calcium channels play a significant role in sustaining the firing activity of DA neurons. In current-clamp conditions, 3 μM nifedipine was sufficient to reduce the spontaneous firing frequency by approximately 60-70% and the AP peak amplitude by 15%. The relative contribution of L-type versus nifedipine-insensitive currents in action potential generation was estimated using action potential clamp commands. Concerning the detection of the secretory activity from isolated dopaminergic neurons (7 DIV), somatodendritic release of dopamine and Ca^{2+}-dependence of secretion were measured by means of depolarization-evoked membrane capacitance increases, using pulses of increased duration (10-300 ms). Under these conditions, secretory responses increased up to 57 fF. This value was confirmed by double-pulse protocols that allowed to estimate the mean size of the ready releasable pool (~65 fF; RRP). Three-fold potentiation of the RRP occurred following incubation with L-DOPA, while the treatment had no effect on the quantal charge flowing through Ca^{2+} channels.

Disclosures: G. Tomagra: None. **M. Bonardi:** None. **F. Picollo:** None. **B. Picconi:** None. **A. Pasquarelli:** None. **P. Olivero:** None. **P. Calabresi:** None. **E. Carbone:** None. **A. Marcantoni:** None. **V. Carabelli:** None.

Poster

119. Calcium Channels

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 119.06/B30

Topic: B.04. Ion Channels

Support: NIH Grant T32GM007308
NIDA Grant R01 DA040484-01
NINDS Grant T32NS086750

Title: L-type calcium channels cooperate with NMDA receptors to signal to the nucleus from dendrites

Authors: *N. MANDELBERG[1], S. D. SUN[2], B. LI[4], R. W. TSIEN[3];
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Abstract: Neurons perform a remarkable range of tasks by altering their physiology as a result of external stimuli. Excitation-transcription (E-T) coupling is the process by which neurons alter their gene expression in response to these inputs. The most important effector of E-T coupling in neurons is calcium- and cAMP response element binding protein (CREB), a transcription factor

whose role in learning and memory has been firmly established in numerous organisms. L-type voltage-gated calcium (Ca_V1) channels are critical for several forms of neuronal plasticity largely because of their privileged role in regulating CREB. This privilege stems from Ca_V1 channels' special relationship with important downstream signaling molecules, such as calcium/calmodulin (CaM)-dependent kinase II (CaMKII). Though the importance of E-T coupling in neuronal plasticity is well established, the process by which it is initiated remains poorly understood. Specifically, it is unclear where on the cell membrane molecules like Ca_V1 channels initiate this process, a question that in turn determines the types of stimuli capable of altering neurons' genetic program.

To better understand the initiation of E-T coupling, we investigated the spatial profile of Ca_V1 channel activity and the biochemical mechanisms Ca_V1 channels use to control signaling to CREB in the nucleus. First, we show evidence that miniature excitatory post-synaptic potentials (mEPSPs) are capable of driving CREB activation in the absence of action potentials and require Ca_V1 channels and *N*-methyl-D-aspartate receptors (NMDARs) to do so. We isolated the activation of Ca_V1 channels at specific anatomical regions of the neuronal membrane to show that Ca_V1 channels can signal to the nucleus from dendritic spines. We then tested Ca_V1 channels' ability to act in concert with NMDARs. We pharmacologically isolated calcium flux through Ca_V1 channels from its voltage dependent conformational signal (VDC) and found that Ca_V1 VDC can synergize with calcium influx through NMDARs to signal to activate CREB via CaMKII. Finally, we provide evidence that synergy between Ca_V1 channels and NMDARs is exaggerated in a mouse model of Timothy Syndrome, a genetic form of autism spectrum disorder, resulting in an exaggerated CREB response to synaptic activity. Together, these results suggest that Ca_V1-mediated E-T coupling can begin from the dendritic spine and is based on the interplay between several local signals with important implications for neurons' ability to convert synaptic inputs into changes in gene expression.

Disclosures: **N. Mandelberg:** None. **S.D. Sun:** None. **B. Li:** None. **R.W. Tsien:** None.

Poster

119. Calcium Channels

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Topic: B.04. Ion Channels

Support: NIH Grant R37AG008796
NIH Grant RF1AG017139

Title: Effects of cyclopiazonic acid (CPA) on total calcium accumulation observed in apical dendrites of CA1 pyramidal neurons from young adult and aged rats

Authors: T. S. JEFFERSON, J. F. DISTERHOFT, *M. M. OH;
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Abstract: The increase in the postburst afterhyperpolarization (AHP) of CA1 pyramidal neurons has been hypothesized to be a main source of the learning and memory impairments observed in aging subjects (Disterhoft & Oh 2006). The postburst AHP is mainly comprised of Ca^{2+}-dependent potassium conductances and thus is triggered by a rise in cytosolic Ca^{2+} levels ($[Ca^{2+}]$). Hence, many studies have shown that the postburst AHP is significantly reduced by blockade of L-type voltage-dependent Ca^{2+} channels (LVCC) and of Ca^{2+}-induced Ca^{2+} release (CICR) in CA1 pyramidal neurons (reviewed in Disterhoft & Oh 2006, 2007). In addition, increased LVCC activity (Thibault & Landfield 1996) and larger CICR capacity (with use of cyclopiazonic acid (CPA) or ryanodine: Kumar & Foster 2004; Gant et al. 2006) have been suggested in CA1 neurons of aged animals. We have provided Ca^{2+}-imaging data that demonstrated Ca^{2+} influx is enhanced in CA1 neurons from aged rats (Oh et al. 2013). However, it remains unclear if the reductions in the postburst AHP following blockade of LVCC or CICR are due to reduced $[Ca^{2+}]$. Hence, the present study was designed to address the relative contribution of CICR (by bath application of CPA) to the rise in cytosolic Ca^{2+} levels evoked with trains of action potentials (APs).
Hippocampal slices were prepared from young adult (3-4 mo) and aged (27-30 mo) male F344xBN rats. Whole-cell current clamp and Ca^{2+}-imaging (Oregon Green BAPTA-6F and Alexa 594) data evoked with 100Hz AP trains were collected using a custom built 2-photon laser scanning microscope system before and 20 min after 10μM CPA was added to the perfusate. Our preliminary data suggest that bath application of CPA increased evoked $[Ca^{2+}]$ by ~15% in young adult (n=5) and ~20% in aged (n=6) CA1 neurons. Vehicle control resulted in similar increase in $[Ca^{2+}]$ (~10%) in both young adult (n=15) and aged (n=9) CA1 neurons. These preliminary findings are surprising and contrary to the suggested reduction of $[Ca^{2+}]$ by antagonizing SERCA function with CPA.
Ongoing studies are designed to explore the contribution of LVCCs to the total Ca^{2+} rise evoked with AP trains. In addition, potential learning- and aging-related differences in the contribution of LVCC and CICR to the total Ca^{2+} rise are also being examined.

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Poster

119. Calcium Channels

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 119.08/B32

Topic: B.04. Ion Channels

Support: BT/PR12754/INF/122/2/2016

Title: Expression and localization of P/Q type calcium channel Cav2.1 in iron-induced experimental epilepsy

Authors: *C. PRAKASH, D. SHARMA;
Sch. of Life Sci., Jawaharlal Nehru Univ., New Delhi, India

Abstract: Epilepsy is a chronic neurological disorder characterized by the occurrence of spontaneous and recurrent seizures. The importance of Ca^{2+} currents in seizure generation and the establishment of epilepsy are well known. Voltage-gated calcium channels are important for signal transmission in neurons. Cav2.1 is dominant and most effective calcium ion channel in triggering action potential-evoked release at synapses. Thus, the present study was executed to explore the expression and localization of Cav2.1 in epilepsy. Rats were made epileptic by intracortical injection of $FeCl_3$. Epileptic seizures were confirmed by analyzing electroencephalographic and multiple unit activity recordings after 30 days of $FeCl_3$ injection. Next, we evaluated the mRNA and protein expression of Cav2.1 using real time-PCR and western blot analysis. Localization and protein expression was also confirmed by immunofluorescence analysis respectively. Our results demonstrated the occurrence of discrete epileptiform episodes and concurrently increased MUA counts. The mRNA and protein levels of Cav2.1α were down-regulated in the cortex and hippocampus of epileptic rats. Moreover, these changes were more in the hippocampus region as compared to the cortex. The study also demonstrated that Cav2.1 channels were dominant in the presynaptic neurons which were the sites with higher changes in neurons. In conclusion, the study indicates that expression and localization of Cav2.1 might be an important mechanism for the modulation of neuronal functions in epilepsy. Thus, targeting Cav2.1 expression may provide a new therapeutic strategy for the treatment of epilepsy.

Disclosures: C. Prakash: None. **D. Sharma:** None.

Poster

119. Calcium Channels

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 119.09/B33

Topic: B.04. Ion Channels

Support: PICT 2015 3330

Title: Ghrelin receptor (GHSR) and dopamine receptor type 2 (D2R) co-expression modifies each receptor's effects on voltage gated calcium channel $Ca_V2.2$

Authors: *E. R. MUSTAFA[1], S. CORDISCO GONZALEZ[2], S. S. RODRIGUEZ[2], J. RAINGO[2];

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Abstract: Presynaptic $Ca_V2.2$ are activated by action potentials, and their calcium current induces neurotransmitter release. In this context, the regulating of $Ca_V2.2$ activity is critical, and one of the most important mechanisms for doing so is through G-protein coupled receptor (GPCR) activity. Two members of GPCRs able to modulate Cav activity are the ghrelin receptor (GHSR) and the dopamine receptor type 2 (D2R). We have previously demonstrated that GHSR constitutive activity reduces $Ca_V2.2$ trafficking to the plasma membrane and that ghrelin-induced GHSR activity inhibits $Ca_V2.2$ current. On the other hand, dopamine-induced D2R activity also inhibits $Ca_V2.2$ current. It has been recently shown that D2R and GHSR are able to hetero-dimerize in hypothalamic neurons. Here we explore how co-expression of GHSR and D2R modulates the effect that each GPCR has individually on $Ca_V2.2$. We found that GHSR-D2R co-expression increases the basal inhibition of $Ca_V2.2$ by GHSR constitutive activity, since less GHSR is needed to reduce $Ca_V2.2$ current when D2R is co-transfected. We tested if this effect is trough GHSR constitutive activity using as a tools an inverse agonist SPA (Substance-P analog) and a mutated version of GHSR (A204E) lacking of constitutive activity, and we were able to occlude the inhibitory effect. Next, we explore the signaling cascade implied in this effect on $Ca_V2.2$ and we found that co-expression of a Gq dominant negative mutant or co-expression of t $G_{\beta\gamma}$ buffer peptide (Mas-GRK2-ct) completely block the reduction in the amplitude of $Ca_V2.2$ currents. By contrast, the acute inhibitory effect of ghrelin on $Ca_V2.2$ current is unaffected by GHSR-D2R co-expression. Meanwhile, GHSR-D2R co-expression decreases inhibition of $Ca_V2.2$ by dopamine-evoked D2R activity (increase in EC_{50}), since a higher dopamine concentration is needed to inhibit $Ca_V2.2$ current when GHSR is co-transfected. This last effect depends on GHSR constitutive activity, since it is occluded by pre-incubation with SPA, and is coupled to G_q protein. Currently, we are exploring if this novel effect of GHSR-D2R hetero-dimerization has an impact on native calcium currents on hypothalamic primary cultures.

Disclosures: E.R. Mustafa: None. **S. Cordisco Gonzalez:** None. **S.S. Rodriguez:** None. **J. Raingo:** None.

Poster

119. Calcium Channels

Location: Hall A

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Program #/Poster #: 119.10/B34

Topic: B.04. Ion Channels

Support: R00MH099405

Title: Detection of a Cacna1b ($Ca_v2.2$) splice variant in brain tissue

Authors: *A. S. ANDRADE[1], A. BUNDA[1], B. LACARUBBA[1], M. AKIKI[2];
[1]Biol. Sci., [2]Molecular, Cell. and Biomed. Sci., Univ. of New Hampshire, Durham, NH

Abstract: Presynaptic $Ca_V2.2$ (N-type) channels are fundamental for transmitter release across the nervous system. The gene encoding $Ca_V2.2$ channels, *Cacna1b*, contains alternatively spliced exons that originate functionally distinct splice variants (e18a, e24a, e31a and 37a/37b). Alternative splicing of the cassette exon 18a generates two mRNA transcripts (+e18a-*Cacna1b* and Δe18a-*Cacna1b*). In this study, using novel mouse genetic models and in situ hybridization (BaseScope™), we confirmed that +e18a-*Cacna1b* splice variants are expressed in monoaminergic regions of midbrain. We expanded these studies and identified +e18a-*Cacna1b* mRNA in deep cerebellar cells and spinal cord motor neurons. Furthermore, we determined that +e18a-*Cacna1b* is enriched in cholecystokinin expressing interneurons. Our results provide key information to understand cell-specific functions of $Ca_V2.2$ channels.

Disclosures: A.S. Andrade: None. **A. Bunda:** None. **B. Lacarubba:** None. **M. Akiki:** None.

Poster

119. Calcium Channels

Location: Hall A

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Program #/Poster #: 119.11/B35

Topic: B.04. Ion Channels

Support: PICT 2015 0330
PICT 2017 0602
PUE IMBICE

Title: Agonist independent activity of dopamine receptor type 1 (D1R) increases $Ca_V2.2$ current in prefrontal cortex neurons

Authors: C. I. MCCARTHY, S. S. RODRIGUEZ, C. CHOU-FREED, V. MARTINEZ DAMONTE, ***J. RAINGO**;
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Abstract: Dopamine and its receptors play an important yet not fully understood role in the prefrontal cortex (PFC) function. Alterations in dopamine receptor type 1 (D1R) density and sensitivity to dopamine are associated with cognitive deficits of aging and schizophrenia. In pyramidal neurons of the PFC, Kisilevsky and col. (2008) showed that D1R co-localizes and physically interacts with voltage-gated calcium channels type 2.2 ($Ca_V2.2$) and that this interaction modulates $Ca_V2.2$ density in postsynaptic sites. Furthermore D1R was reported to display constitutive activity through coupling to Gs protein in absence of dopamine, but the impact of this agonist-independent activation of D1R on Ca_V currents has never been studied.

Our aim here is to understand the role of agonist independent D1R activity on the stimulatory effect on $Ca_V2.2$ surface expression. To do it we transfected HEK293t cells with increasing D1R:$Ca_V2.2$ molar ratios (MR) and verified expression levels using YFP-tagged D1R. We recorded whole-cell calcium currents and found an increase in $Ca_V2.2$ current density by D1R co-expression (152.6% of control at 0.1 D1R:$Ca_V2.2$ MR, P=0.0029). To explore the role of D1R constitutive activity on this effect, we treated cells with Haloperidol (D1R inverse agonist) and Cholerotoxin (Gs protein inhibitor). Results indicate that the increase in current density depends on D1R constitutive activity. We next aimed to understand the scope of this effect on pyramidal neurons of the PFC, where $Ca_V2.2$ has critical post-synaptic functions contributing to firing and plasticity. We recorded voltage gated calcium currents from PFC neurons in brain slices from 4 to 6 week old mice injected with intra-peritoneal haloperidol (1mg/kg) or vehicle, and found that native calcium currents from haloperidol treated mice were significantly smaller than currents from control mice. Moreover we found that the current reduced by haloperidol treatment is mainly $Ca_V2.2$ subtype since the sensitivity to ω conotoxin GVIA 1 μM of calcium currents is dramatically reduced in haloperidoI treated mice. In this context, our study could contribute to understand the mechanism involved in cognitive deficits of aging and schizophrenia associated with changes in D1R expression levels at the PFC.

Disclosures: C.I. McCarthy: None. **S.S. Rodriguez:** None. **C. Chou-Freed:** None. **V. Martinez Damonte:** None. **J. Raingo:** None.

Poster

119. Calcium Channels

Location: Hall A

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Program #/Poster #: 119.12/B36

Topic: B.04. Ion Channels

Support: NIH Grant HL130987
NIH Grant AR059397

Title: Serotonin-mediated modulation of CaV2.2 currents by 5HT-1 receptor stimulation in rat sensory neurons

Authors: L. ANSELMI[1], *H. L. PUHL, III[2], V. RUIZ-VELASCO[1];
[1]Anesthesiol., Penn State Col. of Med., Hershey, PA; [2]NIH, Rockville, MD

Abstract: It is well known that the sympathetic nervous system plays an important role in regulating blood flow in skeletal muscle during exercise and that N-type Ca^{2+} ($Ca_V2.2$) channels are expressed in primary afferents of dorsal root ganglia (DRG). DRG sensory neurons are key mediators in sensing a variety of noxious stimuli that are released under mechanical stress or inflammatory conditions. It has been shown that release of serotonin (5-HT), a peripheral

inflammatory mediator, is increased substantially in ischemic or injured muscle. Previously, we reported that $Ca_V2.2$ channels are the major type of voltage-gated Ca^{2+} channels in rat DRG neurons innervating triceps surae muscle. In the present study, we examined the 5-HT-mediated modulation of $Ca_V2.2$ channels via 5-HT receptors in rat DRG neurons innervating this muscle type three days following injection of the retrograde tracer, DiI. Whole-cell patch-clamp recordings of $Ca_V2.2$ currents (I_{Ca}) were made from isolated DiI-labeled DRG neurons. Whole-cell I_{Ca} were evoked with the "triple-pulse" protocol. Application of 5-HT (0.1 μM - 30 μM) blocked the I_{Ca} in a voltage-dependent manner. The 5-HT pharmacological profile showed a dose-dependent inhibition of the prepulse current with an IC_{50} of approximately 2.6 μM. Furthermore, the 5-HT-mediated I_{Ca} modulation was blocked following overnight pretreatment with pertussis toxin (PTX, 500 ng/ml) indicating that 5-HT receptors couple with $Ca_V2.2$ via $G\alpha_{i/o}$ G protein subunits. This further suggests that the 5-HT1 receptor subfamily was responsible for the I_{Ca} modulation. To ascertain the 5-HT1 receptor subtype that couples to $Ca_V2.2$, we employed selective 5-HT1B and 5-HT-1C receptor blockers. Exposure of either SB216641 (30 μM, 5-HT1B blocker) or LY310762 (30 μM, 5-HT1D blocker) alone led to partial agonist effects. That is, SB216641 and LY310762 blocked I_{Ca} $60\pm11\%$ (n=4) and $38\pm4\%$ (n=4), respectively, suggesting that these specific blockers exhibit intrinsic activity. Overall, these results suggest that 5-HT modulation of I_{Ca} in rat DRG innervating triceps surae muscle occurs following stimulation of 5-HT1 receptor subfamily. These findings contribute to our understanding of how the release of 5-HT onto inflamed or injured tissues can contribute to peripheral sensitization of nerve fibers and pain transmission.

Disclosures: L. Anselmi: None. **H.L. Puhl:** None. **V. Ruiz-Velasco:** None.

Poster

119. Calcium Channels

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 119.13/B37

Topic: B.04. Ion Channels

Title: *In vivo* efficacy of an orally available N-type calcium channel blocker for the potential treatment of neuropathic pain

Authors: *A. WILLUWEIT[1,4], G. GUZMAN[2], P. HIDALGO[2], D. WILLBOLD[3,4,5], J. KUTZSCHE[3];
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Abstract: Aim of Investigation: Neuropathic pain is caused by somatosensory injury or various diseases affecting the somatosensory nervous system. Millions of people worldwide suffer from neuropathic pain with an estimated prevalence up to 8% of the population. Neuropathic pain is chronic, severe and opioid-resistant and its treatment is as urgent as challenging. The N-type calcium channel $Ca_V2.2$ was shown to be an attractive target for therapeutic intervention concerning chronic and neuropathic pain conditions. The well-known $Ca_V2.2$ inhibitor omega-Conotoxin (Ziconotide, Prialt, GVIA) is clinically used for treatment of pain, but needs to be delivered intrathecally, which is a very invasive application route. We have identified the orally available drug candidate PRI-004, which specifically inhibits the $Ca_V2.2$ at nano-molar concentrations. Aim of the current study was to explore PRI-004´s in vitro and in vivo efficacy as therapeutic substance against neuropathic pain.
Methods: We used whole-cell patch-clamp recordings to investigate PRI-004´s potential to inhibit $Ca_V2.2$, heterologously expressed in tsA201 cells. The spinal nerve ligation and the sciatic inflammatory neuritis rat models were used to investigate in vivo efficacy.
Results: Application of PRI-004 in vitro reduced the peak current amplitude by more than 50% without altering the voltage-dependence of the channel activation. No effect was observed on $Ca_V1.2$ L-type channel that is the predominant isoform in the heart. Using both the spinal nerve ligation and the sciatic inflammatory neuritis models in rat we could show that oral application of PRI-004 exerts therapeutic reversion of tactile allodynia.
Conclusions: PRI-004 is inhibiting the N-type calcium channel at nanomolar concentrations without affecting L-type calcium channels and exerts therapeutic activity in two rat models of neuropathic pain. Because PRI-004 is orally available, it might be a promising new drug candidate for treatment of neuropathic pain.

Disclosures: **A. Willuweit:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Priavoid GmbH. **G. Guzman:** None. **P. Hidalgo:** None. **D. Willbold:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Priavoid GmbH. **J. Kutzsche:** None.

Poster

119. Calcium Channels

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 119.14/B38

Topic: B.04. Ion Channels

Support: NIH NS090644
MDA 603852
NIH AG051470

Title: Exploration of Cav2 channel mutations predicted to affect GV-58-mediated slowed deactivation

Authors: *S. ALDRICH[1], R. LAGHAEI[3], H. CHENG[2], I. BAHAR[2], S. D. MERINEY[1]; [1]Neurosci., [2]Computat. and Systems Biol., Univ. of Pittsburgh, Pittsburgh, PA; [3]Pittsburgh Supercomputing Ctr., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: We recently developed a number of Cav2-selective agonist gating modifiers (e.g. GV-58), based on the parent molecule *R*-roscovitine, that have therapeutic potential to treat a number of diseases and conditions that result in neuromuscular weakness (including Lambert-Eaton Myasthenic Syndrome, Spinal Muscular Atrophy, and botulinum toxin A poisoning). These first-in-class drugs act selectively on Cav2 calcium channels within milliseconds after channel opening to stabilize the open state, prolonging their mean open time and slowing deactivation in whole-cell currents. However, it is currently unknown which amino acids within the Cav2 channel structure are important for the effects of these agonist gating modifiers. To explore this question, we made chimeric calcium channels (with a mix of Cav2 and Cav1 domains) and found that drug activity resides within domain III of the Cav2 channel structure (consistent with previous work demonstrating that the parent molecule (*R*-roscovitine) Cav2 effects were dependent on domain III (Yarotsky et al., 2012)). We then generated open and closed state homology models of the Cav2.1 calcium channel. Using these models, we performed *in silico* drug docking simulations to compare predicted binding sites in open and closed channel configurations. We explored predicted binding sites that were present in open, but not closed channels, and compared amino acids in drug-sensitive Cav2 vs. drug-insensitive Cav1 channels to generate target amino acids to evaluate in mutagenesis experiments. Candidate amino acids were mutated using site-directed mutagenesis from their Cav2 identity to the homologous amino acids within Cav1. Mutant channels were expressed in HEK293 cells for functional analysis using patch clamp electrophysiology. Depolarization-evoked currents through mutant calcium channels were recorded in the absence and presence of GV-58 or related analog to evaluate effects on channel deactivation. By calculating the effect of GV-58 and related analogs on the deactivation kinetics of the mutant channels compared to its effect on wild-type channels, we determined the extent to which each mutation disrupted drug-induced gating modification and, therefore, the extent to which the wild-type residues are important for drug agonist activity.

Disclosures: S. Aldrich: None. **R. Laghaei:** None. **H. Cheng:** None. **I. Bahar:** None. **S.D. Meriney:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent holder.

Poster

119. Calcium Channels

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 119.15/B39

Topic: B.04. Ion Channels

Support: GM102525
GM118197

Title: $Ca_V3.1$ isoform of t-type calcium channels is an important molecular target for hypnotic effect of neurosteroid analogue

Authors: *T. TIMIC STAMENIC[1], S. FESEHA[1], F. MANZELLA[1], K. KRISHNAN[2], D. F. COVEY[2], V. JEVTOVIC-TODOROVIC[1], S. TODOROVIC[1];
[1]Anesthesiol., CU Denver, Anschutz Med. Campus, Aurora, CO; [2]Dept Developmental Biol., Washington Univ. Sch. Med., Saint Louis, MO

Abstract: Our previous studies established that T-type calcium channels in the thalamus are inhibited by neurosteroid analogue (3β,5β,17β)-3-hydroxyandrostane-17-carbonitrile (3β-OH). Here we performed *in vitro* and *in vivo* electrophysiological studies from central medial nucleus of thalamus (CeM) in order to interrogate the specific mechanisms underlying the role of $Ca_V3.1$ channels in thalamocortical excitability *in vitro* and oscillations *in vivo* during 3β-OH induced unconsciousness. We first assessed the effects of intra-peritoneal (i.p.) injections of 3β-OH on loss of righting reflex (LORR) in adult male wild type (WT) and $Ca_V3.1$ knockout (KO) mice. We found that after i.p. injections of 80 mg/kg of 3β-OH, half of WT mice exhibited LORR while $Ca_V3.1$ KO animals were completely resistant. We next performed patch clamp excitability experiments in acute brain slices from male juvenile WT and $Ca_V3.1$ KO mice. Although tonic firing was significantly inhibited in both WT (84% at 100 pA; $p<0.001$) and $Ca_V3.1$ KO mice (53% at 100 pA; $p<0.05$), 3 μM 3β-OH hyperpolarized neurons only in WT animals (-5 mV; $p<0.01$). Rebound bursting after cell hyperpolarization was observed only in WT mice and 3β-OH reduced (for about 30%) low threshold spike amplitude (LTS, $p<0.01$) and increased the threshold for LTS (for -9 mV). We next recorded local field potentials (LFPs) from the mice after i.p. injections of 80 mg/kg 3β-OH. Although we found no differences under baseline conditions between WT and mutant mice, we noticed after i.p. injections of 3β-OH there was a prominent increase in δ (0.5-4 Hz) oscillations in WT cohort ($p<0.001$). Similarly, θ (4-8 Hz) and α (8-13 Hz) oscillations were increased in WT animals ($p<0.01$ and $p<0.05$, respectively). Spectral analysis after 3β-OH determined that power density was much higher in WT mice than in KO mice, particularly in 2-10 Hz frequency range ($p<0.001$) and 12 Hz ($p<0.01$). We propose that inability of 3β-OH to hyperpolarize neurons and to increase slow oscillations (mostly in δ frequency range) in $Ca_V3.1$ KO mice can explain inability of 3β-OH to induce LORR in the mutant mice. Observed effect on tonic excitability in both strains, even though more prominent in WT mice, could implicate a different target for 3β-OH effects besides $Ca_V3.1$ T-channels. However, this reduced excitability alone in $Ca_V3.1$ KO mice could not induce unconsciousness state after injections of 3β-OH. Our results demonstrate for the first time the importance of $Ca_V3.1$ T-channels in thalamocortical excitability and oscillations that underlie neurosteroid-induced unconsciousness.

Disclosures: T. Timic Stamenic: None. **S. Feseha:** None. **F. Manzella:** None. **D.F. Covey:** None. **V. Jevtovic-Todorovic:** None. **S. Todorovic:** None. **K. Krishnan:** None.

Poster

119. Calcium Channels

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 119.16/B40

Topic: B.04. Ion Channels

Title: Functional importance of the CACHD1 MIDAS site in the modulation of $Ca_V3.1$ voltage-gated calcium channels

Authors: G. S. COTTRELL, S. INCE, ***G. J. STEPHENS**;
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Abstract: We have recently identified the Ca^{2+} channel and chemotaxis receptor (cache) domain containing protein 1 (CACHD1) as a protein with structural similarities to the $\alpha_2\delta$ voltage-gated Ca^{2+} channel (VGCC) auxiliary subunit that modulates the activity of low-voltage-activated (LVA) CaV3 (T-type) VGCCs. In high-voltage-activated (HVA) VGCCs, $\alpha_2\delta$ subunits contain a metal ion-dependent adhesion site (MIDAS) (DxSxS) within their von Willebrand factor A domains that dictates functional effects including an increase of forward trafficking of HVA VGCCs to the cell surface. Here, we investigate the role of the variant CACHD1 MIDAS (DxGxS) motif on $Ca_V3.1$ trafficking.

Subcellular localization, trafficking and expression levels of Myc-CACHD1 and GFP-$Ca_V3.1$-HA proteins were determined in HEK-293 cells by proximity ligation assays, biotinylation assays, western blotting and immunofluorescence and confocal microscopy. Mutations in the variant MIDAS motif of CACHD1 (D^{234}xGxS to AxAxA, termed CACHD1-AAA) were generated by PCR using standard techniques. Data were analyzed by one-way ANOVA with Tukey's post-hoc test or paired Student's t-test as appropriate.

CACHD1 was prominently expressed at the cell surface and underwent constitutive internalization. CACHD1 promoted cell-surface localization of $Ca_V3.1$ (1.7±0.3 fold increase, n=4, $p<0.05$) in biotinylation assays. Proximity ligation assays revealed that CACHD1 and $Ca_V3.1$ are in close proximity at the cell-surface (<40 nm) and co-immunoprecipitation studies supported presence of $Ca_V3.1$•CACHD1 protein complexes. When expressed in HEK-293 cells, CACHD1-AAA was largely restricted to intracellular compartments with little apparent trafficking to the cell surface, being expressed at significantly lower levels compared to CACHD1 (14±1%, n=4, $p<0.05$). Notably, CACHD1-AAA did not promote the cell surface localization of $Ca_V3.1$ (1.1±0.2%, fold increase n=4, $p<0.05$ compared to CACHD1).

These data suggest that CACHD1 is a constitutively trafficking membrane protein and that residues within the putative MIDAS motif are critical for the trafficking of CACHD1 and $Ca_V3.1$ to the cell surface. It will be of interest to determine the role of other CACDH1 sequence motifs on trafficking and other Ca_V3 functionality in regard to these data.

Disclosures: **G.S. Cottrell:** None. **S. Ince:** None. **G.J. Stephens:** None.

Poster

119. Calcium Channels

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 119.17/B41

Topic: B.04. Ion Channels

Support: NIH U01 NS090340
AHA 19CDA34660056
NIH NS29709

Title: Cav3.1 and 3.2 T-type Ca^{2+} channels collectively contribute to oscillatory excitation of nucleus ambiguus neurons

Authors: *I. AIBA, J. L. NOEBELS;
Baylor Col. of Med., Houston, TX

Abstract: Cholinergic neurons in the nucleus ambiguus (NAm) contribute to cardiac vagal and a part of laryngeal nerves to regulate cardiorespiratory tones. We have recently characterized these cholinergic NAm neurons in acute brainstem slices prepared from adult mouse and identified a prolonged rebound oscillatory firing pattern which can last for seconds. This rebound excitation pattern is mediated by T-type calcium current (ICaT), as ICaT specific inhibitors effectively abolishes it. In mammals, ICaT is generated by Cav3.1 (Cacna1g), Cav3.2 (Cacna1h) and Cav3.3 (Cacna1i) channels. These channels are differentially expressed in pacemaking neurons and other excitable cells including cardiac and vascular myocytes. Currently, specific T-type channel genes responsible for rebound excitation of the NAm neurons have not yet been identified. We explored Cav3 genes responsible for rebound excitation pattern by using transgenic mice that lack Cav3.1 (Cacna1g) and Cav3.2 (Cacna1h) in the NAm. The Cav3.1 gene deletion in the cholinergic NAm neurons was achieved by crossing mice expressing Chat-cre and conditional floxed Cacna1g deletion. Cav3.1 deletion eliminated rebound excitation in 65% (11/17) of cholinergic NAm neurons, while intact rebound excitation was detected in the rest of neurons. Further deletion of Cav3.2 KO by crossing the Cav3.1 conditional deletion mouse with Cav3.2 KO mouse eliminated rebound excitation in the majority of NAm neurons (88%, 7/8 cells), while intact rebound excitation was still detected in a single NAm neuron. These results indicate Cav3.1 and Cav3.2 are the major T-type calcium channels in the NAm neurons, while a minor subset of neurons may express Cav3.3 or other related channels that allow generation of rebound excitation. Because of all or none effect of sensitivity to Cav3 gene deletion, each NAm neuron may differently express predominant Cav3 channel genes.

Disclosures: **I. Aiba:** None. **J.L. Noebels:** None.

Poster

119. Calcium Channels

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 119.18/B42

Topic: B.04. Ion Channels

Support: NIH Grant R15 GM119099-01
PKD Foundation Research Grant

Title: Alternative splicing at the C-terminus of CACNA1G, the spinocerebellar ataxia-42 gene, modulates the activity of the low-voltage activated Cav3.1 calcium channel

Authors: R. WANG, Z. WANG, M. HOSSAIN, J. MIRKOVIC, S. SABZANOV, Y. YU, ***M. RUGGIU**;
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Abstract: Spinocerebellar ataxias (SCAs) are a heterogeneous group of neurodegenerative disorders characterized by brainstem and cerebellum degeneration. They are autosomal dominant genetic diseases with approximately 44 known subtypes, and dominant mutations in the *CACNA1G* gene, encoding the alpha-1G subunit of the low-voltage-gated T-type calcium channel $Ca_v3.1$, cause SCA42. $Ca_v3.1$ plays crucial roles in cardiac and smooth muscle cells and neurons by regulating intracellular Ca^{2+} signaling and modulating transmembrane potential, and mice that are null for this gene present severe motor coordination defects, abnormal electrical conductance in the heart, aberrant action potentials in the brain, disrupted sleeping patterns, and absence epilepsy. *CACNA1G* undergoes extensive alternative splicing, and $Ca_v3.1$ splice variants display altered channel kinetics, localization, and cytosolic Ca^{2+} trafficking. In this work, we analyze the function of two exons, termed E34 and E35. They are found immediately after domain IV in the intracellular C-terminus, but their function is still largely unknown. We have discovered that these exons are alternatively spliced in all possible combinations in mouse tissues, giving rise to four different splice variants. E34 and E35 are preferentially included in nerve tissue postnatally, while they are mostly skipped in embryonic tissues. To investigate the mechanism of E34 and E35 splicing regulation, we generated minigene reporters and tested them against specific splicing factors that may modulate their inclusion and/or skipping. To examine the physiological properties of $Ca_v3.1$ C-terminal splice variants, *Xenopus* oocytes were injected with cRNAs corresponding to specific splice variants, and channel activity was measured using two-electrode voltage clamp technique. We also investigated the effects of the calcium binding protein calmodulin on the channel activity of the different splice variants. Taken together, our data show that alternative splicing at E34 and E35 of $Ca_v3.1$ may modify Ca^{2+} influx through the channel and regulate excitability and intrinsic firing pattern of neurons.

Disclosures: **M. Ruggiu:** None. **R. Wang:** None. **Z. Wang:** None. **M. Hossain:** None. **J. Mirkovic:** None. **S. Sabzanov:** None. **Y. Yu:** None.

Poster

119. Calcium Channels

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 119.19/B43

Topic: B.04. Ion Channels

Support: R01 AA027023
R01 DA03913
R01 MH122729
R21 MH112117
NSF 1650113

Title: Voltage-dependent and intracellular store-dependent contributors to calcium dynamics at the axon initial segment of neocortical pyramidal cells

Authors: *A. M. LIPKIN[1], M. CUNNIFF[1], P. W. SPRATT[2], S. M. LEMKE[3], K. J. BENDER[4]; [1]Univ. of California San Francisco, San Francisco, CA; [2]Neurosci., Univ. Of California San Francisco, San Francisco, CA; [3]Univ. of California, San Francisco, San Francisco, CA; [4]Dept. of Neurol., UCSF, San Francisco, CA

Abstract: The axon initial segment (AIS) is a key site for synaptic integration and action potential initiation in central neurons. In addition to the high density of sodium and potassium channels that are localized to the AIS, the array of calcium channels present here can affect action potential (AP) generation, timing, and the generation of high frequency bursts of APs. In a range of cell classes, low voltage-activated calcium channels are enriched in the AIS. However, in the neocortex, the complement of calcium channels present at the AIS remains unclear. Some studies suggest that high voltage-activated calcium channels are the primary contributors to calcium influx at the AIS, while other studies highlight the importance of low voltage-activated calcium channels. In addition to plasma membrane calcium channels, the AIS of pyramidal neurons also contains cisternal organelles that may release calcium in response to AP-evoked Ca influx.

Here, we examine the interactions between plasma membrane calcium channels and intracellular calcium stores in pyramidal neurons in mouse prefrontal cortex. We characterized the contribution of calcium channels present at the AIS of Layer 5 pyramidal neurons to AP-evoked calcium influx using whole-cell current-clamp electrophysiology, selective pharmacology, and two-photon linescan-based microscopy. We further explored the sub-structure of calcium influx with high-speed, diffraction limited calcium imaging using low-affinity dyes. Interestingly, we identified “hotspots” of calcium influx that occurred periodically along the AIS. Calcium influx

at these hotspots occurred predominantly during the falling phase of the AP, similar to calcium tail currents measured at synaptic boutons. This suggests that calcium channels may be clustered within the AIS in discrete regions, perhaps in close proximity to cisternal organelles.

Disclosures: A.M. Lipkin: None. **M. Cunniff:** None. **P.W. Spratt:** None. **S.M. Lemke:** None. **K.J. Bender:** None.

Poster

119. Calcium Channels

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 119.20/B44

Topic: B.04. Ion Channels

Support: NIH Grant GM 58055

Title: Effect of isoflurane on axonal endoplasmic reticulum calcium dynamics in hippocampal neurons

Authors: *V. OSMAN[1], H. C. HEMMINGS JR[2];
[1]Weill Cornell Med., New York, NY; [2]Dept Anesthesiol & Pharm, Joan and Sanford I Weill Med. Col. of Cornell Univ., New York, NY

Abstract: Volatile anesthetics are essential to modern medicine, but despite their widespread clinical use, their cellular and molecular mechanisms of action remain unclear. Volatile anesthetics depress synaptic transmission by both pre- and post-synaptic effects including inhibition of activity-dependent Ca^{2+} influx into the presynaptic nerve terminal. However, the principal sites of action upstream of Ca^{2+} entry are unknown. Axonal endoplasmic reticulum (ER) controls presynaptic Ca^{2+} through sequestration, and decreased ER Ca^{2+} has been linked to reductions in presynaptic Ca^{2+} influx as well as reductions in synaptic vesicle (SV) exocytosis. ER Ca^{2+} efflux and influx mechanisms are essential for cytoplasmic Ca^{2+} regulation. They provide possible targets for anesthetic action, as mutations in the RyR1 ryanodine receptor sarcoplasmic reticulum (SR) efflux channel in skeletal muscle lead to isoflurane-induced malignant hyperthermia (MH), a pharmacogenetic condition characterized by hyperthermia and muscle rigidity. While we understand MH in skeletal muscle in reasonable detail, the effects of MH mutations on neuronal function are unknown. In this study, primary cultures of rat hippocampal neurons were used to test isoflurane-induced changes in ER Ca^{2+} dynamics measured using transfection of the genetically encoded fluorescent ER Ca^{2+} sensor ER-GCaMP6-150. Preliminary data indicate that isoflurane decreases ER Ca^{2+} in neurons. **We hypothesize that depression of presynaptic Ca^{2+} entry and SV exocytosis by isoflurane involves effects on ER Ca^{2+} dynamics**. This project will further our understanding of the neuronal mechanisms of action of isoflurane in an MH relevant pathway. This will contribute to

the development of more selective anesthetics with fewer side effects, decreasing patient risk, as well as improved treatment for MH patients.
Supported by NIH grant GM 58055.

Disclosures: V. Osman: None. **H.C. Hemmings Jr:** None.

Poster

119. Calcium Channels

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 119.21/B45

Topic: B.04. Ion Channels

Support: NIH R01NS094665

Title: Calcium channel-embedded transcription factors facilitate direct calcium signaling

Authors: *E. RAO[1], D. HEJAZI PASTOR[2], X. DU[1], C. GOMEZ[1];
[1]Univ. of Chicago, Chicago, IL; [2]The Univ. of Chicago, Chicago, IL

Abstract: Calcium is a crucial second messenger in the central nervous system, acting both pre- and post-synaptically to drive several important physiological processes, including neurotransmitter release, modulating signaling pathways, and inducing post-synaptic responses related to learning and memory. Our lab has shown previously that the L-, T-, and P/Q-type voltage-gated calcium channel (VGCC) genes *CACNA1C, CACNA1H,* and *CACNA1A,* respectively, are bicistronic, meaning that they encode two structurally unrelated proteins with distinct functions from the same mRNA. These three genes each encode a VGCC subunit through canonical, cap-dependent translation and a transcription factor through utilization of a cryptic Internal Ribosome Entry Site (IRES) within the open reading frame. Our lab has previously characterized the *CACNA1A* secondary protein α1ACT extensively, elucidating its critical effects during normal cerebellar development and the pathogenic effects of its variants in disorders such as spinocerebellar ataxia 6 (SCA6). The secondary proteins produced by *CACNA1C* and *CACNA1H*, termed α1CCT and α1HCT respectively, were only recently identified by our lab and others, and their function and regulation as transcriptional regulatory units is poorly understood. We hypothesize that these secondary proteins function as activity-coupled transcription factors that regulate an ensemble of genes coincident with calcium channel activity. Using fluorescence microscopy coupled with calcium uncaging in primary neuronal cultures, we have been able to quantify the translocation kinetics of these VGCC secondary proteins following local increases in calcium concentration in real time. Additionally, using RNA-seq, CHiP-seq, and ATAC-seq, we have fully characterized the transcriptional effect each of these secondary proteins has on neural progenitor cells (NPCs), driving genes that are crucial for proper neuronal differentiation and development. Furthermore, we propose that dysfunction

of these novel secondary proteins could contribute to the complex phenotypes observed in neuropsychiatric diseases related to mutations in *CACNA1C* and *CACNA1H* and could therefore potentially be attractive therapeutic targets.

Disclosures: E. Rao: None. **D. Hejazi Pastor:** None. **X. Du:** None. **C. Gomez:** None.

Poster

119. Calcium Channels

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 119.22/B46

Topic: B.04. Ion Channels

Support: CONACYT

Title: Initial characterization of the CACNG2 gene antisense promoter-driven transcript

Authors: *D. MUÑOZ-HERRERA[1], A. CALDERÓN-RIVERA[1], N. ZARCO[2], R. GONZÁLEZ-RAMÍREZ[3], R. FELIX[1];
[1]Cell Biol., CINVESTAV-IPN, Mexico City, Mexico; [2]Mayo Clin., Jacksonville, FL; [3]Dept. of Mol. Biol. and Histocompatibility, "Dr. Manuel Gea Gonzalez" Gen. Hosp., Mexico City, Mexico

Abstract: The $Ca_V\gamma_2$ auxiliary subunit, also referred to as Stargazin, is a protein that possesses four transmembrane domains with cytoplasmic N- and C-termini, exclusively localized in the central nervous system. The protein is expressed in the presynaptic membrane where it inhibits voltage-gated Ca^{2+} (Ca_V) channel functional expression, as well as in the postsynaptic membrane where it controls the targeting of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors towards the plasma membrane. As can be anticipated from its location and function, alterations in the expression of the $Ca_V\gamma_2$ subunit may give rise to various neurological disorders including epilepsy. Despite the relevance of the $Ca_V\gamma_2$ subunit in health and disease, its regulation at transcriptional level has not been intensively addressed. Recently, it has been reported that the *CACNG2* gene promoter (encoding $Ca_V\gamma_2$) can direct transcriptional activity both in sense and antisense directions. In addition, the promoter could regulate the transcription of a long non-coding RNA (lncRNA) in antisense direction. In this work, we further investigated the *CACNG2* gene promoter in antisense direction. Our results showed that this promoter region has three possible transcription start sites and a TATA box that may regulate the lncRNA transcription. Interestingly, the lncRNA may increase the expression of $Ca_V\gamma_2$, providing insight into the molecular mechanisms that regulate the expression of this intriguing protein.

Disclosures: D. Muñoz-Herrera: None. **A. Calderón-Rivera:** None. **N. Zarco:** None. **R. González-Ramírez:** None. **R. Felix:** None.

Poster

119. Calcium Channels

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 119.23/B47

Topic: B.04. Ion Channels

Support: CONACyT

Title: Epidermal growth factor regulates the expression of the calcium channel alpha2delta-1 auxiliary subunit via the ERK/ELK-1 signaling pathway

Authors: *P. DURAN[1], A. SANDOVAL[2], R. GONZALEZ-RAMIREZ[3], N. ZARCO[4], R. FELIX[1];
[1]Cinvestav-IPN, Mexico City, Mexico; [2]FES Iztacala UNAM, Tlalnepantla de Baz, Mexico; [3]Dept. of Mol. Biol. and Histocompatibility, "Dr. Manuel Gea Gonzalez" Gen. Hosp., Mexico City, Mexico; [4]Mayo Clin., Jacksonville, FL

Abstract: Diverse growth factors including the epidermal growth factor (EGF) regulate the expression of ion channels. In this context, previous studies have shown that chronic treatment with EGF increases calcium currents through high voltage-activated (HVA) channels, probably by increasing the number of functional channels at the plasma membrane of GH3 pituitary adenoma cells. An upregulation in prolactin (PRL) secretion associated with an increase in the number of PRL-secreting cells has also been reported in the GH3 cultures in response to EGF. Here, we investigated whether the treatment with EGF increases the expression of the $\alpha_2\delta$-1 auxiliary subunit, a key molecular component of the HVA channel complex. Given that this protein promotes the membrane localization of HVA channels, an increase in its expression would explain the changes in calcium current density observed in GH3 cells after EGF treatment. Our results showed that EGF activates the Ras/Raf/MEK/ERK signaling pathway and significantly increases $\alpha_2\delta$-1 expression at transcriptional and translational levels. Sequence analysis of the *CACNA2D1* gene promoter (encoding $\alpha_2\delta$-1) revealed several binding sites for transcription factors activated by the signaling pathway of interest including CREB and ELK-1. Chromatin immunoprecipitation (ChIP) and site-directed mutagenesis assays showed that ELK-1 is important for the transcriptional regulation of the *CACNA2D1* promoter in response to EGF. Furthermore, biotinylation assays suggested that the treatment with the growth factor increases the membrane expression of the $\alpha_2\delta$-1 subunit. In addition, patch-clamp recordings in GH3 cells showed that ELK-1 overexpression significantly increases current density through HVA channels, while ELK-1 knockdown decreased the functional expression of these channels. Last, by using an enzyme-linked immunosorbent assay, we found that $\alpha_2\delta$-1 overexpression in GH3 cells increases PRL secretion. These results suggest that EGF by activating the

Ras/Raf/MEK/ERK/ELK-1 signaling pathway may influence the functional expression of HVA channels and the secretory behavior of pituitary GH3 cells.

Disclosures: P. Duran: None. **A. Sandoval:** None. **R. Gonzalez-Ramirez:** None. **N. Zarco:** None. **R. Felix:** None.

Poster

119. Calcium Channels

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 119.24/B48

Topic: B.04. Ion Channels

Support: NS057499

Title: Store-operated Orai1 channels regulate Ca^{2+} signaling in dendritic spines, synaptic plasticity, and cognition

Authors: *M. MANESHI[1], A. B. TOTH[2], T. ISHII[4], J. M. RADULOVIC[3], G. T. SWANSON[1], M. PRAKRIYA[5];
[1]Pharmacol., [3]Psychiatry & Behavioral Sci., [2]Northwestern Univ., Chicago, IL; [4]Physiol., Nippon Med. Sch., Tokyo, Japan; [5]Northwestern Univ. - Chicago, Chicago, IL

Abstract: Synaptic plasticity, and in particular, long-term potentiation (LTP) is widely considered to be a major cellular mechanism underlying the formation and acquisition of memories. Alterations in LTP are linked to diseases such as Asperger syndrome, autism and Alzheimer's Disease, highlighting the importance of this process for human health. Most forms of synaptic plasticity require elevations of intracellular Ca^{2+} as an early and obligate step and for many types of LTP, this is thought be mediated by the opening of NMDA receptors. However, the role of other Ca^{2+} pathways including intracellular stores and store-operated Ca^{2+} entry (SOCE) remains obscure. SOCE is a wide-spread mechanism for mobilizing intracellular Ca^{2+} elevations in many non-excitable cells and is mediated by the Ca^{2+} channel, Orai1, and its activator, the ER Ca^{2+} sensor, STIM1. However, the role of SOCE in regulating Ca^{2+} signaling in the brain is largely unknown. Here, using brain-specific constitutive and cell-specific knockouts of Orai1 and STIM1, we sought to understand the role this pathway for neuronal Ca^{2+} signaling and cognitive function. Analysis of Orai1 KO mice showed significant defects in many learning and memory tasks, including Y-maze, Morris-water maze, and fear conditioning, but no detectable changes in sensorimotor function. Measurements of CA3-CA1 LTP in brain slices revealed a striking impairment of LTP in the Orai1 KO mice. To determine the mechanistic basis of these phenotypes, we studied postsynaptic Ca^{2+} elevations evoked by glutamate uncaging in dendritic spines using the genetically-encoded Ca^{2+} indicator, jGCaMP7f. These measurements showed that deletion of Orai1 significantly diminishes the amplitude of Glu-uncaging evoked

Ca^{2+} rises in dendritic spines. Further, this loss of Ca^{2+} signaling is accompanied by decrease in the activation of the key effector enzyme, CaMKII. These results identify a novel role for Orai1 channels in regulating dendritic Ca^{2+} signaling and synaptic plasticity, and raise the possibility of targeting Orai1 channels for developing therapeutics for cognitive dysfunction.

Disclosures: **M. Maneshi:** None. **A.B. Toth:** None. **T. Ishii:** None. **J.M. Radulovic:** None. **G.T. Swanson:** None. **M. Prakriya:** None.

Poster

119. Calcium Channels

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 119.25/B49

Topic: B.04. Ion Channels

Support: T32 HL007638
T32 GM067795
NIH Grant NS087068
NIH Grant NS096246

Title: Li^{+} differentially affects mitochondrial Ca^{2+} efflux in central and peripheral neurons

Authors: *J. RYSTED[1], G. WALTERS[1], Z. LIN[1], M. NOTERMAN[2], G. LIU[3], E. B. TAYLOR[2], S. STRACK[1], Y. M. USACHEV[1];
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Abstract: During neuronal activity, Ca^{2+} entering the cell is buffered by mitochondria and subsequently released back to the cytosol, primarily via mitochondrial Na^{+}/Ca^{2+} exchanger (mtNCX). This process contributes to shaping Ca^{2+} responses and regulating many neuronal processes including synaptic transmission, gene expression and neuronal survival. It is thought that $Na^{+}/Ca^{2+}/Li^{+}$ exchanger (NCLX) is the major mediator of mitochondrial Ca^{2+} efflux. However, some reports suggest that the Na^{+}/Ca^{2+} exchanger family members NCX1-3 are also involved in this transport in neurons. The hallmark of NCLX not shared by NCX1-3, is its ability to use Li^{+} to effectively drive transmembrane Ca^{2+} transport in the absence of Na^{+}. To better understand the molecular and pharmacological properties of mtNCX in neurons, we exploited this unique property of NCLX and examined how Li^{+} affects mitochondrial Ca^{2+} transport in central and peripheral neurons, represented by mouse hippocampal and dorsal root ganglia (DRG) neurons, respectively. By simultaneously monitoring depolarization-induced changes in mitochondrial and cytosolic Ca^{2+} substitution with Li^{+} dramatically slowed mitochondrial Ca^{2+} efflux in hippocampal, but not DRG, neurons. The mtNCX inhibitor CGP37157 blocked mitochondrial Ca^{2+} efflux in both types of neurons. Quantitative RT-PCR showed similar NCLX

expression in adult DRG, hippocampus, cortex, and neonatal hippocampal cultures. NCLX knockdown using shRNA did not significantly change the rate of mitochondrial Ca^{2+} efflux in either DRG or hippocampal neurons. Notably, Li^{+}-induced Ca^{2+} efflux from isolated brain mitochondria was significantly slower than that induced by Na^{+}. A similar effect was found in isolated liver and heart mitochondria. Collectively, our findings suggest that the properties of mitochondrial Ca^{2+} efflux differ between central and peripheral neurons, and that besides NCLX, other proteins may contribute to mitochondrial Ca^{2+} efflux in the brain.

Disclosures: J. Rysted: None. **G. Walters:** None. **Z. Lin:** None. **M. Noterman:** None. **G. Liu:** None. **E.B. Taylor:** None. **S. Strack:** None. **Y.M. Usachev:** None.

Poster

120. Potassium Channels I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 120.01/B50

Topic: B.04. Ion Channels

Title: Regulation of Eag1 potassium channel biosynthesis by a RING E3 ubiquitin ligase

Authors: *Y.-C. FANG[1,2], C.-Y. TANG[1], C.-J. JENG[2];
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Abstract: The ether-à-go-go (Eag) K^{+} channel is a voltage-gated K^{+} channel. Two mammalian Eag isoforms, Eag1 (Kv10.1) and Eag2 (Kv10.2), have been shown to be primarily expressed in the brain. Eag1 may contribute to regulate the nervous system development and the neuronal signaling transduction besides the potassium current in neuronal cells. Mutations in the gene encoding human Eag1 K^{+} channel (*KCNH1*) have been associated with the congenital neurodevelopmental diseases Temple-Baraitser syndrome and Zimmermann-Laband syndrome. Some of the disease-associated Eag1 mutations may lead to enhanced degradation of the K^{+} channel protein, the detailed molecular mechanism of which remains obscure. Here, we aim to determine the molecular machinery responsible for the ubiquitin-dependent regulation of Eag1 K^{+} channel. By employing yeast two-hybrid screening of a rat brain cDNA library, we identified a specific RING E3 ubiquitin ligase, MKRN1 (also known as RNF61), as a potential binding partner of Eag1. The interaction between Eag1 and MKRN1 was confirmed by co-immunoprecipitation, GST pull-down, and proximity ligation assays. Detailed biochemical analyses revealed that MKRN1 interacted mostly with immature Eag1 proteins at the endoplasmic reticulum (ER). Interestingly, similar to the effect of treatment with the proteasomal inhibitor MG132, co-expression with MKRN1 resulted in the presence of a low-molecular-weight protein band that corresponds with the non-glycosylated form of Eag1. Moreover, MKRN1 over-expression promoted the ubiquitination and degradation of Eag1, whereas shRNA

knockdown of MKRN1 notably enhanced Eag1 protein expression. Altogether, our data suggest that MKRN1 may contribute to the ER quality control of Eag1 K^+ channel.

Disclosures: **Y. Fang:** None. **C. Tang:** None. **C. Jeng:** None.

Poster

120. Potassium Channels I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 120.02/B51

Topic: B.04. Ion Channels

Support: Marquette University Committee on Research

Title: The contribution of BK channels to regulation of interneuron firing rates changes across the first postnatal week in the superior region of the hippocampus

Authors: *M. HUNSBERGER, M. MYNLIEFF;
Biol. Sci., Marquette Univ., Milwaukee, WI

Abstract: Large conductance Ca^{2+} and high-voltage activated K^+ channels (BK channels) are critical determinants of neuronal firing and are important for action potential (AP) repolarization and maintenance of neuronal firing frequencies. Dysfunction of BK channels can have wide ranging consequences including predisposing an individual to seizures. We demonstrate that the temporal activity of BK channels in rat hippocampal neurons changes across the first postnatal week in the context of AP firing. This finding may explain in part why neonates are at increased risk of seizures.

Whole cell patch clamp recording in current clamp mode was used to record APs from dissociated, heterogeneous cultures of CA1 hippocampal neurons. Single APs were evoked by 8 nA, 0.1 ms depolarizations. When comparing neurons with similar AP duration (2-3 ms), perfusion with the BK antagonist Iberiotoxin (Ibtx, 100nM) increased AP duration by 0.46±0.12 ms (n=27) in neurons from 1-2 day old rats and by 0.17±0.05 ms in neurons from 6-7 day old rats (n=47; $p<0.05$, Mann-Whitney rank sum test). To determine the importance of BK channels in maintaining firing frequency we compared the effects of IbTx on successive firing rates. IbTx had a greater effect on the early firing frequency, the change in duration of the first inter-AP interval in a 100 ms train, in neurons from 1 day old rats (n=13) than in neurons from 7 day old rats (n=25; t-test, $p<0.01$), but did not have a significant impact on the last inter-AP interval. Preliminary data using a 1000 ms pulse in cells with ≥20Hz firing rate (putative interneurons) suggest a greater effect of IbTx in reducing early firing frequency in AP trains recorded in neurons from 1 day old rats, and a greater reduction in late firing frequency in AP trains recorded in neurons from 7 day old rats.

Together our data suggest that BK channels have fast, transient activity in the early neonatal

period that is replaced by slower-onset sustained activity by the end of the first postnatal week. This is supported by previous expression studies demonstrating that the BK isoform STREX, a fast-activating, transiently activating variant, is principally expressed in prenatal development, and is gradually replaced over the first postnatal week by the ZERO isoform, which is slower-activating and sustained (MacDonald SH-F, et al., BMC Dev Biol. 2006, 6:37). Changes in BK channel activity could facilitate sustained, high-frequency firing in hippocampal interneurons, allowing for proper regulation of hippocampal excitability and more effective suppression of seizure activity as development progresses.

Disclosures: M. Hunsberger: None. **M. Mynlieff:** None.

Poster

120. Potassium Channels I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 120.03/B52

Topic: B.04. Ion Channels

Title: Interplay between β3 integrins and Ca^{2+}-activated K^+ channels in cortical pyramidal neurons

Authors: *C. VITALE[1,2], F. JAUDON[1], A. THALHAMMER[1], L. A. CINGOLANI[1];
[1]Ctr. for Synaptic Neurosci. and Technol. (NSYN), Italian Inst. of Technology-IIT, Genoa, Italy;
[2]Univ. of Genoa, Genoa, Italy

Abstract: Integrins are cell adhesion heterodimers that interact with extracellular matrix (ECM) receptors. They regulate various cellular functions in the central nervous system such as the localization of ion channels, Ca^{2+} homeostasis and synaptic transmission. In this study, we found that β3 integrin interacts with small conductance (SK) Ca^{2+}-activated K^+ channels. SK channels are responsible for the medium afterhyperpolarization current (mIAHP) that regulates excitability and firing pattern in many neurons. We investigated firing properties of layer V pyramidal neurons of the medial prefrontal cortex (mPFC) in WT and β3 integrin KO mice. In layer V, two populations of pyramidal neurons can be distinguished: corticopontine (CPn) and commissural (COM). By using electrophysiological recordings and pharmacology, we identify a mIAHP in both types of neurons and in both genotypes, albeit with different characteristics: mIAHP is larger and faster in CPn than in COM neurons; in the KO, the mIAHP is smaller in CPn neurons, as compared to WT. Furthermore, the SK channel-specific blocker apamin affects differently the firing pattern of CPn and COM neurons; it increases adaptation in CPn neurons, while having no significant effect in COM neurons. To complement the electrophysiological results, we used viral retrograde labelling to investigate expression of β3 integrin and SK channel in both neuronal types. Altogether, our findings suggest that β3 integrin regulates the mIAHP

and the firing properties of layer V pyramidal neurons, although to different degrees in CPn and COM neurons.

Disclosures: C. Vitale: None. **F. Jaudon:** None. **A. Thalhammer:** None. **L.A. Cingolani:** None.

Poster

120. Potassium Channels I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 120.04/B53

Topic: B.04. Ion Channels

Title: The Ca^{2+}-activated potassium channel KCa3.1 as a therapeutic target in microglia-mediated neuroinflammation

Authors: *J. DI LUCENTE[1], H. M. NGUYEN[2], H. WULFF[2], L.-W. JIN[1], I. MAEZAWA[1]; [1]Pathology and Lab. Med., UC Davis, Sacramento, CA; [2]Pharmacol., Univ. of California Davis, Davis, CA

Abstract: Microglia play key roles in both CNS homeostasis and immune defense. Microglia activation following the detection of danger signals results in the release of factors involved in neuronal damage. Microglia express a number of cell surface receptors and channels that can activate microglia upon environmental changes. We have previously demonstrated that blocking the calcium-activated KCa3.1 potassium channel reduces microglial activation and neurotoxicity in both Alzheimer's disease and ischemic stroke. We also showed that KCa3.1 blockers inhibit pro-inflammatory cytokine production in cultured microglia after stimulation with lipopolysaccharides (LPS). In the current study, our goal is to determinate if KCa3.1 is active and required for microglial M1-like pro-inflammatory activation following intracerebroventricular injection (ICV) of LPS in 3 months old C57BL/6J mice. ICV-LPS enhanced cerebral Iba-1 immunoreactivity, indicating microglial activation. Using microglia freshly isolated from these mice, we found that LPS increased microglial KCa3.1 current density and mRNA expression. LPS also upregulated both mRNA expression and protein levels of pro-inflammatory mediators such as IL-1β, TNF-α, IL-6, and iNOS. To assess whether KCa3.1 is required for pro-inflammatory response of microglia *in vivo*, two complementary approaches, genetic knockout (KCa3.1-/-) and selective pharmacological blockage (Senicapoc), were used. We found that both KCa3.1 knockout and Senicapoc reduced Iba-1 immunoreactivity and expression of proinflammatory mediators, and mitigated LPS-induced long-term potentiation (LTP) impairment. These results support our hypothesis that KCa3.1 plays a pivotal role in the activation of microglia and the KCa3.1 blocker Senicapoc is a therapeutic candidate for neurological diseases where microglia-mediated neurotoxicity is implicated in the pathogenesis.

Disclosures: **J. Di Lucente:** None. **H.M. Nguyen:** None. **H. Wulff:** None. **L. Jin:** None. **I. Maezawa:** None.

Poster

120. Potassium Channels I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 120.05/B54

Topic: B.04. Ion Channels

Title: Small conductance Ca^{2+} activated K^{+}channels modulate the expression of ketamine-induced cognitive impairments in C57BL/6J mice

Authors: *C. A. RICE[1], R. W. STACKMAN, Jr.[2];
[1]Florida Atlantic Univ. Dept. of Psychology, Jupiter, FL; [2]Dept. of Psychology, Florida Atlantic Univ., Jupiter, FL

Abstract: Small conductance $^{Ca2+}$-activated K+ (SK) channels, expressed throughout the CNS, constrain the intrinsic excitability of neurons by enhancing the afterhyperpolarization, and shape glutamatergic postsynaptic potentials and limit the induction of NMDA receptor (NMDAR)-dependent synaptic plasticity, by affecting NMDAR activation. Homomeric or heteromeric SK channels are comprised of subtypes SK1, SK2 and SK3. Behaviorally, SK channels modulate the encoding of memory dependent upon several forebrain regions, including the hippocampus. Hippocampal memory and physiology is compromised in several neurological and psychiatric conditions including schizophrenia, depression, epilepsy as well as aging and Alzheimer's disease. It is hypothesized that drugs that influence SK channel function may modulate the expression of cognitive impairments associated with hippocampal compromise including executive functioning, working memory, and selective attention. Studies are in progress to establish the effect of the SK2 activator, CyPPA, and the SK1 activator, GW542573X, on cognitive impairments associated with a repeated systemic ketamine treatment regimen. Consistent with previous reports, we found that daily systemic administration of ketamine (KET) for 7 days led to the expression of cognitive deficits in long-term memory tasks, and impaired latent inhibition in male C57BL/6J mice. Chronic KET- or saline-treated mice received systemic CyPPA or vehicle and were placed in a familiar arena containing two identical novel objects. A test session was presented 24 h later to assess strength of object memory. KET/VEH-treated mice demonstrated significantly less novel object exploration compared to SAL/VEH-treated mice, indicating KET-induced object memory impairment. Consistent with previous work, SAL/CyPPA-treated mice exhibited impaired object memory. Mice that received KET/CyPPA showed significantly more novel object recognition compared to KET/VEH-treated mice, indicating that CyPPA influenced KET-induced memory impairment. These results suggest that SK2 channels modulate the expression of cognitive deficits associated with the repeated

ketamine model. Experiments testing the effects of systemic CyPPA and GW542573X on trace fear conditioning and a 5-choice serial reaction time task in KET-treated C57BL/6J mice are planned.

Disclosures: C.A. Rice: None. **R.W. Stackman:** None.

Poster

120. Potassium Channels I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 120.06/B55

Topic: B.04. Ion Channels

Support: NIH grant NS091836
SGCI NSF award, ACI-1547611

Title: Simulations of SK2 and SK3 currents in spinal motoneurons

Authors: *M. H. MOUSA[1], A. A. MAHROUS[2], S. M. ELBASIOUNY[1,2];
[1]Biomedical, Industrial and Human Factors Engin., [2]Dept. of Neuroscience, Cell Biology, and Physiol., Wright State Univ., Dayton, OH

Abstract: Small conductance Ca^{2+}-activated potassium (SK) channels mediate medium after-hyperpolarization (mAHP) in motoneurons (MN) and are responsible for regulating the motoneuronal excitability by modulating the MN discharge rate. It has been shown that fast and slow MNs differ in their intrinsic expression level of SK channels, and its SK2 and SK3 isoforms, and were correlated to their AHP differences (Deardorff et al., 2013). Specifically, slow MNs express both SK2 and SK3 isoforms, whereas fast MNs express only the SK2 isoform. However, it is unknown whether the ionic currents mediated by the SK2 and SK3 isoforms are similar or different? Given that SK2 and SK3 currents cannot be experimentally isolated using pharmacological agents, we employed computer simulations to investigate their kinetic properties. Therefore, the goal of the present work is to reverse engineer the SK2 and SK3 currents from the electrophysiological recordings obtained from fast and slow mouse MNs. The developed computer models of these currents would then be used to compare their properties and channel kinetics.
To achieve that, intracellular recordings were first obtained from spinal MNs in the whole-cord preparation of adult mice, and MNs were split into their respective type using their AHP ½ decay (Deardorff et al., 2013). Following the methodology of Elbasiouny et. al (2005), high-fidelity computer models of fast and slow MNs were then developed based on the 3D reconstructed morphology of MN types. For each cell model, the model parameters were adjusted and optimized until they matched 18 different electrical properties (i.e., input resistance, time constant, rheobase, AP height and width, AHP size and duration, FI-gain, etc.) measured

experimentally from slow and fast MNs.
Our simulations suggest that SK2 and SK3 currents have different properties with respect to their Ca^{2+} sensitivity. Specifically, SK3 currents activate at low Ca^{2+} concentrations, whereas SK2 currents require higher Ca^{2+} concentrations in order to activate. Additionally, our simulations indicate that SK3 current is faster than SK2 current, as suggested by the difference in their activation time constants. Furthermore, the simulations indicate that SK3 currents in slow cells contribute more to the cell AHP than SK2 currents. Taken together, the developed SK2 and SK3 models simulate motoneuronal SK currents more accurately than existing unified SK models that do not differentiate between the currents of both isoforms. The difference in kinetics of these currents underlie the difference in mAHP between fast and slow MNs.

Disclosures: **M.H. Mousa:** None. **A.A. Mahrous:** None. **S.M. Elbasiouny:** None.

Poster

120. Potassium Channels I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 120.07/B56

Topic: B.04. Ion Channels

Support: NNSF Grant 31630029
NNSF Grant 31661143037

Title: Axonal A- and D-type potassium currents regulate action potential waveform and dopamine release of midbrain dopamine neurons

Authors: *Y. XIAO[1], W. JI[2], Q. HE[1], L. MAO[2], Y. SHU[1];
[1]Beijing Normal Univ., Beijing, China; [2]Beijing Natl. Lab. for Mol. Sciences, CAS Key Lab. of Analytical Chem. for Living Biosystems, Inst. of Chemistry, Chinese Acad. of Sci., Beijing, China

Abstract: The neuromodulator dopamine plays key roles in regulating important brain functions including motor control, motivation and cognition. Dopamine is mainly released by midbrain dopaminergic neurons (DANs). Alteration of spiking activities or selective loss of these neurons could cause changes in dopamine release in the midbrain and other target brain regions, leading to severe brain disorders including Parkinson's disease, depression, and schizophrenia. Voltage-gated K^+ (Kv) channels determine neuronal excitability and regulate neurotransmitter release. Previous studies focus on Kv channels at the somatodendritic compartments. However, it remains largely unclear about the biophysical properties and the function of Kv channels distributed at the axon of DANs. In this study, we performed whole-cell recordings from the axons of DANs in acute midbrain slices from tyrosine hydroxylase (TH)-GFP mice. We observed the rapidly activating and inactivating A-type K^+ currents in DAN axons. In addition,

we also detected the rapidly activating but slowly inactivating D-type currents in the axon. Blocking these currents respectively could substantially prolong axonal APs, indicating the involvement of both currents in axonal AP repolarization. Further experiments with carbon-fiber electrochemical amperometry demonstrated that, in the presence of A- or D-current blockers, dopamine release in the dorsal striatum induced by optogenetic stimulation of dopaminergic axons was substantially enhanced. In conclusion, we show that Kv channels that mediate A- and D-currents shape AP waveform in DAN axons, and subsequently regulate dopamine release in the target brain region. Therefore, these axonal potassium channels could be potential drug targets for the modulation of DAN excitability and the treatment of related brain disorders.

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Poster

120. Potassium Channels I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 120.08/B57

Topic: B.04. Ion Channels

Support: NIH GM007324
NIH DC01919
NIH NS18492

Title: Activation of Slack potassium channels (KCNT1) triggers an increase in mRNA translation

Authors: *T. J. MALONE, P. LICZNERSKI, E. A. JONAS, L. K. KACZMAREK;
Yale Univ. Sch. of Med., New Haven, CT

Abstract: The Slack ion channel is a member of a family of large conductance sodium-activated potassium channels. Several gain-of-function Slack mutations are associated with early-onset epilepsy and severe intellectual disability in humans. Misregulation of mRNA translation is a possible explanation for the intellectual disability phenotype. FMRP, an important regulator of mRNA translation, binds both Slack mRNA and the Slack protein. The association of Slack with FMRP stimulates channel activity, raising the possibility that activation of Slack channels may also regulate the function of FMRP. Our laboratory has previously identified Slack as required for a translation-dependent recovery from an extended period of inhibition in Aplysia neurons following stimulation, further suggesting that Slack may play a role in the regulation of mRNA translation. Using FRAP experiments to visualize real-time translation in a stable Slack-expressing HEK cell line and in mouse cortical neurons, together with pharmacological manipulation and silencing RNA knockdowns, we have found that Slack activation causes an increase in translation that is enhanced in the absence of FMRP. This increase in translation

persists in the presence of the Slack channel blocker quinidine, indicating that it does not require ion flux through the channel. We also found that the R455H Slack mutation, one of the clinical mutations affecting human patients, causes a baseline increase in translation levels. This mechanism of Slack-dependent translation potentially represents the first instance of the direct modulation of mRNA translation by activation of an ion channel. The increased translation levels caused by the Slack mutation indicate that misregulation of translation is a possible mechanism to explain the intellectual disability experienced by patients with Slack mutations.

Disclosures: T.J. Malone: None. **P. Licznerski:** None. **E.A. Jonas:** None. **L.K. Kaczmarek:** None.

Poster

120. Potassium Channels I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 120.09/B58

Topic: B.04. Ion Channels

Support: NSFC Grant

Title: Kcnt1 mutants related morbidity of epilepsy and cardiac arrhythmia is attributed to same channelpathy

Authors: *Z. ZHANG, Y. LIU, Z.-S. SHEN, Y. XU, Q.-Y. TANG;
Xuzhou Med. Univ., Xuzhou, China

Abstract: In recent years, more and more genetic KCNT1mutants that are associated with epilepsy, Brugada syndrome and cardiac arrhythmia have been identified from patients with epilepsy. However, whether the mutants related to cardiac arrhythmia share similar biophysical properties with mutants associated with epilepsy remains elusive. In this abstract, we characterized the biophysical properties of 13 epilepsy associated genetic mutants of KCNT1channels, including two mutants associated with cardiac arrhythmia. We found that 12 of total 13 mutants possess enhanced maximum Po while some of them also have enhanced sodium sensitivity. Furthermore, the patient who carried the only one mutant that has decreased Po and lower maximum Po actually has other factor that may induce epilepsy. Thus, the enhanced maximum Po and increased sodium sensitivity could be a standard to judge if a Slack channel mutant is really associated with epilepsy. Furthermore, KCNT1 channel KO mice are resistent to PTZ induced epilepsy, while the H777H KI mice has the spontaneus seizure. Furthermore, the cardiac arrythemia can be attributed to the expression patterns of KCNT1 channel in heart.

Disclosures: Z. Zhang: None. **Y. Liu:** None. **Z. Shen:** None. **Y. Xu:** None. **Q. Tang:** None.

Poster

120. Potassium Channels I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 120.10/B59

Topic: B.04. Ion Channels

Title: Suppression of macro- and micro-scopic Kv1.1 channel activity by amyloid peptide fragments

Authors: *K. DEBOEUF, M. ISLAM, N. THELEN, J. FARLEY;
Indiana Univ., Bloomington, IN

Abstract: Past studies have linked the beta amyloid peptide (Aβ) to the disruption of Ca^{2+} homeostasis, synaptic communication, and long-term potentiation (LTP), but the underlying mechanism(s) is still largely unclear. Because Kv1.1 and related channels are activated during an action potential, regulate depolarization Ca^{2+} influx, and the inhibition of Kv1 channels can be neurotoxic, we speculate that Aβ-suppression of Kv1 channels may be early targets in Alzheimer's Disease (AD) pathogenesis. Stage V and VI *Xenopus laevis* oocytes [Ecocyte Bioscience (Austin, TX)] were injected with Kv1.1 cRNA. The effects of bath application of Aβ fragments (1-42) and (25-35) on macroscopic currents were assed using standard two electrode voltage-clamp (TEVC) methods, whereas direct single channel effects were assessed using patch clamp and artificial membrane techniques ["tip-dip" and black lipid membrane (BLM)]. Bath application of 1 μM Aβ(1-42) produced 45% suppression of macroscopic Kv1.1 currents, whereas Aβ(25-35) produced ~38% suppression within 30 min. Aβ suppression of Kv1.1 was partially Ca^{2+}- and PP2B-dependent, with only ~25% Aβ suppression taking place when cells were loaded with BAPTA-AM or exposed to the PP2B-inhibitor cyclosporine A (CsA). Reduction in macroscopic currents was not dependent on a reduction in the number of channels present, as western blot analyses did not reveal any detectable differences in band intensities between conditions. Application of Aβ to the intracellular face of Kv1.1 channels in both patch clamp and tip dip experiments produced dramatic reductions in *p*(open), with no observable current ~2 min post-addition. BLM experiments also showed reductions in *p*(open) in response to intra- and extra-cellular Aβ application but did not fully eliminate channel activity (~45% reduction). Suppression of Kv1.1 and related K^+ channels presynaptically could lead to larger and longer action potentials, thus allowing a greater influx of Ca^{2+} and subsequent increase in glutamate release. Postsynaptically, the increased glutamate release, through activation of AMPA and NMDA receptors, may contribute to excitotoxicity.

Disclosures: K. Deboeuf: None. **M. Islam:** None. **N. Thelen:** None. **J. Farley:** None.

Poster

120. Potassium Channels I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 120.11/B60

Topic: B.04. Ion Channels

Title: Generation of a human disease model for KCNA2-related developmental epileptic encephalopathy using iPSC-derived neurons

Authors: *B. UYSAL, N. SCHWARZ, H. LÖFFLER, H. KOCH, U. HEDRICH, H. LERCHE; Dept. of Neurol. and Epileptology, Hertie Inst. for Clin. Brain Res., Tübingen, Germany

Abstract: *De novo* mutations in the *KCNA2* gene have recently been identified as a causative factor for developmental epileptic encephalopathies (DEE). The *KCNA2* gene encodes the voltage-gated potassium channel $K_V1.2$ α-subunit which is widely expressed across the brain, in both excitatory and inhibitory neurons. The experiments performed in heterologous expression systems indicated gain- or loss-of-function effects caused by some of the identified *KCNA2* mutations, whereas a number of those mutations, such as the p.Thr374Ala mutation, showed both gain- and loss-of-function effects on the channel function. In this study, developmental epileptic encephalopathy caused by the *KCNA2*-p.Thr374Ala mutation, which was identified in patients with the most severe phenotype among the *KCNA2*-related DEEs, was investigated in an iPSC-derived neuronal model system. To do so, human iPSCs were differentiated into excitatory neuronal cultures via overexpression of the neuronal transcription factor Neurogenin-2 and co-cultured with astrocytes for up to 8 weeks to obtain mature neuronal networks. Electrophysiological experiments were performed to compare passive and active electrical properties of patient-derived neurons to those of healthy controls. We found that patient-derived neurons expressing $K_V1.2$-p.Thr374Ala mutant channels showed a decreased outward current density and were hypoexcitable compared to neurons from healthy controls. Half-width and rising phase of action potentials were prolonged in patient-derived neurons, which may explain the significantly reduced frequency of evoked action potentials, and at the same time raises the question whether prolonged action potentials could be the reason for increased neurotransmitter release from the presynaptic terminals leading to hyperexcitability of the network. On the other hand, spike rate adaptation was impaired in patient-derived neurons, which may lead to a hyperexcitability on the network level. Further studies, on both single cell and network level, including interneuron differentiation protocols are needed to gain deeper insight into the mechanism of *KCNA2* -related DEE.

Disclosures: B. Uysal: None. **N. Schwarz:** None. **H. Löffler:** None. **H. Koch:** None. **U. Hedrich:** None. **H. Lerche:** None.

Poster

120. Potassium Channels I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 120.12/B61

Topic: B.04. Ion Channels

Support: NIH NS100294

Title: Functional interaction between the voltage-gated potassium channel Kv1.3 and purinergic P2X receptors in pro-inflammatory microglia

Authors: *H. M. NGUYEN[1], Y.-J. CHEN[1], J. DI LUCENTE[2], L.-W. JIN[2], I. MAEZAWA[2], H. WULFF[1];
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Abstract: As the main immunocompetent cells of the brain microglia directly regulate many aspects of the CNS, from synapse formation during early development to maintenance of neuronal health thereafter. Under pathophysiological conditions, microglia are activated by danger associated molecular patterns (DAMPs) derived from local tissue damage and ischemia. We have previously showed that brain microglia express potassium ion channels, whose differential expression profile is associated with and regulates distinct microglial activation states. For example, microglia stimulated with the classical M1 stimulus lipopolysaccharides (LPS) consistently display increased Kv1.3 channel expression and their treatment with the Kv1.3-selective PAP-1 inhibitor reduced proinflammatory markers such as IL-1β, iNOS and COX2. As potassium ion efflux repolarizes membrane potential, and hence determines the driving force for calcium entry, we further investigated the relationship between microglial Kv1.3 channels and P2X purinergic receptors. Using patch-clamp recordings of cultured primary microglia, we observed that in addition to the increase in Kv1.3 currents, LPS also reduces the constitutive expression of ATP-induced currents. In agreement with our patch-clamp recording results, LPS-stimulated microglia displayed reduced ATP-induced calcium signals in calcium imaging experiments compared to non-treated control cells and cells stimulated with IL-4. We further confirmed these changes at the gene expression level using qPCR on both cultured microglia and microglia acutely isolated from mice receiving LPS via intracerebroventricular injection. Functionally, pharmacological inhibition of Kv1.3 negatively affected calcium response to ATP in Kv1.3-expressing microglia, affirming its role in modulating the driving force for calcium entry. Interestingly, microglia from mice experiencing ischemic stroke after MCAO procedure displayed an increase in both Kv1.3 and P2X currents, suggesting specificity in the respective DAMPs triggered by different models of inflammation. Since Kv1.3 inhibitors effectively reduced microglia-associated inflammatory responses *in vitro* and *in vivo*, our data

suggest a possible mechanism for their mode of action via reducing calcium entry mediated by the P2X receptors to regulate intracellular calcium.

Disclosures: H.M. Nguyen: None. **Y. Chen:** None. **J. Di Lucente:** None. **L. Jin:** None. **I. Maezawa:** None. **H. Wulff:** None.

Poster

120. Potassium Channels I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 120.13/B62

Topic: B.04. Ion Channels

Title: Control of neuronal excitability by palmitoylation-dependent ion channel clustering at the axon initial segment

Authors: S. S. SANDERS[1], L. M. HERNANDEZ[2], H. SOH[3], S. S. KARNAM[2], A. TZINGOUNIS[4], ***G. THOMAS**[2];
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Abstract: Precise subcellular clustering of neurotransmitter receptors and ion channels is essential for normal synaptic transmission and neuronal excitability. Perhaps the best-studied example of such clustering is at excitatory synapses, where the ‘scaffold’ protein PSD-95 controls the clustering of glutamate receptors. It is well established that PSD-95 critically requires the protein-lipid modification palmitoylation for its clustering activity. However, although PSD-95’s close paralog PSD-93 is also palmitoylated, the functional importance of PSD-93 palmitoylation and the relevant palmitoyl acyltransferase (PAT) are both unknown. We found that a largely unstudied PAT, ZDHHC14, directly binds and palmitoylates PSD-93. Strikingly, rather than target PSD-93 to synapses, palmitoylation targets PSD-93 to another important site of ion channel clustering, the Axon Initial Segment (AIS). Lentiviral-mediated Zdhhc14 knockdown reduced both palmitoylation and AIS targeting of PSD-93 and also Kv1 family potassium channels to which PSD-93 directly binds. Consistent with their co-regulation, ZDHHC14 expression mirrors that of Kv1 channels during neurodevelopment, and Zdhhc14 knockdown lowers the threshold of action potential firing and thereby increases neuronal excitability. Synaptic clustering of glutamate receptors and AIS clustering of Kv1 channels are thus both controlled by similar palmitoylation-dependent mechanisms, but each involves distinct PDZ domain proteins that are in turn regulated by distinct PATs. These findings have broad implications for our understanding of physiological regulation of neuronal excitability and its dysfunction in conditions such as epilepsy.

Disclosures: **S.S. Sanders:** None. **L.M. Hernandez:** None. **H. Soh:** None. **S.S. Karnam:** None. **A. Tzingounis:** None. **G. Thomas:** None.

Poster

120. Potassium Channels I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 120.14/B63

Topic: B.04. Ion Channels

Support: KRF-2019R1A2C2003642

Title: Trafficking and localization of Kv2.1-HCN2 channel complexes in neurons

Authors: ***S. PARK**, J.-Y. HWANG, K.-S. PARK;
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Abstract: The voltage-gated potassium channel Kv2.1 has a critical role in regulating neuronal membrane excitability and is localized to plasma membrane clusters on the soma, proximal dendrites, and axon initial segment (AIS). Hyperpolarization activated cyclic nucleotide-gated channel 2 (HCN2) is localized to the soma, dendrites, and axon terminals in neurons, and these localizations show differences among neuronal cell types. HCN2 channel is implicated in neuropathic pain, febrile seizure, and depression and contributes spontaneous rhythmic activity in brain. Here, we show that Kv2.1 channels affinity-purified from rat brain are associated with HCN2 channels using mass spectrometry. HCN2 and Kv2.1 channel expressions reveal a different distribution among the distinct regions of the brain. Co-expression of HCN2-WT and Kv2.1 in heterologous cells results in plasma membrane localization, while HCN2-N380Q, a trafficking-defective mutant, retains Kv2.1 in the endoplasmic reticulum. In hippocampal neurons, HCN2-WT dramatically reconstitutes the large somatodendritic and axonal clusters of Kv2.1 in plasma membrane, whereas HCN2-N380Q significantly leads to disruption of the clustering and AIS specific localization of Kv2.1. Thus, these results suggest that HCN2 channel regulates the surface localization and axonal trafficking of Kv2.1 in neurons.

Disclosures: **S. Park:** None. **J. Hwang:** None. **K. Park:** None.

Poster

120. Potassium Channels I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 120.15/B64

Topic: B.04. Ion Channels

Support: KRF-2019R1A2C2003642

Title: Nav channel beta3 regulates the expression and localization of Kv3.1b channels in neurons

Authors: *J. SHIM[1], D.-H. KIM[2], Y. CHOI[1], J.-S. CHOI[2], K.-S. PARK[1];
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Abstract: Voltage-gated K^+ channel Kv3.1b play a crucial role in regulating fast-spiking properties of neurons and is wildly expressed in the dendrites, the somatodendritic region, and the axonal nodes of Ranvier in neurons. It has been reported that balance activity of Kv3 and Nav channels is important for inducting and maintaining fast-spiking. Here, we show that Navβ3, an auxiliary subunit of Nav channels, is a part of the Kv3.1b channel complex in rat brain using mass spectrometry. Co-expression of Navβ3 results in changes in the steady-state expression levels and stability of Kv3.1b channels. Interestingly, Kv3.1b and Navβ3 are differentially expressed between distinct regions of the brain and have a different expression pattern during brain development. Navβ3 regulates Kv3.1b channel trafficking to the cell surface and reduces the localization and expression of Kv3.1b in dendrites in hippocampal neurons. In addition, Navβ3 dramatically decrease the current densities of Kv3.1b channel in a voltage-dependent manner and induces a hyperpolarizing shift in the voltage dependence of steady-state inactivation. Therefore, these data suggest an unexpected role for Navβ3 in regulating the biophysical characteristics and localization of Kv3.1b channel.

Disclosures: J. Shim: None. **D. Kim:** None. **Y. Choi:** None. **J. Choi:** None. **K. Park:** None.

Poster

120. Potassium Channels I

Location: Hall A

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Topic: B.04. Ion Channels

Support: NIH grant DC 01919 (L.K.K)
NIDA Keck Center
National Ataxia Foundation

Title: Tank binding kinase-1 interacts with the cytoplasmic C-terminus of the Kv3.3 potassium channel

Authors: *Y. ZHANG[1], L. VARELA[1], K. SZIGETI-BUCK[1], M. CHATTERJEE[1], T. HORVATH[1], L. K. KACZMAREK[2];
[1]Yale Univ. Sch. Med., New Haven, CT; [2]Yale, New Haven, CT

Abstract: Spinocerebellar Ataxia type 13 (SCA13), a condition that lead to cerebellar degeneration, is caused by mutations in the *KCNC3* gene, which encodes the voltage-dependent potassium channel Kv3.3. These channels are expressed at particularly high levels in Purkinje cells in the cerebellum, and directly bind Hax-1, a cell survival protein required for survival of the cerebellum. To investigate how KCNC3 mutations lead to neurodegeneration, we generated mice bearing the G592R Kv3.3 mutation using CRISPR Cas9 gene editing. G592R Kv3.3 channels are fully functional as potassium channels but have abnormal interactions with cytoplasmic signaling pathways and lead to cerebellar degeneration in humans. A screen for protein kinases activated by this mutation revealed that the activity of Tank-Binding protein 1 (TBK1), an enzyme that plays a key role in the formation of multivesicular bodies, autophagy and mitophagy, is much higher in the cerebellum of G592R Kv3.3 knock-in mice than in that of wild type animals. Total levels of TBK1 and of phosphorylated S6 protein were not altered by the mutation, and no differences in TBK1 activation were detected in the forebrain of mutant and wild type animals. We found that TBK1 can be coimmunoprecipitated with Kv3.3 channels and that this binding is significantly enhanced by the G592R mutation. To determine sites on the Kv3.3 channel required for interaction with TBK1, we carried out a series of gene truncations C-terminus of Kv3.3 and determined that a stretch of amino acids in the center of the cytoplasmic C-terminus, including polyproline rich-region that contains G592R is required for tight association of TBK1 with the channel. Using transfected cell lines, we found that G592R Kv3.3 channels increases TBK1 activation basally and that depolarization of the mutant channels further stimulates TBK1 activity over that produced by depolarization of wild type channels. By immunoelectron microscopy and western blotting, we find that the enhanced activation of TBK1 in G592R Kv3.3 knock-in mice is associated with increased numbers of intracellular multivesicular bodies containing Hax-1, increased levels of CD63, a molecular marker for multivesicular bodies, and increased LAMP2 expression. No change was found in levels of LC3B, a protein associated with autophagy. Our findings suggest that Kv3.3 channels both physically and functionally interact with TBK1, and that this protein complex regulate the trafficking of Kv3.3 together with the survival protein Hax-1 into multivesicular bodies and lysosomes for degradation.

Disclosures: Y. Zhang: None. **L. Varela:** None. **K. Szigeti-Buck:** None. **M. Chatterjee:** None. **T. Horvath:** None. **L.K. Kaczmarek:** None.

Poster

120. Potassium Channels I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 120.17/B66

Topic: B.04. Ion Channels

Title: Pin1 isomerization of Kv4.2 regulates neuronal excitability in the mouse hippocampus

Authors: *C. MALLOY[1], J.-H. HU[1], D. A. HOFFMAN[2];
[1]Eunice Kennedy Shriver Natl. Inst. of Child Hlth. and Human Develop., [2]NIH, Bethesda, MD

Abstract: The A-type potassium current (I_A) is a vital regulator of neuronal excitability. In rodent hippocampal CA1 pyramidal cells, this current is carried primarily by voltage-gated Kv4.2 K+ channels. Along with other voltage-gated ion channels localized in the somatodendritic compartment of pyramidal cells, Kv4.2 contributes to the firing mode of the cell. Kv4.2 function is regulated by associated auxiliary subunits in complex with the channel and dysfunctions in this regulation have been implicated in pathological conditions characterized by aberrant neuronal excitability, including epilepsy. To date, modulation of complex formation and stability has not been fully addressed. To identify potential modifiers of the Kv4.2 complex, we utilized tandem affinity purification with mass spectrometry to screen additional Kv4.2 binding partners. We identified the cis/trans peptidyl-prolyl isomerase, Pin1, as a novel Kv4.2 binding partner. To probe the role of Pin1 in regulating Kv4.2 function, Crispr-cas9 technology was utilized to generate Pin1-Kv4.2 binding mutant knockin mice (Kv4.2TA). We confirmed disruption of endogenous Kv4.2-Pin1 binding by co-immunoprecipitation from whole brain lysates. We investigated the physiological consequence of this reduced binding on neuronal excitability using whole-cell patch clamp electrophysiology in acute hippocampal slices from adult mice. Kv4.2TA mice displayed reduced action potential (AP) firing frequency in response to stepped somatic current injections. Additionally, enhanced after-hyperpolarization and increased interspike intervals were observed in AP trains in Kv4.2TA mice. This reduction in neuronal excitability was replicated by pharmacological blockade of Pin1 activity in wild type (WT) mice. As Kv4.2-containing channels are known to reduce excitability in pyramidal neurons by shunting depolarization and providing repolarization, these results suggest enhanced Kv4.2 function in Kv4.2TA mice. To confirm a direct influence by Pin1 on Kv4.2 function, we performed voltage-clamp recordings from outside-out somatic patches pulled from CA1 pyramidal cells. Isolation of I_A current revealed a significant increase in current density in patches pulled from Kv4.2TA cells relative to WT. Additionally, a leftward shift in the normalized recovery from inactivation curve was identified in Kv4.2TA cells relative to WT suggesting faster recovery of conductance from a non-conducting state. Therefore, we show that Pin1 modulates CA1 pyramidal cell excitability through regulation of Kv4.2 channel availability and recovery from inactivation kinetics.

Disclosures: **C. Malloy:** None. **J. Hu:** None. **D.A. Hoffman:** None.

Poster

120. Potassium Channels I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 120.18/DP02/B67

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: B.04. Ion Channels

Support: NIH Z01-HD008755
NIH OxCam Scholars program
Gates Cambridge program

Title: Intermittent channel trafficking in dendrites

Authors: *A. BELLOTTI[1,2], J. MURPHY[2], F. FORNI[1], D. HOFFMAN[2], T. O'LEARY[1];
[1]Dept. of Engin., Univ. of Cambridge, Cambridge, United Kingdom; [2]Natl. Inst. of Child Hlth. and Human Develop., NIH, Bethesda, MD

Abstract: Neurons are excitable cells that contain processes that can extend hundreds or thousands of microns away from the cell body. This unique morphology presents several logistical obstacles regarding the function of the cell as a cohesive unit. One such challenge is the transport and regulation of ion channel concentrations throughout the dendritic arborization of pyramidal cells. To compound this issue, Kv4.2 is a voltage-gated potassium channel that is endogenously expressed with increasing density with distance from the soma. Previous modeling studies confirm that such cargo demand profiles create transport bottlenecks and result in critical speed-precision tradeoffs.

We propose that ion channels such as Kv4.2 are trafficked on microtubules intermittently in discrete on/off states to improve system stability. This hypothesis is based on 200+ hours of dendrite imaging following transfection of Kv4.2-GFP. Dendritic transport was observed in only 30% of total recording time, and dendritic activity appears to follow a dichotomous random process with puncta appearing in clustered bursts. Extended-time recordings reveal on-states with puncta trafficking for over 1 hr in duration and off-states lasting up to 2.5 hrs. Interestingly, axonal trafficking is present during 90% of total recording time, and puncta transport in axons appear to be continuous with no distinct off-state. Inferential statistical analysis of the dataset is performed to determine which stochastic process best describes the underlying intermittent phenomenon. Prospective models include the inhomogenous Poisson point process, the telegraph process, and combinations of these processes.

The observed intermittent phenomenon has been replicated in a mathematical model to assess its feasibility in improving system stability. Initial open-loop simulations reveal a speed-stability tradeoff governed by the frequency of the intermittent phenomenon. In closed-loop simulations,

linear controllers with increasing gains result in instability, whereas an on/off nonlinear switch controller can reduce instability.

Disclosures: A. Bellotti: None. **T. O'Leary:** None. **D. Hoffman:** None. **F. Forni:** None. **J. Murphy:** None.

Poster

120. Potassium Channels I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 120.19/B68

Topic: B.04. Ion Channels

Title: Interrogating voltage-gated ion channel currents and gene expression from the same cell via a modified Patch-seq experimental protocol

Authors: *B. LEE, R. MANN, L. NG, A. BUDZILLO, B. KALMBACH, K. BAKER, R. MORSE, A. OLDRE, H. ZENG, G. MURPHY, J. BERG;
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Abstract: Patch-seq is a powerful approach to characterize the electrophysiological, morphological and transcriptomic features from single mouse and human neurons. Using this technique at scale has allowed us to establish the correspondence between a neuron's intrinsic electrical properties and its transcriptomic 'type'. Understanding how specific ion channels contribute to differences in the electrical signature of different transcriptomic types can allow us to better understand neuronal function. To study these channels, we modified the patch-seq protocol to include a 'nucleated patch' recording following characterization of the cell's intrinsic electrical properties by whole cell current clamp recording. Due to its roughly spherical geometry, this configuration is ideal to characterize ion channel currents and densities of the perisomatic region. Here we relate these macroscopic voltage-gated channel currents to the cell's transcriptomic signature. Using well-established voltage clamp protocols, we isolate the amplitude and kinetics of inactivating and non-inactivating potassium currents. In general, we find distinct potassium channel amplitude and kinetics between cells identified within and between transcriptomic cell classes. Ongoing analysis seeks to relate these differences to specific ion channel genes, intrinsic firing pattern differences, and cell morphologies. Ultimately these independent data modalities create a synergistic, more comprehensive, and refined taxonomy of neuronal diversity in the mouse and human cortex.

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Poster

121. Neurotransmitter Release and Vesicle Recycling

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 121.01/B69

Topic: B.05. Neurotransmitter Release

Support: NIH Grant NS032385

Title: Activity-evoked and spontaneous opening of synaptic fusion pores

Authors: *D. BULGARI[1], D. L. DEITCHER[2], B. F. SCHMIDT[3], M. A. CARPENTER[4], C. SZENT-GYORGYI[3], M. P. BRUCHEZ[3,4,5], E. S. LEVITAN[1];
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Abstract: Synaptic release of neuropeptides packaged in dense-core vesicles (DCVs) regulates synapses, circuits and behaviors including feeding, sleeping and pain perception. Exocytosis of DCVs has been avidly studied in non-neuronal cells, but not in native synapses. Presynaptic DCVs differ from their non-neuronal counterparts in many respects. For example, they are smaller (~100 nm instead of 300-1000 nm), allowing them to fit in thin axons and small boutons. Therefore, they cannot form the very large fusion pores produced by neuroendocrine secretory granules. Neuropeptide release at the *Drosophila* neuromuscular junction is slow and dynamin dependent. However, fusion pore gating properties and permeability for molecules as large as neuropeptides (0.65-2 kDa for the NMJ neuropeptides proctolin, MIP and CCAP) remain unknown. Fusion pore opening can be detected by imaging extracellular fluorescent dyes entering docked vesicles, but this approach would label DCVs and small synaptic vesicles, which mediate small molecule co-transmission. Here we selectively image DCV fusion pores at *Drosophila* type Is synaptic boutons by genetically loading DCVs with a proneuropeptide fused to a fluorogen-activating protein (FAP). Specifically, we used an antibody-derived light chain variable domain whose fused homodimer (an atypical scFv) binds malachite green with picomolar affinity to generate far red fluorescence. Applying a variety of membrane impermeant malachite green derivatives allowed us to image when and where fusion pores open at synapses and to probe their conduction properties. We report that activity-evoked kiss and run exocytosis opens synaptic DCV fusion pores away from active zones that readily conduct molecules larger than most native neuropeptides (i.e., at least 4.5 kDa). Remarkably, these synaptic fusion pores also open spontaneously in the absence of stimulation and extracellular Ca^{2+}. SNARE perturbations demonstrate different mechanisms for activity-evoked and spontaneous fusion pore openings, with the latter sharing features of spontaneous small molecule transmitter release by

active zone-associated small synaptic vesicles. Fusion pore opening at resting synapses provides a mechanism for activity-independent peptidergic transmission.

Disclosures: D. Bulgari: None. **D.L. Deitcher:** None. **B.F. Schmidt:** None. **M.A. Carpenter:** None. **C. Szent-Gyorgyi:** None. **M.P. Bruchez:** None. **E.S. Levitan:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 121.02/B70

Topic: B.05. Neurotransmitter Release

Support: NIH Grant EY010542
NIH Grant F30 NEI EY028848

Title: Vesicular calcium sensor synaptotagmin-1 mediates neurotransmission from mammalian rods and cones

Authors: *W. B. THORESON[1], J. J. GRASSMEYER[1], C. L. HAYS[1], A. CAHILL[1], M. J. VAN HOOK[1], N. BABAI[2];
[1]Univ. of Nebraska Med. Ctr., Omaha, NE; [2]Friedrich Alexander Univ., Erlangen, Germany

Abstract: Synaptic exocytosis from amphibian photoreceptors does not use the Ca^{2+} sensor Synaptotagmin-1 (Syt1) and shows unusually low Ca^{2+} cooperativity. However, Syt1 is present in synaptic terminals of mouse rods and cones suggesting it may serve as the exocytotic Ca^{2+} sensor in mammals. To test a role for Syt1 in mouse rods and cones, we generated mice with loxP sites flanking exon 6 in Syt1 and crossed them with *Rho-iCre* or *HRGP-Cre* mice that express Cre only in rods or cones, respectively. We confirmed selective Syt1 elimination from rods or cones by immunohistochemistry. Consistent with diminished rod output, eliminating Syt1 from rods suppressed ERG b-waves (reflecting bipolar cell responses) evoked by dim flashes. Consistent with loss of release from cones, eliminating Syt1 from cones reduced b-waves evoked by bright flashes and abolished responses to high frequency flicker. Neither ERG a-waves (reflecting photoreceptor light responses) nor presynaptic Ca^{2+} currents (I_{Ca}) were altered. Removal of Syt1 also did not alter synaptic anatomy or promote ectopic sprouting of bipolar and horizontal cell dendrites. Anion currents accompanying presynaptic glutamate transporter activity were used to measure glutamate release from individual rods and cones. Release from cones was also assessed from the inhibition of I_{Ca} by vesicular protons. Eliminating Syt1 abolished release from cones evoked by 2-25 ms depolarizing steps. Loss of Syt1 abolished release from rods evoked by 5 ms steps but did not fully eliminate release evoked by 25 or 500 ms steps. Spontaneous release persisted in both rods and cones. To measure Ca^{2+} cooperativity of release, we varied extracellular Ca^{2+} and recorded post-synaptic currents evoked in horizontal or

ganglion cells by extracellular stimulation of cones or cone bipolar cells, respectively. Like amphibians, mouse cones showed a low cooperativity of N=2.7 whereas bipolar cells showed a more typical high cooperativity of 5.
These results indicate that Syt1 is the Ca^{2+} sensor that regulates fast synaptic release by mouse rods and cones while other sensors contribute to spontaneous and slower forms of release. Although salamander photoreceptors do not use Syt1, mouse and salamander show similarly low Ca^{2+} cooperativity unlike most other synapses, suggesting this property is not due to use of a particular Syt. The undisturbed synaptic anatomy seen after deletion of Syt1 suggests a limited role for neurotransmission in maintaining photoreceptor synapse integrity, emphasizing the importance of proteins involved in active zone organization.

Disclosures: W.B. Thoreson: None. **J.J. Grassmeyer:** None. **C.L. Hays:** None. **A. Cahill:** None. **M.J. Van Hook:** None. **N. Babai:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 121.03/B71

Topic: B.05. Neurotransmitter Release

Support: NIH grant MH116580
NIH grant NS097362
NIH grant MH061876
HHMI Investigator Grant

Title: Syt1-associated developmental disorder: Molecular pathogenesis and new insights into the Ca^{2+}-sensing mechanism of Syt1

Authors: *M. M. BRADBERRY[1], N. A. COURTNEY[1], E. R. CHAPMAN[2,1];
[1]Univ. of Wisconsin-Madison, Madison, WI; [2]Neurosci., Howard Hughes Med. Inst., Madison, WI

Abstract: Objective & Rationale C2 domains are common in eukaryotic proteins and often occur as tandem pairs. At neuronal synapses, the tandem-C2 domain protein synaptotagmin-1 (syt1) acts as a fast Ca^{2+} sensor that synchronizes exocytosis with Ca^{2+} influx during action potential firing. Heterozygous missense mutations in syt1 have recently been associated with severe but heterogeneous developmental delay, highlighting the importance of this protein for normal brain function. Syt1 triggers exocytosis via Ca^{2+}-dependent penetration of the presynaptic plasma membrane by its tandem C2 domains, and a significant body of work suggests that these tandem domains act cooperatively via direct interactions with one another. However, while each C2 domain of syt1 contains a Ca^{2+}-binding pocket, it is unknown whether these binding pockets

function cooperatively but distinctly (as in hemoglobin binding oxygen) or act as a unified Ca^{2+} binding site. Moreover, well-defined pathogenic mechanisms and the basis for phenotypic heterogeneity in syt1-associated developmental disorder remain unknown. **Methods & Results** Here, we report the clinical, physiologic and biophysical characterization of three syt1 mutations from human patients of varying disability. Release of both GABA and glutamate was differentially and severely impaired in cultured neurons expressing mutant variants. Strikingly, in biophysical studies, Ca^{2+}-dependent membrane penetration was always coupled between C2A and C2B regardless of mutation, establishing a unified mechanism for Ca^{2+}-dependent membrane penetration by syt1. **Conclusions** Our results establish the molecular cause of, and basis for phenotypic heterogeneity in, syt1-associated developmental disorder. Our results also define a fundamental mechanistic aspect of syt1 that is essential for synaptic transmission *in vivo*.

Disclosures: M.M. Bradberry: None. **N.A. Courtney:** None. **E.R. Chapman:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 121.04/B72

Topic: B.05. Neurotransmitter Release

Support: ARC Discovery Project DP160100849
NHMRC Project Grant APP1122351
UQ Foundation Research Excellence Awards
NARSAD Young Investigator Grant 24980

Title: Ca^{2+}-triggered neurotransmitter release requires two Ca^{2+}sensors in *C. elegans*

Authors: *L. LI, H. LIU, Y. YU, J. TANG, Z. HU;
Queensland Brain Inst., Brisbane, Australia

Abstract: Synaptic vesicles (SVs) fuse with plasma membrane to release neurotransmitters. Several members in Synaptotagmin family proteins have been recognized as Ca^{2+} sensors to mediate SV release through their tandem C2 domains (C2A and C2B). Here we report that SV release at the *C. elegans* neuromuscular junctions (NMJs) requires two Ca^{2+} sensors, SNT-1 and SNT-3. Stimulus-evoked excitatory postsynaptic current (EPSC) was reduced by 60% in *C. elegans* mutant lacking *snt-1*, a mouse Syt1 homolog. Electrophysiological screening of all other Synaptotagmin isoforms have identified SNT-3 as a second Ca^{2+} sensor required for Ca^{2+}-triggered neurotransmitter release. The remaining evoked release in *snt-1* mutant was completely abolished in *snt-1;snt-3* double mutants. However, SV release was normal in *snt-3* single mutants, suggesting that the SNT-3 serves as a Ca^{2+} sensor only when SNT-1 is absent. Compared to SNT-1 which has an N-terminal transmembrane domain (TM), SNT-3 lacks a TM,

leading to a diffused expression in the axons. Biochemical evidence showed that SNT-3 interacts with plasma membrane, and the C2 domains have a similar Ca^{2+}-binding affinity with SNT-1. These findings raised new questions of how SNT-3 exerts a Ca^{2+} sensor function, and why SNT-3 is not functional when SNT-1 exists.

Disclosures: L. Li: None. **H. Liu:** None. **Y. Yu:** None. **J. Tang:** None. **Z. Hu:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 121.05/B73

Topic: B.05. Neurotransmitter Release

Support: KOREA-NSF2017M3C7A1048268
KOREA-NSF2017R1A2B4007019
KOREA-NSF2018R1A6A1A03025124

Title: SV2A is essential for distinct synaptic vesicle recycling in excitatory and inhibitory nerve terminals

Authors: *W. LEE[1], S. HWANG[1], S. KOH[1], S. KIM[2];
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Abstract: Proper brain function requires a balance between excitatory and inhibitory neuronal activity. This balance, which is disrupted in various neural disorders, ultimately depends on the functional properties of both excitatory and inhibitory neurons; however, how the physiological properties of presynaptic terminals are controlled in these neurons is largely unknown. In this study, we generated pHluorin-conjugated, synaptic vesicle-specific tracers that are preferentially expressed in excitatory or inhibitory nerve terminals. We found that synaptic vesicle recycling is ~1.8-fold slower in inhibitory nerve terminals than excitatory nerve terminals, resulting in reduced efficacy of synaptic vesicle trafficking in inhibitory presynaptic terminals during repetitive activities. Interestingly, this relative difference in trafficking efficiency is mediated by synaptic vesicle protein 2A (SV2A), which is more highly expressed in inhibitory synapses and interacts in a phosphorylation-dependent manner with synaptotagmin I. These findings indicate that SV2A coordinates distinct properties of synaptic vesicle trafficking between excitatory and inhibitory synapses.

Disclosures: W. Lee: None. **S. Hwang:** None. **S. Koh:** None. **S. Kim:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 121.06/B74

Topic: B.05. Neurotransmitter Release

Support: EY10542
RPB Senior Scientific Investigator Award
EY012128
Frederic B Asche Foundation
F30EY28848

Title: Multiquantal release from rod ribbons is facilitated by syntaxin3B

Authors: *C. L. HAYS[1], J. GRASSMEYER[2], R. JANZ[4], R. HEIDELBERGER[5], W. B. THORESON[3];
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Abstract: Ribbon synapses, including those of hair cells and retinal bipolar cells, show a remarkable capability for synchronous release of multiple vesicles. We examined the frequency of synchronous multiquantal release in ribbon-bearing rod photoreceptor cells and tested the hypothesis that multiquantal (MQ) release involves homotypic fusion among neighboring vesicles on synaptic ribbons promoted by the SNARE protein syntaxin3B. To measure release presynaptically in salamander rods, we recorded anion currents that accompany glutamate transporter activity ($I_{A(Glu)}$). To amplify $I_{A(Glu)}$, we used a pipette solution with SCN^- as the principal anion. The control pipette solution contained 5 mM EGTA as the Ca^{2+} buffer; we also tested 0.05 mM EGTA and 5 mM BAPTA. For recordings from horizontal cells, we used a CsGluconate-based pipette solution. In rod/horizontal cell pairs, spontaneous $I_{A(Glu)}$ events recorded presynaptically were correlated in amplitude with simultaneous miniature post-synaptic currents (r^2=0.25±0.05, N=7 pairs) indicating that glutamate release can be detected both pre- and post-synaptically. The quantal content of spontaneous $I_{A(Glu)}$ and mEPSCs averaged 1.57±0.08 (N=7) and 1.46±0.12 (N=5), respectively, indicating that many rod release events are multiquantal. The frequency of MQ events (>15 pA) did not differ with 0.5 mM and 5 mM EGTA but was almost completely abolished after 4 min with 10 mM BAPTA ($p<0.0001$). This suggests that MQ fusion occurs within 100 nm of Ca^{2+} channels beneath synaptic ribbons. The overall frequency of large and small events was reduced by 10 mM BAPTA and slightly reduced by 5 mM EGTA relative to 0.05 mM EGTA, consistent with a combination of ribbon and non-ribbon release of uniquantal events. Damaging synaptic ribbons by fluorophore-assisted laser

inactivation of a FITC-RIBEYE binding peptide introduced through the patch pipette reduced the frequency of spontaneous MQ events (N=7, p=0.02) as well as the amplitude of $I_{A(Glu)}$ evoked by 2 ms steps to -10 mV (p=0.0008). These changes were not seen with a scrambled control peptide. Introduction of a peptide that mimics the SNARE-binding region of syntaxin 3B speeded time-dependent decline of MQ events. 15 min after patch rupture, the proportion of large (>15 pA) to small events declined by 87±6%, significantly more than with a scrambled control peptide (57±10%, p =0.03). The overall frequency of spontaneous currents was not reduced by the syntaxin3B peptide (p = 0.88). Our results suggest that a substantial fraction of spontaneous release from rods is MQ, occurring preferentially at ribbons and promoted by syntaxin3B, perhaps by facilitating homotypic fusion of vesicles.

Disclosures: C.L. Hays: None. **J. Grassmeyer:** None. **R. Janz:** None. **R. Heidelberger:** None. **W.B. Thoreson:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 121.07/B75

Topic: B.05. Neurotransmitter Release

Support: NIH R21NS085665
UIUC funding

Title: Nano-resolved study of acetylcholine neurotransmission on single B1/B2 *Aplysia* cells

Authors: *S. N. PHAM, R. CHEN, S. E. MURPHY, C. H. OH, J. V. SWEEDLER, M. SHEN; Chem., Univ. of Illinois At Urbana-Champaign, Urbana, IL

Abstract: Acetylcholine is an important neurotransmitter that has been found to be linked to learning, memory, and neural plasticity, which leads this molecule to be important in understanding neurodegenerative diseases such as Alzheimer's disease. Because of that, the rationale behind this study is to be able to analyze acetylcholine release with high spatiotemporal precision. Currently, there are electrochemical methods that are able to detect redox active neurotransmitters such as dopamine and serotonin using carbon fiber electrodes. However, because acetylcholine is a non-redox active molecule, many of the electrochemical studies on it use complex enzyme-bound electrodes. Here, we present a study that uses a nano-interface between two immiscible electrolyte solutions (nano-ITIES) electrode1 that is able to selectively detect acetylcholine with nanometer resolution; these electrodes can be made to be 5-500 nm in radius and have a fairly simple and robust design principle that involves pulling capillaries and adding an immiscible liquid to the tip. Using scanning electrochemical microscopy, we are able to place the electrode within a nanometer-scale distance from the surface of a neuron. For this

study, Aplysia californica B1/B2 neurons were cultured on a poly-L-Lysine coated silicon wafer. As these neurons are known to be cholinergic, they are an ideal initial model system. Using feedback mode and chronoamperometry, we were able to see acetylcholine release upon potassium stimulation at the nano-tip placed near the surface of the cell membrane. We see picoamp currents, and high (>20:1) signal to noise ratios upon stimulation. To summarize the results, we putatively determine, based on previous work,2 that these are vesicular exocytosis events which have an average t1/2 of 9.2 ± 4.9 s and an average total release of 3.7 x 107 ± 2.4 107 molecules, which corresponds to a concentration of 6.5 ± 1.9 µM. We are coupling this exploratory electrochemical data with mass spectrometry data, specifically using capillary electrophoresis-electrospray ionization-mass spectrometry to provide a second analytical control for acetylcholine detection. Our current efforts are to qualitatively detect whether acetylcholine is present in the B1/B2 single neurons, which the data supports. The significance of our results demonstrates the design of a useful nanometer spatially resolved and millisecond temporal resolved electrochemical platform for detecting acetylcholine on single cells.
Shen, M.*; Colombo, M. L. Analytical Methods, 2015, 7, 7095-7105.
Welle, T. M.; Alanis, K.; Colombo, M. L.; Sweedler, J. V.; Shen, M.* Chem. Sci., 2018, 9, 4937-4941.

Disclosures: **S.N. Pham:** None. **R. Chen:** None. **S.E. Murphy:** None. **C.H. Oh:** None. **J.V. Sweedler:** None. **M. Shen:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 121.08/B76

Topic: B.05. Neurotransmitter Release

Support: UQ Foundation Research Excellence Awards
NARSAD Young Investigator Grant 24980
NIH/NEI R21 1R21EY029450-01

Title: A dual role for the UNC-13 M domain in Ca^{2+}-triggered neurotransmitter release

Authors: H. LIU[1], L. LI[1], Y. YU[1], J. TANG[1], S. SHEORAN[2], J. E. RICHMOND[2], *Z. HU[1]; [1]The Univ. of Queensland, Queensland Brain Inst., Brisbane, Australia; [2]Biolog Sci., Univ. Illinois Chicago, Chicago, IL

Abstract: Synaptic vesicle (SV) priming and fusion require the Munc13 family of proteins, several of which (e.g. Munc13-1, Munc13-2, and ubMunc13-2) have been shown to be essential in regulating short-term synaptic plasticity. However, the underlying molecular mechanisms remain unclear. The nematode *C. elegans* expresses two UNC-13 isoforms, UNC-13L and UNC-

13S (also called UNC-13MR). Here we report a novel dual function of the N-terminal M domain in *C. elegans* UNC-13MR, a Munc13-2 ortholog. Deleting the M domain in UNC-13MR led to a significant increase in tonic and evoked neurotransmitter release, as well as the size of the readily releasable vesicle pool, revealing an inhibitory function of the M domain in SV priming and fusion. The inhibitory effects of the M domain were eliminated in the absence of the C1 and C2B domains. This suggests that the M domain inhibits the C1-C2B module during synaptic transmission. Interestingly, we found that the M domain directly promoted SV fusion when fused to the MUNC2C fragment, which has been shown to be the minimal region required for priming and fusion. These findings reveal that the M domain regulates synaptic transmission via dual modes. However, it is still unclear how it switches between these modes under physiological conditions.

Disclosures: H. Liu: None. **L. Li:** None. **Y. Yu:** None. **J. Tang:** None. **S. Sheoran:** None. **J.E. Richmond:** None. **Z. Hu:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

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Program #/Poster #: 121.09/B77

Topic: B.05. Neurotransmitter Release

Support: DFG Individual research grant (LU 1819/2-1)

Title: Munc13 critically affects the architecture of the presynaptic terminal

Authors: C. PAPANTONIOU[1], C. IMIG[3], N. BROSE[4], W. BAUMEISTER[2], B. H. COOPER[4], *V. LUCIC[2];
[2]Mol. Structural Biol., [1]Max Planck Inst. of Biochem., Martinsried, Germany; [3]Mol. Neurobio., [4]Max Planck Inst. of Exptl. Med., Goettingen, Germany

Abstract: Synaptic neurotransmitter release is mediated by a complex and highly regulated molecular machinery. Munc13 protein family members orchestrate SNARE complex assembly and are critical for rendering synaptic vesicles functionally release-competent for calcium-evoked fusion during synaptic activity. Previous electron tomography analyses of synapses from Munc13-deficient mutants using high-pressure freezing fixation and automated freeze substitution on plastic sections indicated that in this preparation Munc13s have an essential role in attaching or “docking” vesicles to the plasma membrane and that therefore synaptic vesicle docking and priming may represent morphological and functional manifestations of the same process. To address the question of how Munc13s execute their function on the molecular level, we established a methodological workflow that allows us to isolate synaptosomes from hippocampal organotypic slice cultures of Munc13-1 and -2 double KO mice and image them by

cryo-electron tomography, a method that utilizes faithful sample preservation and direct imaging of fully hydrated unlabeled biological material to provide an accurate representation of molecular architecture of cells at a single nanometer resolution. The automated detection and analysis of synaptic vesicle-bound complexes showed that genetic ablation of Munc13-1 and -2 proteins increases the distance between the membrane-proximal vesicles and the plasma membrane without changing the vesicle number and has profound effects on the properties of tethers that link synaptic vesicles to the plasma membrane.

Disclosures: C. Papantoniou: None. **C. Imig:** None. **N. Brose:** None. **W. Baumeister:** None. **B.H. Cooper:** None. **V. Lucic:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 121.10/B78

Topic: B.05. Neurotransmitter Release

Support: NIH R56MH107387

Title: SphK1/S1P pathway is important for synaptic vesicle endocytosis

Authors: *Z.-J. JIANG, T. L. DELANEY, L.-W. GONG;
Univ. of Illinois at Chicago, Chicago, IL

Abstract: Sphingosine-1-phosphate (S1P), a bioactive phospholipid, is implied to be involved in many neurological diseases such as Alzheimer's disease and multiple sclerosis. SphK1, a major kinase responsible for S1P production in the brain, is concentrated within presynaptic terminals and important for synaptic transmission. However, it remains largely undefined whether the SphK1/S1P pathway is important for synaptic vesicle endocytosis, a process that is critical to maintain synaptic transmission by generating synaptic vesicles locally within the synaptic terminals. Using pHluorin-based live-cell imaging, we studied the potential roles of SphK1/S1P pathway in synaptic vesicle recycling in neurons in culture by examining the effects of $SphK1^{G82D}$ ($SphK1^{DN}$), a dominant negative catalytic inactive mutant of SphK1. Consistent with previous reports, we observed a defect in exocytosis induced by $SphK1^{DN}$, suggesting a role of SphK1/S1P in exocytosis. Interestingly, $SphK1^{DN}$ also slowed down the signal decay of pHluorin tagged to synaptophysin, indicating a critical role of SphK1/S1P pathway in synaptic vesicle endocytosis. The importance of SphK1/S1P pathway in endocytosis is further validated by the fact that $SphK1^{DN}$ has no effect on the reacidification rate of newly formed endocytic vesicles. Furthermore, by detecting single vesicle endocytosis in chromaffin cells using cell-attached capacitance measurements, we found that $SphK1^{DN}$ increased the duration of vesicle fission, a rate-limiting step in endocytosis, indicating an importance of SphK1/S1P pathway in

vesicle fission during endocytosis. Taken together, our study supports a critical role of SphK1/S1P pathway in synaptic vesicle endocytosis. Based on the close relationship between malfunctions in synaptic vesicle endocytosis and neurological disorders, our data may provide new insights into pathological mechanisms of neurological disorders related to the SphK1/S1P pathway.

Disclosures: Z. Jiang: None. **T.L. Delaney:** None. **L. Gong:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

Location: Hall A

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Program #/Poster #: 121.11/B79

Topic: B.05. Neurotransmitter Release

Support: Deutsche Forschungsgemeinschaft (DFG), CRC 894

Title: Functional analysis of calcium dependent activator protein for secretion 1 and 2 differential subcellular localization

Authors: *A. STAUDT[1], A. H. SHAIB[2], O. RATAI[1], A. SHAABAN[1], H. BZEIH[1], R. MOHRMANN[3], J. RETTIG[1], U. BECHERER[1];
[1]Ctr. for Integrative Physiol. and Mol. Med., Saarland Univ., Homburg, Germany; [2]Max Planck Inst. for Exptl. Med., Göttingen, Germany; [3]Inst. für Physiologie, Otto-von-Guericke Univ. Magdeburg, Magdeburg, Germany

Abstract: The calcium-dependent activator protein for secretion (CAPS) is an established priming factor for large dense core vesicles (LDCVs) and has been reported to prime synaptic vesicles (SVs) as well. It is a multi-domain protein consisting of two family members CAPS1 and CAPS2. Recently, we showed that in dorsal root ganglion (DRG) neurons CAPS1 exclusively mediates SV exocytosis, while CAPS2 only promote neuropeptide release from LDCVs. We further showed that their differential function is due to distinct localization. In fact, CAPS1 is highly enriched at synapses while CAPS2 has a more diffuse mainly somatic localization. To establish and maintain this differential localization, the subcellular transport mechanism of both CAPS paralogs must differ and be tightly controlled. In the peripheral nervous system, cell soma and synaptic contacts are separated by a great distance, which poses a challenge for synaptic targeting of cytoplasmic proteins. Active zone proteins overcome this problem by their association with so-called piccolo/bassoon transport vesicles. We propose that CAPS1 but not CAPS2 is able to bind to these vesicles to be directed to synapses. We investigated this question by targeted mutagenesis of CAPS2. We verified that our mutant CAPS2/CAPS1 was correctly expressed and functional. Using immunocytochemistry together with confocal microscopy we could show that the CAPS2/CAPS1 chimera protein was re-

localized from the soma to synapses. With this approach we identified a specific domain responsible for CAPS1 synaptic localization.

Disclosures: **A. Staudt:** A. Employment/Salary (full or part-time):; Saarland University. **A.H. Shaib:** None. **O. Ratai:** None. **A. Shaaban:** None. **H. Bzeih:** None. **R. Mohrmann:** None. **J. Rettig:** None. **U. Becherer:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 121.12/B80

Topic: B.05. Neurotransmitter Release

Support: SFB958
TRR186

Title: Analysis of essential and modulatory domains of Syntaxin-1 in murine hippocampal synapses

Authors: *G. VARDAR, M. BROCKMANN, A. SALAZAR LÁZARO, M. WEBER-BOYVAT, T. TRIMBUCH, C. ROSENMUND;
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Abstract: Fusion of the neurotransmitter filled vesicles with neuronal plasma membrane is commenced and accomplished by the intricate interactions of the components of the vesicular release machinery. Not only the vesicle fusion process itself, but also the preceding steps, vesicle docking and priming, are carried through by the sequential events of these interactions. Among the proteins involved in all the steps leading to neurotransmitter release, it is the SNARE complex which form the backbone of these sequential reactions. Furthermore, a great body of data points towards the binary complex composed of the SNARE protein Syntaxin-1 (Stx1 collectively refers to Stx1A and Stx1B) and its close binding partner Munc18-1 as one of the initiation factors of the assembly of the vesicular release machinery. Crystallographic investigations on this binary complex have revealed two distinct binding sites on Stx1 for its interaction with Munc18-1: a short fragment at its N-terminal end, called N-peptide, and the H_{abc}-domain together with the SNARE motif when in closed conformation. Powerful evidence driven from in vitro studies show that both N-peptide binding and H_{abc}-domain binding modes are essential for an unimpaired Stx1−Munc18-1 complex function. On the other hand, a consensus about whether these binding modes occur collaboratively or in isolation from one another and also a consensus about whether they perform distinct and indispensable functions in the sequential events leading to neurotransmitter release in vivo are still not achieved. To address these issues, we used our Stx1A/1B double knockout (DKO) mouse model system in which we

can provide a complete loss of vesicle priming and fusion, and a severe impairment of vesicle docking in hippocampal neurons. By means of a rescue approach, we determined the electrophysiological characteristics of vesicle priming and fusion in Stx1A/1B DKO neurons lentivirally expressing Stx1A mutants which lack either N-peptide or H_{abc}-domain, or which are rendered locked in an open conformation. Even though neither N-peptide nor H_{abc}-domain of Stx1A has proven to be absolutely required for vesicle priming and vesicle fusion per se, we could show independent modulatory effects of these two Munc18-1 binding modes on neurotransmitter release.

Disclosures: G. Vardar: None. **A. Salazar Lázaro:** None. **M. Weber-Boyvat:** None. **T. Trimbuch:** None. **C. Rosenmund:** None. **M. Brockmann:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

Location: Hall A

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Topic: B.05. Neurotransmitter Release

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Title: Exocytosis of GABAergic synaptic vesicles tracked by real-time three-dimensional microscopy

Authors: *C. PARK[1], C. FAN[1], K. PARK[1], C. LEE[3], H. PARK[1,2];
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Abstract: The dynamic balance between excitation and inhibition in synaptic transmission is necessary for proper functions of the brain. Inhibitory synaptic transmission, which is mediated mainly by GABA and glycine, regulates the neuronal excitability. Inhibitory synaptic transmission is reported to considerably differ from excitatory synaptic transmission. However, the inhibitory synaptic transmission compared to the excitatory synaptic transmission remains poorly understood. In particular, the precise and dynamic motion of synaptic vesicles containing inhibitory neurotransmitters up to the moment of exocytosis remains unknown. In this study, we investigated the dynamics of GABAergic synaptic vesicles in mature rat hippocampal neurons by real-time three-dimensional tracking of single synaptic vesicles till the moment of exocytosis. To specifically label single GABAergic vesicles, we used quantum dot-conjugated antibodies

against the luminal domain of vesicular GABA transporter (VGAT), co-labeling inhibitory presynaptic terminals with CypHer5E-tagged anti-VGAT. Using a partial quenching of the quantum dot signal as a reporter of kiss-and-run fusion, we found that the prevalence of kiss-and-run fusion in the GABAergic vesicles was distinct from that of synaptic vesicles labeled by synaptotagmin-1 (Syt1) antibodies conjugated with quantum dots. The net displacement of GABAergic synaptic vesicles undergoing fusion was significantly different from that of Syt1-labeled synaptic vesicles. Based on the results, we suggest that GABAergic synaptic vesicles may have dynamics distinct from Syt1-labeled synaptic vesicles.

Disclosures: **C. Park:** None. **C. Fan:** None. **K. Park:** None. **C. Lee:** None. **H. Park:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 121.14/B82

Topic: B.05. Neurotransmitter Release

Support: 1R01 NS105810-01A1
1DP2NS111133-01
Alfred P. Sloan Foundation
Johns Hopkins Startup Fund
McKnight Foundation

Title: The organization and occupancy of synaptic vesicle fusion sites

Authors: G. KUSICK, M. CHIN, ***S. WATANABE**;
Johns Hopkins Univ., Baltimore, MD

Abstract: Synaptic vesicles fuse with the plasma membrane to release neurotransmitter following an action potential, after which new vesicles must ‘dock’ to refill vacated release sites. How many vesicles can fuse at a single active zone, where they fuse within the active zone, and how quickly they are replaced with new vesicles is not well-established. To capture synaptic vesicle exocytosis at cultured mouse hippocampal synapses, we induced single action potentials by electrical field stimulation then subjected neurons to high-pressure freezing to examine their morphology by electron microscopy. During synchronous release, multiple vesicles can fuse at a single active zone; this multivesicular release is augmented by increasing the extracellular calcium concentration. Synchronous fusions are distributed throughout the active zone, whereas asynchronous fusions are biased toward the center of the active zone. Immediately after stimulation, the total number of docked vesicles across all synapses decreases by ~1/3. Between 8 and 14 ms, new vesicles are recruited to the plasma membrane and fully replenish the docked pool, but docking of these vesicles is transient and they either undock or fuse within 100 ms.

These results demonstrate that recruitment of synaptic vesicles to release sites is rapid and reversible.

Disclosures: S. Watanabe: None. **G. Kusick:** None. **M. Chin:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 121.15/B83

Topic: B.05. Neurotransmitter Release

Support: American Heart Association [17GRNT33661156]

Title: The serotonin transporter modulates the number and quantal size of vesicular release events in sympathoadrenal chromaffin cells

Authors: R. L. BRINDLEY, ***K. P. CURRIE**;
Cooper Med. Sch. of Rowan Univ., Camden, NJ

Abstract: The serotonin (5-HT) transporter (SERT) is an important regulator of 5-HT signaling in the CNS. SERT is also highly expressed in adrenal chromaffin cells which comprise the neuroendocrine arm of the sympathetic nervous system. Based on our current and previous work we hypothesize that adrenal chromaffin cells are a previously unrecognized peripheral hub for serotonergic control of the sympathoadrenal stress response. To test this hypothesis we used carbon fiber amperometry to investigate how 5-HT / SERT modulates catecholamine secretion from isolated mouse adrenal chromaffin cells. We show that 5-HT_{1A} receptors inhibited catecholamine secretion by reducing the number of evoked vesicular release events. This pathway was enhanced by blocking SERT activity using escitalopram (an SSRI antidepressant) or in cells isolated from $SERT^{-/-}$ mice. SERT had a second distinct mechanism of action independent of 5-HT_{1A} receptor activation. The half-width (duration) and charge (amount of oxidizable transmitter released) of individual amperometric spikes (vesicular fusion events) was significantly smaller (~35%) in $SERT^{-/-}$ cells compared to wild-type cells. The same effect was recapitulated in wild-type cells by *in vitro* pharmacological block of SERT (~72hrs culture with escitalopram) or by restricting the amount of extracellular 5-HT available for uptake (~72hrs culture dialyzed serum media). HPLC showed the 5-HT content of adrenal glands from wild-type mice only accounts for a small fraction (~0.13%) of the total monoamines, which are predominantly epinephrine and norepinephrine. Although the 5-HT content was significantly reduced (50-80%) in glands isolated from $SERT^{-/-}$ mice, the catecholamine content was unaltered making it unlikely that the reduced size of quantal release events (35% decrease of amperometric spike charge) is simply due to reduced vesicular monoamine content. Ongoing work will dissect

the underlying mechanisms by which adrenal SERT / 5-HT regulate catecholamine secretion and hence the sympathoadrenal stress response.

Disclosures: R.L. Brindley: None. **K.P. Currie:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

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Program #/Poster #: 121.16/B84

Topic: B.05. Neurotransmitter Release

Support: T32-GM007767
NIH Grant GM111997

Title: Synaptotagmin-7 endows a population of dense core vesicles with distinct calcium sensing and fusion properties

Authors: *A. CHAPMAN-MORALES[1], M. BENDAHMANE[1], A. J. B. KREUTZBERGER[2], N. SCHENK[1], V. KIESSLING[2], D. CASTLE[2], L. TAMM[2], D. R. GIOVANNUCCI[3], P. M. JENKINS[1], A. ANANTHARAM[1];
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Abstract: Synaptotagmin-7 (Syt7) is one of two major calcium sensors for regulated exocytosis in adrenal chromaffin cells. Its high sensitivity allows tunable secretory responses to a range of stimuli that result in graded increases in intracellular calcium. Despite the undoubted importance of Syt7, questions remain as to whether the protein operates from the vesicle or plasma membranes and to what degree the functions of chromaffin cell Syts are redundant. Here, these issues were examined using two distinct experimental preparations - primary mouse chromaffin cells lacking endogenous Syt7 and a reconstitution assay employing cell-derived granules expressing either Syt7 or Syt1. First, we find that endogenous Syt7 is punctate in appearance, consistent with its sorting to organelles. Antibody-based staining reveals it to be co-localized with plasminogen activator inhibitor 1 (PAI1) - a protein of the vesicle dense core - but rarely with Syt1. Functionally, mouse chromaffin cells lacking Syt7 exhibit properties that readily distinguish them from WT cells. For example, lumenal cargo proteins are released at faster rates from Syt7 KO cells than WT cells. KO cells also exhibit deficits in fusion efficacy, both in response to elevated KCl depolarization and cholinergic stimulation. To further distinguish between the roles of Syt7 and Syt1 in fusion, purified dense core vesicles expressing only one of the two proteins were triggered to fuse on reconstituted planar supported bilayers bearing t-SNAREs. These studies demonstrate that Syt7 confers substantially greater calcium sensitivity to granule fusion than Syt1 and slows that rate at which cargos are released, just as in primary cells

employing overexpressed Syts. By virtue of its sorting and biochemistry, Syt7 serves unique roles in the biology of the chromaffin cell secretory response in ways that distinguish it from Syt1.

Disclosures: A. Chapman-Morales: None. **M. Bendahmane:** None. **A.J.B. Kreutzberger:** None. **N. Schenk:** None. **V. Kiessling:** None. **D. Castle:** None. **L. Tamm:** None. **D.R. Giovannucci:** None. **P.M. Jenkins:** None. **A. Anantharam:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 121.17/B85

Topic: B.05. Neurotransmitter Release

Support: ISF 1427/12

Title: Activity dependent modulation of synaptic transmission and of presynaptic calcium dynamics by α-synuclein

Authors: A. STAVSKY[1], M. ATIAS[2], J. KAHN[2], ***D. GITLER**[1];
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Abstract: Recent work suggests that α-synuclein (α-syn) is a physiologic attenuator of neurotransmission and that it exhibits functional interactions with key presynaptic proteins (synapsin, VAMP2) known to participate in different segments of the synaptic vesicle (SV) cycle. Nevertheless, the specific role of α-syn in the SV cycle and the mechanism by which it constrains it remain unknown, partly due to the fact that deletion of α-syn in mice has been reported to produce only mild effects. By varying stimulation protocols, we report that contrary to previous findings, deletion of α-syn and α-syn overexpression produce opposite but varying effects on SV recycling as imaged using synaptophysin-pHluorin. Furthermore, using genetically encoded calcium indicators, we find that the expression level of α-syn alters the magnitude of calcium transients in both the cytosolic and mitochondrial compartments of the presynaptic terminal. Finally, we report that altering α-syn expression changes the resting distribution of SVs within the terminal. Our findings thus support an activity and calcium-dependent role for α-syn in modulating neurotransmitter release and in defining the structure of the presynaptic terminal.

Disclosures: A. Stavsky: None. **M. Atias:** None. **J. Kahn:** None. **D. Gitler:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 121.18/B86

Topic: B.05. Neurotransmitter Release

Support: NIH Grant 5R25GM096161

Title: VIP immunohistochemical reactivity in rat dorsal horn is increased by prolonged vaginocervical stimulation (VCS) that "exhausts" VCS-produced analgesia

Authors: *J. A. RIVERA, E. VENTURA-AQUINO, **B. R. KOMISARUK**;
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Abstract: In previous studies in rats, 1) vaginocervical mechanostimulation (VCS) produced analgesia, 2) the analgesia was "exhausted" (i.e., habituated) by prolonged (1 hour) VCS; vasoactive intestinal peptide (VIP) is: 3) concentrated in rat sacral spinal cord, 4) released into superfusates of spinal cord during VCS or 5) electrical stimulation of the pelvic nerve; 6) the pelvic nerve provides vaginocervical afferents; 7) intrathecal administration of VIP produces analgesia. Presently, we utilized immunohistochemical reactivity of VIP to visualize the levels of VIP in the sacral-3 dorsal horn after VCS-induced exhaustion of analgesia, compared to a no-VCS control condition. Methods: Sprague-Dawley rats (150-190g bw) were ovariectomized and treated with estradiol benzoate (5ug/d sc x 2; n=6) or sesame oil (n=6). VCS (100g) was applied for up to one hour and tail flick latency (TFL) to radiant heat was measured each 10 min as the indicator of analgesia. Rats were sacrificed by ketamine/xylazine overdose, the experimental group upon analgesia exhaustion, the control group upon neither stimulation nor testing. Rats were perfused with buffered formalin and the spinal cords removed by spine/spinal cord transection plus air pressure. Immunohistochemistry (40um sections) using VIP antibody was prepared by FD Neurotechnologies. Image analysis used a Nikon microscope with NIS BRS 11.00 software. We constructed ROIs of laminae 1-2 for each image, set a constant brightness (density) threshold, omitted size or circularity criteria, and measured the proportion of the immunoreactive area to the entire ROI. Results: Initial unstimulated baseline TFL was 3.6±0.5sec, which increased significantly to 7.6±0.6sec during VCS ($t=-1.85$, $p<0.05$). After continuous VCS to analgesia exhaustion (approx 1 hour), as designed, the TFL had returned to 2.7±0.2sec (NS versus initial TFL). The VCS-to-exhaustion group showed a significantly higher VIP immunoreactive proportion of the ROI (0.30±0.1) than the control group (0.08±0.1; $t=-2.8$, $p<0.01$). VIP immunoreactivity between the estrogen and oil control groups was NS ($t=-0.03$, $p=0.5$). While depletion of the primary afferent neuropeptide VIP in laminae 1-2 could be expected to accompany exhaustion of the analgesic response to the peptide, the present finding of increased VIP immunoreactivity could be a consequence of: a) accumulation of VIP in the

synaptic space prior to enzymatic degradation and/or clearing, b) released VIP being bound to the postsynaptic membranes, c) internalization of VIP into postsynaptic neurons, d) relative inaccessibility to antibody of VIP in presynaptic vesicles yielding apparently low levels prior to VCS.

Disclosures: J.A. Rivera: None. **E. Ventura-Aquino:** None. **B.R. Komisaruk:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 121.19/B87

Topic: B.05. Neurotransmitter Release

Support: NIH Grant R01DC016324

Title: Presynaptic Na^+ regulates synaptic vesicle recycling at the calyx of Held synapse

Authors: Y. ZHU, D. LI, *H. HUANG;
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Abstract: Retrieval of synaptic vesicles via endocytosis is essential for maintaining repetitive synaptic transmission. At the calyx of Held, a giant glutamatergic synapse in the auditory brainstem that fires continuous action potentials at high-frequency up to several hundred hertz, efficient vesicle recycling is especially crucial to meet the demand for intense synaptic transmission. Using two-photon Na^+ imaging, we found the Na^+ accumulated robustly at the calyceal terminals during high-frequency firing. By directly measuring the membrane capacitance at calyx of Held, we found that increase of presynaptic Na^+ concentration facilitated both slow and rapid forms of endocytosis, as well as endocytosis overshoot, while did not affect the readily releasable pool size, calcium influx, or exocytosis. To examine whether this facilitation is related to the Na^+ dependent vesicular content change, we dialyzed the calyceal terminal with different concentrations of glutamate or blocked the vesicular glutamate uptake with bafilomycin and found the rate of endocytosis was not affected by regulating the glutamate contents. Since endocytosis is critically dependent on intracellular Ca^{2+} and the activity of Na^+/Ca^{2+} exchanger (NCX) might have been altered when we changed the Na^+ gradient, we then lowered the extracellular Na^+ concentration, which inhibiting the NCX function and mimicking the concentration gradient change induced by increased intracellular Na^+ concentration. However, no differences in endocytosis rate were observed. Therefore, during high-frequency synaptic transmission, when large amounts of synaptic vesicles are fused, Na^+ accumulated in terminals, facilitated vesicle recycling and sustained reliable synaptic transmission.

Disclosures: Y. Zhu: None. **D. Li:** None. **H. Huang:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 121.20/B88

Topic: B.06. Synaptic Transmission

Support: NIH P20GM103554
UNR VPRI Research Enhancement Grant

Title: Presynaptic loss of DRP1 affects cytosolic calcium load and inhibits endocytosis at the calyx of Held

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[3]Physiol. and Cell Biol., Univ. of Nevada, Reno Sch. of Med., Reno, NV

Abstract: Dynamin-Related Protein 1 (DRP1) is essential for mitochondrial fission, autophagy and localization, as well as for the regulation of synaptic vesicle (SV) recycling. However, the mechanisms by which DRP1 contributes to the latter process remain incompletely understood. Our recently published work shows that impaired transmission is due in part to increased cytosolic Ca^{2+} load during activity. Alternatively, other prior work suggests that DRP1 is involved in SV endocytosis directly. Here, we sought to determine whether presynaptic mitochondrial Ca^{2+} uptake, and/or SV endocytosis are altered due to DRP1 loss from the giant calyx of Held synapse.
DRP1 was selectively eliminated in the presynaptic terminal by injecting Floxed-DRP1 (DRP1fl/fl) mice with a recombinant adeno-associated virus expressing Cre recombinase and GFP reporter, at postnatal day 1 (P1). To monitor Ca^{2+} flux in the intact presynaptic terminal, a genetically encoded cytosolic calcium indicator (jRGECO1a) or a mitochondrial calcium indicator (mito-LAR-GECO1.2) were co-injected. Fluorescence changes were monitored in response to electrical stimulation.
Activity-dependent increases in cytoplasmic calcium were larger in DRP1-KO mice versus controls, with slower clearance kinetics. Surprisingly, mitochondrial calcium following stimulation was also significantly increased in DRP1-KO terminals versus controls. Whereas mitochondria were able to retain calcium after prolonged stimulus (400 Hz, 5 seconds) in control terminals, mitochondrial Ca^{2+} dispersed immediately following stimulus cessation in DRP1-KO terminals. Defective mitochondrial calcium handling contributes to defective SV recycling in DRP-KO, but the mechanism is unclear.
We tested whether DRP1 has a direct role in SV retrieval by measuring presynaptic capacitance changes with direct whole-cell electrophysiology recordings at the calyx terminal. While Ca^{2+} influx and exocytosis were largely normal in DRP1-KO terminals, we observed a near complete

lack of SV endocytosis compared to WT mice ($P<0.05$). This result implicates DRP1 function in a two-step model for SV membrane scission during compensatory endocytosis.
Our data indicate DRP1 supports neurotransmission at the mouse calyx of Held through multifaceted functions: impacting mitochondrial calcium buffering via a novel mechanism, and directly mediating SV retrieval. Further studies into the role of this protein in pathological neurotransmission are necessary.

Disclosures: K.D. Reihl: None. **M. Singh:** None. **R.B. Renden:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 121.21/B89

Topic: B.05. Neurotransmitter Release

Support: NIH Grant 5R01DC004274

Title: Synaptic vesicle endocytosis, spontaneous release and evoked exocytosis at mature glutamatergic and glycinergic synapses

Authors: *A. A. DAGOSTIN[1], H. P. VON GERSDORFF[2];
[1]Vollum Inst., Oregon Hlth. and Sci. Univ., Portland, OR; [2]Vollum Inst., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: The rapid recycling of vesicles in the presynaptic terminal during exocytosis, endocytosis and vesicle trafficking at the active zone maintains proper synaptic function. Vesicle reuptake occurs via clathrin-mediated and clathrin-independent endocytosis, and both rely on dynamin for the final step of membrane fission and vesicle reformation. Not only membrane recycling but also active zone clearance depends on efficient membrane reuptake, making dynamin a key player in continuous neurotransmission, especially at synapses that fire at high frequencies. To study the disruption of endocytosis we blocked dynamin with Dyngo 4A (30 µM or 60 µM) and recorded glutamatergic and glycinergic currents from neurons of MNTB or LSO in brainstem slices from postnatal day 20-25 mice (C57BL/6J). We evoked PSCs by electrically stimulating the afferent fibers of each nucleus at 300 Hz (33-35°C; 1.2mM $[Ca^{2+}]_e$).To ensure proper delivery of Dyngo 4A to the nerve terminals, we sonicated the solution and incubated the slices with Dyngo 4A for at least 20 minutes prior to recordings. We observed a striking effect of Dyngo 4A on MNTB synapses: a large increase in the frequency of the spontaneous EPSCS (sEPSCs; 3.4±0.7 Hz to 38.6±6.8 Hz), indicating the drug was acting on the presynaptic terminal. We also observed a reduction in the evoked EPSC amplitude (~40%), sometimes leading to failures at the end of the stimulation protocol, which may be explained by a reduction in evoked vesicle pool size (smaller readily releasable pool - RRP). In the LSO, we observed a

strong reduction on glycinergic eIPSCs (~70%), without failures at 300Hz stimulation, indicating a reduced RRP. The paired-pulse ratio increased (suggesting a reduction in release probability) and the short-term plasticity (STP) of the eIPSCs trains was altered with Dyngo 4A, indicating a role for dynamin in vesicle recycling and STP at the LSO. For both synapses, under the same paradigms, we show that dynamin is necessary to maintain the RRP size and that it maintains a reliable transmission in MNTB during high-frequency afferent fiber activity. In LSO synapses, dynamin activity regulates RRP size and also shapes STP, which ultimately regulates information coding in the postsynaptic neuron.

Disclosures: A.A. Dagostin: None. **H.P. von Gersdorff:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 121.22/B90

Topic: B.05. Neurotransmitter Release

Support: NIH

Title: TRPC5 positively regulates synaptic vesicle endocytosis

Authors: *T. L. DELANEY, Z. JIANG, L.-W. GONG;
Univ. of Illinois at Chicago, Chicago, IL

Abstract: The transient receptor potential canonical 5 (TRPC5), a nonselective Ca^{2+} permeable cation channel, is linked to neurological diseases such as epilepsy and Huntington's disease. TRPC5 is concentrated in presynaptic terminals and interacts with many endocytic proteins. However, it is unclear whether TRPC5 plays a role in synaptic vesicle recycling. Using synaptophysin-pHluorin based live-cell imaging, we examined effects of a well-established dominant negative TRPC5 mutant ($TRPC5^{DN}$) on synaptic vesicle endocytosis in neurons in culture. There was an increase in the duration of the pHluorin signal decay after stimulation, indicating a potential role of TRPC5 in synaptic vesicle endocytosis, considering no change in the acidification rate of endocytic vesicles was observed. Collectively, our data reveals a physiological function of TRPC5 in synaptic vesicle endocytosis.

Disclosures: T.L. Delaney: None. **Z. Jiang:** None. **L. Gong:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 121.23/B91

Topic: B.06. Synaptic Transmission

Support: NIH Grant R21NS104259
NIH Grant T35HL007649
Jack and Francine Levin Yale-at-MBL Student Research Fellowship

Title: The structural dynamics of actin and presynaptic vesicles in a mammalian synapse

Authors: *S. SUBRAMANIAN[1], J. BURTON[1], M. E. GOODRIDGE[1], D. GOMEZ[1], J. SONG[3,4], J. XIA[3,4], L. K. KACZMAREK[2,4], E. A. JONAS[1,4];
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Abstract: The phenomenon of post-tetanic potentiation occurs in part due to the accumulation of a readily releasable pool (RRP) of vesicles following rapid vesicle depletion. Clustering of the neuronal active zone, mediated by the actin cytoskeleton, is inversely correlated with vesicle cycling. Thus, molecular crowding within the active zone may be a key regulator of RRP size. One mechanism by which actin polymerization may be regulated in an activity-dependent manner is via the voltage-gated potassium channel Kv3.3. This channel is expressed in subsets of rapidly firing neurons and influences action potential repolarization. The C-terminal of Kv3.3 interacts with the Arp2/3 complex and the anti-apoptotic protein Hax1 to assemble actin filaments, which in turn impedes N-type inactivation of the channel. Super resolution microscopy shows that either knockout of the Kv3.3 gene or knock-in of a functional Kv3.3 channel mutant that fails to interact with the Arp2/3 complex result in disruption of the actin cytoskeleton in the calyx of Held presynaptic terminal in the auditory brainstem. Thus, Kv3.3 organizes the actin structure of the presynaptic terminal, offering a possible mechanism for the activity-dependent accumulation of vesicles at the active zone. In order to test this hypothesis, we depolarized synapses and visualized the reorganization of the actin cytoskeleton and the presynaptic vesicles in the period following stimulation. We found that stimulation induced a rapid depolymerization in F-actin at the active zone followed by polymerization of actin in a region distal to the active zone 15 seconds later. By 30 seconds, this polymerized actin had consolidated at the active zone, restoring the original actin architecture of the presynaptic terminal. We are now using three-dimensional reconstruction of these synapses to visualize how the changes in the actin architecture facilitate changes in the size and spread of vesicle pools at the active zone. The activity-dependent structural changes in the presynaptic terminal that we

have identified here offer novel insights into the basic mechanisms of synaptic plasticity and neurodegenerative disease as well as directions for future studies.

Disclosures: S. Subramanian: None. **J. Burton:** None. **M.E. Goodridge:** None. **D. Gomez:** None. **J. Song:** None. **J. Xia:** None. **L.K. Kaczmarek:** None. **E.A. Jonas:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 121.24/B92

Topic: B.05. Neurotransmitter Release

Support: Schram-Stiftung T287/25457
Emmy Noether Young Investigator Award MI-1702/1
Synaptic Systems Fellowship

Title: Regulation of vesicle acidification by Rabconnectin3a at the neuronal synapse

Authors: *S. GOWRISANKARAN[1], A. RAIMONDI[2], Z. FARSI[3], N. DE ROUX[4], I. MILOSEVIC[1];
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Abstract: Acidification of vesicles is achieved by vacuolar ATPases (vATPases), a family of proton pumps that controls the pH gradient across organelle membranes. Despite their critical importance at the synapse and in many intracellular trafficking routes, the regulation of vATPase activity is poorly understood. In a search for the vATPase regulators, we cloned human Dmxl2 gene encoding Rabconnectin-3a (Rbcn-3a). An alteration in the gene dosage of Dmxl2 in human patients resulted in a complex pathology called as poly endocrine-polyneuropathy syndrome (PEPNS). Rbcn-3a encodes a large 340kDa protein, whose function at the mammalian synapse remains largely unknown.
We found it to be present on every organelle that acidifies; including SVs. Loss of Rbcn-3a in mice resulted in early embryonic lethality. When Rbcn-3a is eliminated from neuronal cells in culture, neurons developed normally, yet their activity was perturbed. Neurons without Rbcn-3a failed to fully acidify SVs, and showed altered SV recycling. Curiously, the synapses of neurons without Rbcn-3a also accumulated lysosomes-like structures and lysosomal markers, suggesting an unanticipated connection between the machinery for endocytosis, acidification and cellular homeostasis.

Disclosures: **S. Gowrisankaran:** None. **A. Raimondi:** None. **Z. Farsi:** None. **N. de Roux:** None. **I. Milosevic:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 121.25/B93

Topic: B.05. Neurotransmitter Release

Title: Kinetic dissection of vesicle recycling in the calyx of held synapse at physiological temperature

Authors: Z. LIU[1,2], Y. ZHU[1,2], Y. HU[3], S. ZHANG[1,2], *J. SUN[1,2];
[1]Inst. of Biophysics, Chinese Acad. of Sci., Beijing, China; [2]Sch. of Life Science,, Univ. of Chinese Acad. of Sci., Beijing, China; [3]High Sch. Affiliated to Renmin Univ., Beijing, China

Abstract: Synaptic transmission at mammalian central synapse is undergoing with stochastic activity at physiological temperature. Synaptic recycling vesicle pool with proper kinetic structure ensures sustained synaptic transmission. However, the kinetic structure of recycling vesicle pool has never been quantitatively analyzed so far and most of the studies were performed at room temperature and under resting neuronal status. With combination of presynaptic capacitance measurement and postsynaptic EPSC recording on the calyx of Held synapse at physiological temperature, we studied the vesicle recycling under sustained presynaptic stimulation at physiological temperature. The kinetics of vesicle reuse was revealed by impeding transmitter refilling with folimycin. It was found that more than 90% vesicles involved in recycling at calyceal terminals but they are not homogeneously competent for reusing. A significant surface pool of vesicles was detected corresponding to different intensity of stimulations. We dissected the group of recycling vesicles as kinetically connected subpopulations and obtained the kinetic structure of the recycling vesicle pool in nerve terminal. The sizes and transferring rates among these sub-pools were dynamically regulated by neuronal activity thus to ensure the efficient synaptic transmission. Our work for the first time provides a quantitative description of synaptic vesicles trafficking along a complete recycling pathway, which helps to understand the impact of vesicle recycling on stable and reliable synaptic transmission under variant neuronal activities.

Disclosures: **Z. Liu:** None. **Y. Zhu:** None. **Y. Hu:** None. **S. Zhang:** None. **J. Sun:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 121.26/B94

Topic: B.05. Neurotransmitter Release

Title: Calcium- and phospholipid-dependent regulation of synaptic plasticity by Munc13-1

Authors: *N. LIPSTEIN[1], S. CHANG[3], K.-H. LIN[4], B. H. COOPER[1], A. GHUMAN[1], J. J. JANS[5], H. TASCHENBERGER[2], N. BROSE[2];
[1]Dept. of Mol. Neurobio., Max-Planck Inst. of Exptl. Med., Göttingen, Germany; [2]Dept. of Mol. Neurobio., Max-Planck Inst. of Exptl. Med., Goettingen, Germany; [3]Univ. of Toronto, Toronto, ON, Canada; [4]Max Planck Inst. for Biophysical Chem., Göttingen, Germany; [5]Utrecht Univ. Med. Ctr., Utrecht, Netherlands

Abstract: Synaptic transmission exhibits rapid and dynamic changes in efficacy, a phenomenon termed short-term synaptic plasticity (STP). While it is well-established that STP is fundamental for information processing in neuronal networks, the molecular basis of STP remains unclear. At the presynaptic active zone, neurotransmitter release is tightly coupled in time and space by the Ca^{2+}-dependent, coordinated function of hundreds of proteins and their interplay with membrane lipids of the synaptic vesicle and of the plasma membrane. Munc13 proteins are key regulators of neurotransmitter release, as they mediate the priming step that renders synaptic vesicles fusion-competent, and their genetic elimination causes a complete block of neurotransmission. We use mouse genetics tools and electrophysiology to study how Ca^{2+}- and phospholipid signaling to Munc13s impacts synaptic transmission and STP at the calyx of Held synapse. In addition, we study the molecular mechanisms by which variations in Munc13s leads to abnormal neurotransmission in a novel congenital brain disorder, characterized by autism-spectrum disorder, a dyskinetic movement disorder, and intellectual disability.

Disclosures: N. Lipstein: None. **S. Chang:** None. **K. Lin:** None. **B.H. Cooper:** None. **A. Ghuman:** None. **J.J. Jans:** None. **H. Taschenberger:** None. **N. Brose:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

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Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 121.27/B95

Topic: B.05. Neurotransmitter Release

Support: DFG Grant HE7480/2-1
EU ERA-NET Grant Adkinhumice
DFG Grant RO1296/8-1

Title: Calcium-independent exo-endocytosis coupling at small central synapses

Authors: M. ORLANDO, C. ROSENMUND, ***M. A. HERMAN**;
Inst. of Neurophysiol., Charité-Universitätsmedizin Berlin, Berlin, Germany

Abstract: Successful synaptic transmission requires release of neurotransmitter from the presynaptic terminal by synaptic vesicle exocytosis. The retrieval of synaptic vesicles from the plasma membrane by endocytosis is crucial for maintaining synaptic structure and efficient neurotransmission. Sites of synaptic vesicle fusion must be cleared rapidly; therefore, the coupling of exo- and endocytosis is crucial. Despite many years of research, the exact molecular and biophysical requirements exo- and endocytosis coupling are still debated. The role of Ca^{2+} in exo- and endocytosis coupling has remained particularly enigmatic, in large part because of calcium's necessity in triggering synaptic vesicle fusion. We sought to gain insight into whether Ca^{2+} is required for initiating endocytosis by triggering exocytosis in a calcium-independent manner: exposure of cultured neurons to hypertonic sucrose solution (Rosenmund and Stevens, 1996). Using electron microscopy analysis of high-pressure frozen hippocampal synapses, we found that sucrose application induced formation of membrane invaginations, or endocytic pits, that resolved upon sucrose washout. Sucrose induced endocytic pit formation independent of clathrin or temperature; however, in synapses that were incapable of undergoing exocytosis or where the actin dynamics were shifted towards a depolymerized state by treatment with latrunculin-A, hypertonic sucrose was unable to induce endocytic pits. Furthermore, we investigated whether calcium is necessary for the kinetics of endocytosis by monitoring the sucrose evoked fluorescence signal of the pH sensitive variant of GFP, pHlourin, tagged to synaptophysin (SypHy) with live-cell imaging. We found that that protein recycling occurs in the absence of Ca^{2+} influx. Interestingly, Ca^{2+} chelation by BAPTA slowed the sucrose-evoked SypHy decay, without affecting the formation of endocytic pits, suggesting that while Ca^{2+} influx is not required to initiate endocytosis, it plays a role in modulating the speed of the complete recycling process. Overall, our data clarify the controversial role of Ca^{2+} in the initiation of endocytosis at presynaptic terminals. We suggest that membrane tension, and not Ca^{2+} influx, drives the initial coupling of exo- and endocytosis at small central synapses.

Disclosures: **M. Orlando:** None. **C. Rosenmund:** None. **M.A. Herman:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 121.28/B96

Topic: B.05. Neurotransmitter Release

Support: Johns Hopkins Startup Package
NIH S10 RR026445

Title: Effects of acute ATP shortage on the synaptic vesicle cycle at the ultrastructural level

Authors: *Q. GAN[1], S. WATANABE[2];
[1]Johns Hopkins Sch. of Med., Baltimore, MD; [2]Dept. of Cell Biol., Johns Hopkins Univ., Baltimore, MD

Abstract: Following synaptic vesicle fusion at the presynaptic terminal, vesicular membrane and proteins must be recycled via endocytosis to replenish the vesicle pool. A previous study revealed using the pHluorin assay that local depletion of ATP at presynaptic terminals impairs the rate of vesicle cycling during sustained synaptic activity, possibly by blocking endocytosis. However, due to limitations inherent to the pHluorin approach, it is unknown which exact step in the vesicle cycling process is most sensitive to the availability of ATP. Moreover, it is unclear whether glycolysis or oxidative phosphorylation (OxPhos) is more important for supplying energy to the synaptic vesicle cycle. In the present study, we used time-resolved electron microscopy to capture rapid changes in the presynaptic membrane ultrastructure in cultured hippocampal neurons at various time points following stimulation. We combined this method with drug treatments that acutely block glycolysis and OxPhos and found that acute ATP depletion leads to the arrest of spontaneous as well as activity-induced synaptic vesicle endocytosis at early stages. In addition, blocking OxPhos alone has a weaker effect than blocking both oxidative phosphorylation and glycolysis. This suggests that aerobic glycolysis alone might be able to partially meet the energy demand of vesicle cycling independent of OxPhos. Furthermore, we found that prolonged block of OxPhos leads to the clustering of PFKL, a rate-limiting glycolytic enzyme, towards synaptic sites. Our results shed new light on the energetics of synaptic vesicle endocytosis and may have important implications for the pathophysiology of brain ischemia.

Disclosures: Q. Gan: None. **S. Watanabe:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 121.29/B97

Topic: B.06. Synaptic Transmission

Support: NIH DC01919

Title: The Kv3.3 potassium channel controls endocytosis by organizing the actin cytoskeleton at nerve terminals

Authors: *L. K. KACZMAREK[1], X.-S. WU[2], S. SUBRAMANIAN[1], J. XIA[3], Y. ZHANG[1], L. EL-HASSAR[1], K. SZIGETI-BUCK[1], T. L. HORVATH[1], E. A. JONAS[1], L.-G. WU[2];
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Abstract: Endocytosis, which recycles vesicles and sustains synaptic transmission, employs the force-generating cytoskeletal filamentous actin (F-actin) to bend membrane for vesicle formation. We have now found that F-actin at mouse calyx nerve terminals is organized by Kv3.3 voltage-dependent potassium channels that binds the Arp2/3 actin-nucleating complex. Kv3.3 is expressed at high levels in the calyx of Held presynaptic terminals in the medial nucleus of the trapezoid body. By electron immunomicroscopy, the channel is found to be localized adjacent to clusters of vesicles at presynaptic membranes that face the postsynaptic cell. These terminals have a dense subcortical actin network that is absent in postsynaptic membranes, which lack Kv3.3. This presynaptic actin network in disrupted in Kv3.3 knockout animals and in those that express a mutant channel, G592R Kv3.3, which has normal electrophysiological function but cannot bind the Arp2/3 complex. Both slow (~10-30 s) and rapid (less than ~3 s) endocytosis are inhibited in Kv3.3 knockout animals and in those expressing G592R Kv3.3. We found a similar effect of deletion or mutation of Kv3.3 on synaptic vesicle endocytosis at synapses of hippocampal neurons in culture. These results suggest updating current endocytosis model by including potassium channels in organizing actin cytoskeleton for force generation at nerve terminals. This model not only extends the repertoire of crucial endocytic players unexpectedly to potassium channels, but also may link endocytosis to, and thus improve our understanding of, potassium channel-relevant diseases, particularly human spinocerebellar ataxia type 13 is caused by Kv3.3 mutations, and which are characterized by cerebellar atrophy, motor symptoms, and abnormalities in auditory function.

Disclosures: L.K. Kaczmarek: None. **X. Wu:** None. **S. Subramanian:** None. **J. Xia:** None. **Y. Zhang:** None. **L. El-Hassar:** None. **K. Szigeti-Buck:** None. **T.L. Horvath:** None. **E.A. Jonas:** None. **L. Wu:** None.

Poster

121. Neurotransmitter Release and Vesicle Recycling

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 121.30/B98

Topic: B.05. Neurotransmitter Release

Support: INSERM
Region Normandie
EFS
CHU, Caen

Title: Understanding the neuronal trafficking of tissue type plasminogen activator and its neuronal functions

Authors: *A. VARANGOT[1], S. LENOIR[2], L. LEBOUVIER[1], Y. HOMMET[1], D. VIVIEN[1,3]; [1]INSERM U1237, CAEN, France; [2]INSERM U1216, Grenoble, France; [3]Dept. of Clin. Res., Caen Univ. Hosp., Caen, France

Abstract: The discovery of the neuronal expression of the serine protease tissue type plasminogen activator (tPA) has opened new avenues of research. With important functions in the physiopathology of the central nervous system, tPA can maybe bring answers in understanding neurotransmission in a certain subtype of glutamatergic neurons. For example, its interactions with synaptic receptors (NMDAR, LRP1, Annexin II, and EGFR) and its role in the maturation of BDNF have been reported to mediate its ability to control the synaptic plasticity and of the neuronal survival. Thus, a better description of the mechanisms regulating the neuronal trafficking of tPA may help to further understand how tPA can influence brain functions and dysfunctions.
Using an innovative labelling of the intracellular tPA, we have recently revealed from living primary cultures of cortical and hippocampal neurons, the neuronal distribution of tPA (Lenoir S. et al., *Frontiers Cell. Neurosci*., In press). We have also demonstrated that the neuronal tPA was contained in endosomal vesicles positives for Rabs and in exosomal vesicles positives for synaptobrevin-2 (VAMP2), both in dendrites and axons. tPA-containing vesicles differ in their dynamics, with the dendritic vesicles that are less mobiles than the axonal vesicles, these laters displaying mainly a retrograde transport. Furthermore, we have also demonstrated that the trafficking and the exocytosis of tPA were finely tuned by the neuronal activity through mechanisms that are dependent of Ca^{2+} influx. We have thus observed that the neuronal activity reduced the motion and the velocity of tPA positives vesicles and enhanced the exocytosis of tPA positive vesicles both in axons and dendrites.
TPA is mainly known for its role in the control of the fibrinolysis when present in the blood, but studies on its neuronal physiology are relatively weak. These data provide new insights about the

neuronal trafficking of tPA, contributing to a better knowledge of the neuronal functions of this serine protease.
Key words: tPA, neurons, vesicular trafficking, plasticity.

Disclosures: **A. Varangot:** None. **S. Lenoir:** None. **L. Lebouvier:** None. **Y. Hommet:** None. **D. Vivien:** None.

Poster

122. Structural Plasticity and Circuit Remodeling II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 122.01/B99

Topic: B.07. Synaptic Plasticity

Title: Characterization of PSD lattice-like structure purified from rat forebrain

Authors: ***T. SUZUKI**[1], S. HIGASHIYAMA[2], K. TABUCHI[1], Y. SHIRAI[1], W. LI[3];
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Abstract: "PSD lattice" has been identified in the preparation obtained after treatment of synaptosome, synaptic plasma membrane (SPM) or Triton X-100-PSD with deoxycholate, a relatively strong detergent, and has been considered to be a basic backbone of type I PSDs before the discovery of various scaffold/adaptor proteins. However, major constituents of the PSD lattice and the relationship between the PSD lattice and the scaffold/adaptor protein model for PSD structure have not yet been known. Previously we purified a fraction enriched with PSD lattice-like structures from rat forebrain and discussed the relationship between the PSD lattice structure and the scaffold/adaptor protein model (Suzuki et al., 2018, J. Neurochem., 144, 390-407). However, the whole protein molecules constituting the PSD lattice could not been identified, because some proteins in the preparation were SDS-insoluble. In this paper, we improved the purification protocol and succeeded in avoiding denaturation of component proteins. The newly prepared PSD lattice-like preparation enabled identification and analyzes of the component proteins by SDS-PAGE and western blotting. Indeed, we identified protein components by SDS-PAGE, western blotting and mass spectrometry, and examined localization of the identified proteins in the PSD lattice-like structures by immuno-gold electron microscopy. We found that tubulin, both alpha- and beta-tubulin, are major constituents. Unexpectedly, contents of other cytoskeletal proteins such as fodrin, actin and alpha-internexin, and typical scaffold/adaptor proteins, such as PSD-95, Shank, Homer and GKAP were very few in a typical PSD lattice. Microtubules, microfilaments and intermediate filaments were not present in the preparation and thus, they were not the structures constituting the PSD lattice. Our results suggest that tubulin may construct a PSD lattice-like structure either alone or associated with other proteins at the postsynaptic site of neurons. We propose that non-microtubule tubulin may

form PSD lattice-like structure at the postsynaptic sites. This tubulin may be a long-sought form of tubulin present in the SPM.

Disclosures: T. Suzuki: None. **S. Higashiyama:** None. **K. Tabuchi:** None. **Y. Shirai:** None. **W. Li:** None.

Poster

122. Structural Plasticity and Circuit Remodeling II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 122.02/B100

Topic: B.07. Synaptic Plasticity

Title: New structural evidences on the storage capacity of synapses in hippocampal area CA1 and DG in rat

Authors: *M. SAMAVAT[1], T. M. BARTOL, Jr.[1], C. BROMER[1], D. D. HUBBARD[2], D. C. HANKA[2], K. M. HARRIS[3], T. J. SEJNOWSKI[1];
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Abstract: Numerous electrophysiological evidences have shown that synaptic strengths are highly correlated with synapse sizes via a mechanism called synaptic plasticity. While the distribution of synapse sizes for different areas of the brain has been illustrated earlier in research articles, discovering the potential precision of synapse sizes has not been studied previously. Another fundamental question, assuming we can calculate the precision of synapses, would be the possible distinguishable category of synapse sizes that code information and the possible interpretation of calculated precision. To answer these questions, a detailed experimental analysis of a dense three-dimensional reconstruction of serial section electron microscopy (3DEM) from the middle stratum radiatum in hippocampal area CA1 of rat has been analyzed to determine how much information can be stored at a synapse through synaptic plasticity. In a previous study (TM Bartol Jr, Elife 4 (2015)) the authors measured the coefficient of variation of spine head volumes of 10 same-dendrite same-axon (SDSA) pairs from this data set assuming Signal-to-Noise Ratio (SNR) with a value of 1. Then, with a simulation analysis approach, it was found that 26 Gaussian distributions could span the range of spine sizes of the 10 SDSA pairs, implying that the storage capacity of the rat brain synapses for this region is 4.7 bits of information. We have analyzed the complete data set of 287 spine head volumes using novel clustering approaches and advanced information theory and found a set of distinguishable clusters of synaptic strengths. Here, we have found the new lower bound for the storage capacity of synapses in stratum radiatum in rat hippocampal area CA1 based on experimental data. We further extended our analysis on synapses from 3DEM data set of granule cell dendrites in the middle molecular layer (MML) of the dentate gyrus. We have answered this question whether

the DG calculated capacity for control cases is changed in response to inducing LTP in vivo and if it differs from the capacity of area CA1 synapses. Our analysis shows that inducing LTP increases the number of distinguishable clusters of synaptic strengths compared to the control case. Last but not least, our analysis shows that the CA1 synapses are more precise than the DG synapses.

Disclosures: M. Samavat: None. **T.M. Bartol:** None. **C. Bromer:** None. **D.D. Hubbard:** None. **D.C. Hanka:** None. **K.M. Harris:** None. **T.J. Sejnowski:** None.

Poster

122. Structural Plasticity and Circuit Remodeling II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 122.03/B101

Topic: B.07. Synaptic Plasticity

Support: R56 MH095980

Title: Are synapses in the aging brain too large to learn?

Authors: *L. M. KIRK, P. H. PARKER, K. HARRIS;
Univ. of Texas, Austin, Austin, TX

Abstract: Augmentation of LTP is a current model for the advantages of spaced versus massed learning. In the adult rat hippocampus, LTP can be saturated after one round of repeated bursts of high-frequency stimulation, namely theta-burst stimulation (TBS). Further potentiation, or augmentation of LTP, can only be achieved after a refractory period of at least 90 minutes and requires 4 hours for augmentation to be reliable. In spaced learning, memory consolidation is greatly improved if training is spread across spaced trials rather than clumped together in one "cram session." Spaced learning is better than massed learning for memory consolidation and enhancement of LTP. Interestingly, prior studies in humans used spaced vs massed training protocols to test word-word association recall and found that both older adults (mean age of 65 years) and young adults (mean age of 19 years) show improvement in memory recall when spaced training was used. However, the amount of improvement compared to massed training protocols in older adults was significantly less than in young adults, indicating spaced learning loses efficacy as we age. In models of age-related cognitive decline, LTP is surprisingly intact; however, augmentation of LTP has not been tested. Our preliminary data demonstrate a reduced capacity for augmentation of LTP in aged rats (25-26 months). Aged rats were assessed for cognitive impairment in concurrent experiments alongside young adults using the Morris Water Maze (MWM). Tissue from the stratum radiatum of hippocampal area CA1 from young adults and cognitively impaired aged rats was processed for 3DEM. Initial findings revealed the distribution of synapse size was shifted right (larger) for aged impaired animals relative to young

adults. These findings suggest the intriguing possibility that synapses of cognitively impaired aged animals are unable to be augmented because their synaptic weight is already saturated.

Disclosures: L.M. Kirk: None. **P.H. Parker:** None. **K. Harris:** None.

Poster

122. Structural Plasticity and Circuit Remodeling II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 122.04/B102

Topic: B.07. Synaptic Plasticity

Support: NIH Grant RO1NS062736
NIH Training Grant 5T32GM099608-07

Title: Saturation of structural plasticity at individual CA1 dendritic spines

Authors: *J. C. FLORES, K. M. ZITO;
Ctr. for Neurosci., Univ. of California, Davis, Davis, CA

Abstract: Learning is crucial for survival. One interesting aspect of learning in humans is that efficiency of learning can be improved when there are breaks between episodes of learning. Long-term potentiation (LTP) of synaptic strength has been proposed to be the cellular basis of learning and memory. Notably, LTP can saturate in the hippocampus and that this saturation can lead to deficits in behavioral learning tasks. Intriguingly, saturation of LTP is released over time. Thus, it is possible that saturation of LTP in the hippocampus is the underlying phenomenon that explains the increased efficacy of spaced learning over massed learning. We propose that LTP saturates at individual synapses. In the hippocampus, most excitatory synapses occur at small protrusions on dendrites called dendritic spines. Spines are dynamic and have been shown to stabilize and grow in response to high-frequency stimulation in vitro and during learning in vivo. The size of a spine is strongly correlated with the strength and size of its associated synapse. Here, we use high-frequency 2-photon glutamate uncaging and time-lapse imaging to show that structural LTP can saturate at individual dendritic spines after a single LTP induction. We also show that saturation of LTP is specific to the stimulated spine. We hypothesize that this saturation is due to altered function of specific components of the signaling cascades involved in LTP. Using 2-photon FRET fluorescence lifetime imaging and a molecularly encoded CaMKII FRET probe, we show that 30 minutes following structural LTP induction at single spines, CaMKII activation in response to high-frequency stimulation is significantly reduced. We are currently investigating a potential mechanism for the decreased CaMKII activation. This work will further our understanding of the signaling mechanisms that limit LTP and thus will lead to a better understanding of learning.

Disclosures: **J.C. Flores:** None. **K.M. Zito:** None.

Poster

122. Structural Plasticity and Circuit Remodeling II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 122.05/C1

Topic: B.07. Synaptic Plasticity

Support: R03 AG 052120
LSU-SVM Corp

Title: SK2 modulates synaptic plasticity by tuning CaMKII activity

Authors: A. SHRETHA[1], R. SULTANA[1], C. C. LEE[2], *O. M. OGUNDELE[1];
[1]Comparative Biomed. Sci., Louisiana State Univ., Baton Rouge, LA; [2]Comparative Biomed. Sci., Louisiana State Univ. Sch. of Vet. Med., Baton Rouge, LA

Abstract: NMDAR-linked Ca^{++} current represents a significant percentage of post-synaptic transient Ca^{++} current. It is pertinent to the modulation of synaptic strength that underlies dendritic spine plasticity and cognitive function. In the hippocampus, synaptic potentiation is tuned by CaMKIIα Thr286/287 phosphorylation. On the other hand, post-synaptic transient Ca^{++} current produced by glutamatergic ionotropic neurotransmission repetitively activate small conductance (SK2) channels which in turn abrogates the potentiation event. Here, we demonstrate that the upstream suppression of GluN1 function in the CA1 of the hippocampus suppressed T286/T287 phosphorylation of CaMKII, and was accompanied by an increased SK2 expression, and activity. Consistent with the ablation of GluN1 function, positive modulation of SK2 in wild type hippocampus (normal GluN1) decreased T286/T287 phosphorylation of CaMKII and bursting frequency that is determined - mostly - by T286 CaMKIIα. Together with the suppression of T287 phosphorylation of CaMKIIβ, a significant loss of hippocampal α-actinin suggests that SK2 impact spine localization of CaMKII, and its substrate targeting downstream of NMDAR. Our results demonstrate that positive modulation of SK2 function in the CA1 refines synaptic plasticity by attenuating CaMKII T286/T287 phosphorylation, and synaptic localization.

Disclosures: **A. Shretha:** None. **R. Sultana:** None. **C.C. Lee:** None. **O.M. Ogundele:** None.

Poster

122. Structural Plasticity and Circuit Remodeling II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 122.06/C2

Topic: B.07. Synaptic Plasticity

Support: NIH Grant RO1NS062736

Title: Non ionotropic NMDA receptor signaling mechanisms driving dendritic spine plasticity

Authors: *I. S. STEIN[1], D. K. PARK[1], J. N. JAHNCKE[2], K. M. ZITO[3];
[1]UC Davis, Davis, CA; [2]Neurosci. Grad. Program, Oregon Hlth. & Sci. Univ., Portland, OR;
[3]Univ. of California Davis, Davis, CA

Abstract: Activity driven changes in neuronal connectivity are important for the experience-dependent remodeling of brain circuitry during development, learning and memory. The formation and elimination of dendritic spine synapses are vital for this refinement of synaptic circuits. Notably, the elimination or pruning of excessive and imprecise dendritic spine synapses has been linked to improvements in learning, and increased spine loss has been associated with intellectual disability and behavioral impairment. Morphological changes in dendritic spines are closely linked to changes in synaptic function, and it is now widely established that the shrinkage and elimination of spines can be driven by glutamatergic activity patterns that lead to the long-term depression (LTD) of synaptic strength. However, recent studies surprisingly showed that blocking ion flow through the NMDA-type glutamate receptor (NMDAR) did not prevent spine shrinkage and LTD induction. Instead, these novel findings support a model where glutamate binding to the receptor is sufficient to induce conformational changes in the NMDAR signaling complex, which lead to spine shrinkage and synaptic weakening independent of NMDAR-mediated Ca^{2+}-influx. We have previously shown that activation of p38 MAPK is necessary for this non-ionotropic NMDAR-dependent signaling in activity-dependent spine shrinkage. Using two-photon glutamate uncaging, time-lapse imaging, and whole-cell recordings, we have further investigated the role of non-ionotropic NMDAR signaling during spine structural and functional plasticity. Specifically, we have focused on how glutamate binding to the NMDAR complex in the absence of ion influx drives p38 MAPK activation and on how this novel signaling cascade causes spine structural changes during input-specific synaptic plasticity. Results from these experiments will lead to a better understanding of the signaling mechanisms driving dendritic spine remodeling during neuronal circuit development and plasticity and should provide new insights into how dysregulation of these plasticity mechanisms contributes to altered synaptic connectivity in neurological disorders.

Disclosures: I.S. Stein: None. **D.K. Park:** None. **J.N. Jahncke:** None. **K.M. Zito:** None.

Poster

122. Structural Plasticity and Circuit Remodeling II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 122.07/C3

Topic: B.07. Synaptic Plasticity

Support: NIH Grant RO1NS062736

Title: Non-ionotropic NMDA receptor signaling in structural plasticity of dendritic spines and schizophrenia

Authors: *D. K. PARK, I. S. STEIN, L. J. TOM, K. M. STACHOWICZ, K. ZITO;
Ctr. for Neurosci., Univ. of California, Davis, Davis, CA

Abstract: Learning and memory formation are vital biological processes that involve dynamic rewiring of the neural circuitry. Modification of synaptic connections in the cerebral cortex involves growth, stabilization, and elimination of dendritic spines, all of which depend on the activation of NMDA-type glutamate receptors (NMDARs). Simultaneous binding of glutamate and co-agonist, glycine or d-serine, to the NMDARs allow for opening of the channel. NMDAR activation can result in either long-term potentiation (LTP) and spine growth, or long-term depression (LTD) and spine shrinkage. While calcium influx through the receptor is crucial for LTP and spine growth, binding of glutamate to the NMDAR is sufficient to induce LTD and spine shrinkage in an ion-flux independent manner. Non-ionotropic NMDAR signaling is thought to be vital for memory formation by mediating spine shrinkage and elimination; however, excessive signaling may underlie reduced synaptic connectivity observed in neurological disorders. Notably, schizophrenia is a debilitating disorder with symptoms such as hallucinations and cognitive deficits that is also associated with decreased spine density, decreased d-serine levels, and reduced expression of the enzyme that produces d-serine, serine racemase. Under these conditions, it would be expected that decreased NMDAR co-agonist availability would bias glutamatergic synapses toward non-ionotropic NMDAR signaling, leading to LTD and spine shrinkage. With the use of two-photon imaging, glutamate uncaging, and biochemistry on serine racemase knockout mice, which lack d-serine and exhibit many of the neuropathological features observed in schizophrenia, we test this novel hypothesis that a bias for non-ionotropic NMDAR signaling due to decreased d-serine availability obstructs growth and stabilization of spines, ultimately leading to decreased spine density associated with the disorder. Findings from this study will help further our understanding of the underlying molecular and cellular mechanisms for schizophrenia, and could also increase our understanding of other neurological disorders that stem from dysregulation of synaptic plasticity mechanisms.

Disclosures: D.K. Park: None. **I.S. Stein:** None. **L.J. Tom:** None. **K.M. Stachowicz:** None. **K. Zito:** None.

Poster

122. Structural Plasticity and Circuit Remodeling II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 122.08/C4

Topic: B.07. Synaptic Plasticity

Support: NIH T32 HL007822
R01-MH102338
R01-HL088548

Title: Synaptic crosstalk conferred by a zone of differentially-regulated calcium signaling in the dendritic shaft adjoining a potentiated spine

Authors: P. J. DITTMER, M. L. DELL'ACQUA, ***W. A. SATHER**;
Univ. of Colorado Sch. of Med., Aurora, CO

Abstract: Patterns of postsynaptic activity that induce long-term potentiation of fast excitatory transmission at glutamatergic synapses between hippocampal neurons cause enlargement of the postsynaptic dendritic spine and promote growth in the volume of the potentiated spine's content of endoplasmic reticulum (ER). Such postsynaptic activity patterns also impact Ca^{2+} signaling in the adjoining dendritic shaft, in a zone centered on the spine-shaft junction and that extends ~10-20 μm in either direction along the shaft. Comparing this functionally specialized "paraspinal" zone in the shaft with the dendrite in general, plasticity-inducing stimulation of a single spine (uncaging of MNI-glutamate via 60 laser flashes of 2 msec duration, once per sec, in 0 Mg^{2+} solution and in a 1 μm x 1 μm box adjacent to the spine) causes: (i) more profound and extensive depletion of Ca^{2+} stores in the shaft ER, (ii) a greater degree of interaction between stromal interaction molecule 1 (STIM1) and voltage-gated L-type Ca^{2+} channels in the shaft (as measured by Förster resonance energy transfer between over-expressed fluorescent protein-tagged partners), and (iii) consequently generates stronger feedback inhibition of shaft L-type Ca^{2+} channels by STIM1.
Here we show that the length of the paraspinal zone along the dendritic axis can be approximately doubled through the neuromodulatory action of isoproterenol-stimulated β-adrenergic receptors (βARs). The mechanism of βAR enlargement of the zone arises from protein kinase A-mediated enhancement of L-type Ca^{2+} current. The isoproterenol-enhanced influx of Ca^{2+} through L-type Ca^{2+} channels depletes $[Ca^{2+}]_{ER}$ via ryanodine receptor-dependent, Ca^{2+}-induced Ca^{2+} release along a ~doubled length of the dendrite. This extended zone of Ca^{2+} depletion-activated STIM1 inhibits L-type Ca^{2+} channels along a stretch of dendrite that is also ~doubled in length compared to control. An important function of the dendritic shaft's paraspinal zone is to support crosstalk between spines along its length, such that spines neighboring a strongly stimulated spine (1 Hz uncaging, 60 sec) are enabled to themselves undergo structural

plasticity in response to stimulation (0.167 Hz uncaging, 60 sec) that would otherwise be subthreshold for induction of spine structural plasticity. For the weakly-stimulated neighboring spines, both spine volume and spine ER volume become enlarged. RNAi knockdown of STIM1 or block by nimodipine of L-type Ca^{2+} channels prevents crosstalk, indicating that STIM1 and L channels are required. Finally, activation of βARs extends the range along the shaft over which such spine-to-spine communication can occur.

Disclosures: W.A. Sather: None. **M.L. Dell'Acqua:** None. **P.J. Dittmer:** None.

Poster

122. Structural Plasticity and Circuit Remodeling II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 122.09/C5

Topic: B.07. Synaptic Plasticity

Support: Rappaport mental healt research fellowship

Title: Cs6 clusters: evidence for a role of novel proteoglycan structures in experience-dependent clustered plasticity

Authors: *G. CHELINI[1], C. BERCIU[1], P. DURNING[1], L. BALASCO[3], A. BOYER-BOITEAU[1], J. VALERI[1], K. HUANG[1], Y. BOZZI[3], K. J. RESSLER[2], S. BERRETTA[1]; [1]Basic Neurosci., [2]Div. of Depression and Anxiety Disorders, McLean Hosp. - Harvard Med. Sch., Belmont, MA; [3]Ctr. for Mind/Brain Sci., Univ. of Trento, Trento, Italy

Abstract: The mechanisms underlying experience-dependent learning arguably represent a major challenge in neuroscience. Recent, elegant studies shifted the focus from single synaptic mechanisms to coordinated changes involving several synapses within short dendritic stretches. Spatial organization of locally coordinated synapses was shown to be mediated by the immediate early gene Arc, a key molecular player in activity-dependent plasticity. Recent findings from our group and others may be relevant to these mechanisms. In the human brain, we have shown a novel structure, i.e. CS6 clusters (50-200 μm diameter), enriched in 6-sulfated chondroitin sulfate proteoglycans (CS6-CSPGs), molecules known to play a key role in synaptic plasticity. Our results show that CS6 clusters are altered in psychiatric disorders and are composed of numerous glial cells surrounding a cluster of dendrites. On the basis of their molecular and cellular composition, we postulated that CS6 clusters may correspond to transient microenvironment contributing to coordinated synaptic plasticity. To test this hypothesis, we focused on the mouse barrel cortex and used a combination of somatosensory manipulations, immunohistochemistry, high resolution imaging and quantitative microscopy. Electron microscopy data show that CS6 clusters result from the accumulation of CS6-CSPG within dendritic spines and astrocytes end-feet surrounding them, within a segregated microdomain. In

naïve home-caged animals, we found that dendritic spine densities are higher in dendrites within CS6 clusters as compared to segments of the same dendrite located outside (p=0.04, n=8). Unilateral sensory (whisker) stimulation induced a significant increase of CS6 clusters within 2 hours (p=0.001, n=4). Conversely, sensory deprivation (whisker trimming, 1 week) induced a significant decrease of the number of CS6 clusters in the barrel cortex (p=0.00001, n=5). Finally, numbers CS6 clusters were significantly and positively correlated to the expression of the neuroplastic protein Arc (r=0.76, p=0.02, n=8). Together, these findings indicate that CS6 clusters form in response to specific stimulation with temporal and subcellular characteristics consistent with locally coordinated plasticity. Our results are consistent with the hypothesis that CS6 clusters may correspond to transient, multi-synaptic memory engrams.

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Poster

122. Structural Plasticity and Circuit Remodeling II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 122.10/C6

Topic: B.07. Synaptic Plasticity

Title: Visualization of micro RNA distribution at the functional states of dendritic spines

Authors: *H.-J. KIM, H.-M. LEE, J.-H. KIM;
Life Sci., POSTECH, Pohang, Korea, Republic of

Abstract: microRNAs (miRNAs), small non-coding RNA, have emerged as critical regulators of synapse development and plasticity through their control of gene expression. However, we still do not know the distribution and role of miRNAs at dendritic spines which major loci of excitatory inputs and synaptic plasticity accompanying structural changes. Brain-specific miR-134s likely regulate the morphological maturation of spines, but their subcellular distributions and functional impacts have rarely been assessed. Here, we adapted atomic force microscopy (AFM) to visualize in situ miR-134s, which indicated that they are mainly distributed at nearby dendritic shafts and necks of spines. The abundance of miR-134s varied between morphologically and functionally distinct spine types and their amounts were inversely correlated with their postulated maturation stages. Moreover, spines exhibited reduced contents of miR-134s when selectively stimulated with beads containing a brain-derived neurotrophic factor (BDNF). From these results, in situ visualizations of miRNAs provided unprecedented insights into the “inverse synaptic-tagging” roles of miR-134s that are selective to inactive/irrelevant synapses and potentially a molecular means for modifying synaptic connectivity via structural alteration.

Disclosures: **H. Kim:** None. **H. Lee:** None. **J. Kim:** None.

Poster

122. Structural Plasticity and Circuit Remodeling II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 122.11/C7

Topic: B.07. Synaptic Plasticity

Title: Modulation of synaptic structure and function in primary rodent hippocampal neurons by autism risk genes- A high content imaging analysis

Authors: L. MICHOLT, K. W. YOUNG, ***C. E. BAZENET**, J. RUDOLPH, K. KOTTIG, H. VON DER KAMMER;
Evotec SE, Hamburg, Germany

Abstract: Objective To develop fluorescence-based automated imaging methodologies suitable for the detection and quantification of changes in synapse number and spine morphology upon modulation of disease risk genes and pharmacological treatments. **Methods** Rat primary hippocampal neurons were transfected at DIV7 with eGFP and then fixed at DIV21 for spine identification in rat hippocampal neurons transfected. ASD risk gene delivery to neurons was conducted with Adeno-Associated Viruses (AAV) or lipofectamine-mediated plasmid transfection. Transfected neurons were imaged on a PerkinElmer Opera high-content imaging platform. Automated image analysis of pre- and post-synaptic markers was performed using in-house developed AcapellaTM-based scripts and data were compared to manual counting techniques (Imaris, Bitplane). **Results** Initial experiments used antibodies against synaptophysin and PSD95 as pre- and post-synaptic markers, respectively. Automated detection and analysis demonstrated a decrease in synapse number after pharmacological enhancement of synaptic transmission. This effect could be blocked by pharmacological inhibition of synaptic transmission. The structure of the post-synaptic spine is critical for synaptic transmission. We therefore developed an automated Z-plane analysis protocol for measuring eGFP-labelled primary neurons in multiwell plate format. Data obtained on spine length, volume and density compared favorably to manual analysis methods. To model diseases at a synaptic level we used AAV-mediated shRNAs to knock-down key Autism Spectrum Disease (ASD) related genes. Automated counting of pre- and post-synaptic pairs identified either up- or down-regulation of synapse density depending on the ASD gene being targeted. **Conclusions** Monitoring synaptic integrity in cultured neurons in combination with modulation of risk gene expression by viral transduction could serve as an experimental system for measuring synaptic dysfunction associated with CNS disorders. This robust platform for the automated collection and analysis of both synapse number and spine morphology can thus be used to identify/validate targets and/or

small molecules capable of regulating neuronal synapses. Such information would be beneficial for the development of CNS disease therapies.

Disclosures: C.E. Bazenet: None. **L. Micholt:** None. **K.W. Young:** None. **J. Rudolph:** None. **K. Kottig:** None. **H. von der Kammer:** None.

Poster

122. Structural Plasticity and Circuit Remodeling II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 122.12/C8

Topic: B.07. Synaptic Plasticity

Support: GM055145
NS089578

Title: Dendritic spine abnormalities and behavioral deficits in forebrain-specific MARK1 knockout mice

Authors: *E. C. KELLY-CASTRO[1], M. SUN[1], H. ZHANG[2];
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Abstract: Dendritic spines are dynamic postsynaptic structures that play an important role in cognitive functions, such as learning and memory. The dysregulation of spine size, shape and density may lead to learning and memory deficiencies, as well as neurological disorders such as autism spectrum disorders (ASD). Previous studies from our laboratory have demonstrated that partitioning defective 1 c (Par1c), also known as microtubule affinity regulating kinase 1 (MARK1), regulates dendritic spine morphogenesis and plasticity in cultured hippocampal neurons. Interestingly, studies have found multiple single nucleotide polymorphisms of MARK1 associated with ASD and bipolar disorder. However, the role of Par1c/MARK1 in synaptic plasticity and cognitive functions *in vivo* is still unknown. Therefore, we developed a conditional knockout (cKO) MARK1 mouse model to examine the effects of MARK1 depletion on spine morphogenesis and cognitive functions such as learning and memory. In this mouse model, MARK1 is depleted postnatally from pyramidal neurons of the forebrain including the hippocampus and the cerebral cortex. We found that dendritic spine density is significantly decreased in the MARK1 conditional KO mice compared to wild type controls. Furthermore, MARK1 cKO mice show a defect in spatial learning in the Morris water maze test and significantly reduced anxiety in the Elevated Plus Maze. Together, our studies point to an important role for MARK1 in regulating dendritic spine morphogenesis, spatial learning and anxiety-related behavior *in vivo*.

Disclosures: **E.C. Kelly-Castro:** None. **M. Sun:** None. **H. Zhang:** None.

Poster

122. Structural Plasticity and Circuit Remodeling II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 122.13/C9

Topic: B.07. Synaptic Plasticity

Support: NIH R01-MH106489

Title: The K63-specific deubiquitinase CYLD regulates dendritic spine remodeling, synaptic plasticity, and behavior

Authors: ***A. S. ZAJICEK**[1], Q. MA[1], H. RUAN[1], H. DAI[1], S.-C. SUN[2], S. AKBARIAN[3], W.-D. YAO[1];
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Abstract: Ubiquitination is a reversible post-translational modification involved in nearly every cellular process. Although the ubiquitin proteasome system (UPS) is well established to regulate synaptic protein degradation and turnover and has a major role in synapse remodeling and plasticity, the role of non-proteolytic ubiquitination has not been well studied in the CNS. CYLD is a Lysine 63 (K63) deubiquitinase that can specifically cleave non-proteolytic K63-linked polyubiquitin chains attached to protein substrates. CYLD is best known for its role in regulating the NF-κB signaling pathway in the immune system and its causative involvement in familial cylindromatosis, a benign tumor in the head and neck. However, recent studies from us and others have reported that CYLD is enriched in the post-synaptic density (PSD) of excitatory neurons in rodent brains, mediates the deubiquitination of the synaptic scaffold PSD-95, and is recruited to the PSD by neuronal activity. Here, we investigate the role of CYLD in synaptic remodeling and plasticity using a combination of biochemical, cellular imaging, electrophysiology, and behavior techniques. We found that CYLD reduces mature dendritic spine density and dendrite arborization in rodent hippocampal neurons in vitro and *in vivo*, whereas knockdown of CYLD has the opposite effects. Consistently, Western blotting shows that CYLD regulates the levels and clustering of a number of synaptic proteins. Brain slice electrophysiology experiments on CYLD knockout (KO) mice reveals significantly increased synaptic strength and altered short- and long-term plasticity in the mutant hippocampus. Finally, we investigated the behavior of CYLD KO mice and found an increase of depression and anxiety-like phenotypes, and a decrease in behavioral flexibility in mutant mice. Together, our results indicate that CYLD has an important role in synapse development, remodeling, and plasticity, and is potentially involved in certain neurological and psychiatric disorders.

Disclosures: **A.S. Zajicek:** None. **Q. Ma:** None. **H. Ruan:** None. **H. Dai:** None. **S. Sun:** None. **S. Akbarian:** None. **W. Yao:** None.

Poster

122. Structural Plasticity and Circuit Remodeling II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 122.14/C10

Topic: B.07. Synaptic Plasticity

Title: Input-side dependent left-right asymmetry is critical for the formation of experience-dependent laterality in the hippocampus

Authors: M. J. CASE[1], D. KLEINDIENST[1], K. KOBAYASHI[2], ***R. SHIGEMOTO**[1]; [1]IST Austria, Klosterneuburg, Austria; [2]Natl. Inst. For Physiological Sci., Okazaki, Japan

Abstract: Hemispheric asymmetry is a fundamental feature of the mammalian central nervous system, enabling regional specialization of function. The formation of the asymmetry depends both on genetics and experience. In the hippocampus, the left side is more associated with context-dependent episodic memory and the right with spatial memory formation. We discovered input-side dependent asymmetry of synaptic connections between CA3-CA1 pyramidal cells in the stratum radiatum (*sr*), where projections from the left CA3 make smaller synapses with a higher density of NMDA receptor subunit NR2B than those originating from the right CA3 (Kawakami et al., 2003; Shinohara et al., 2008). Shinohara et al (2013) also identified that environmental enrichment induces a right-side specific increase in synapse density in CA1 *sr* in rats, which may reflect right side-dominant formation of spatial memory. Here, we aim to examine how these two different hippocampal asymmetries that depend on genetics and experience, respectively, are related to each other. Environmental enrichment for 6 weeks after weaning induced a significant right-side specific increase in synapse density in mouse CA1 *sr*, confirming the previous results in rats. To investigate the importance of input-side in the right-side specific synapse increase, we performed unilateral injections of AAV expressing venus-VAMP2 into the CA3 area, and examined the terminal densities by measuring fluorescence intensities and synapse densities at the EM level in the ipsilateral and contralateral *sr* in wild-type mice. We found that the contralateral connections from the left CA3 are solely responsible for the right-side specific synaptic density increase. However, no change in synapse density was induced in the *iv* mutant nor PirB knockout mice, which lack the input-side dependent asymmetry (Kawakami et al., 2008; Ukai et al., 2017). These results suggest that the input-side dependent asymmetry plays a critical role in the formation of experience-driven laterality in the brain.

Disclosures: **M.J. Case:** None. **D. Kleindienst:** None. **K. Kobayashi:** None. **R. Shigemoto:** None.

Poster

122. Structural Plasticity and Circuit Remodeling II

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Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 122.15/C11

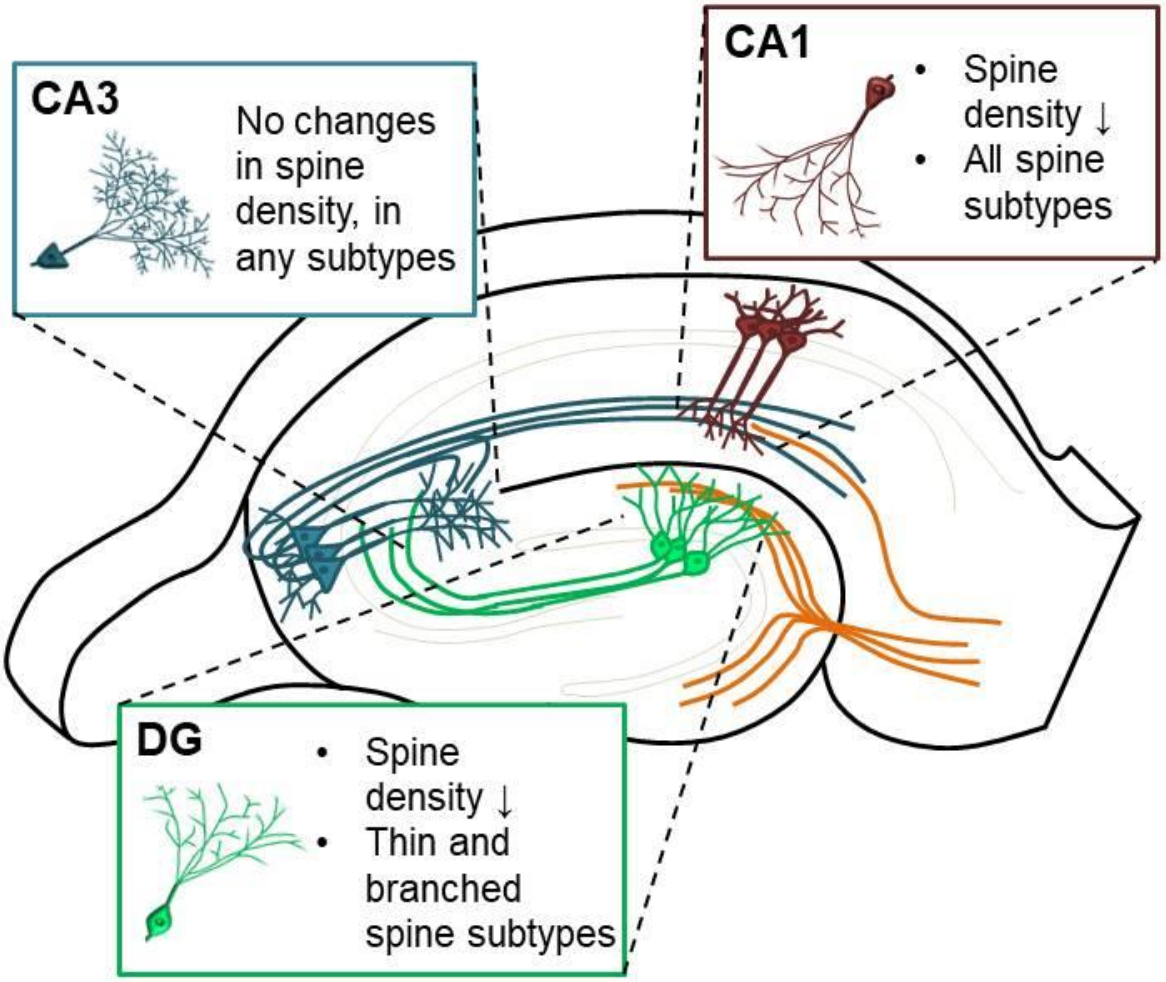
Topic: B.07. Synaptic Plasticity

Support: HFSP Grant RGY0063/2017

Title: A brief period of sleep deprivation leads to dendritic spine loss in the dentate gyrus of the hippocampus

Authors: *F. RAVEN[1], P. MEERLO[1], E. A. VAN DER ZEE[1], T. ABEL[2], R. HAVEKES[1]; [1]Univ. of Groningen, Groningen, Netherlands; [2]Dept. of Mol. Physiol. and Biophysics, Univ. of Iowa, Iowa City, IA

Abstract: Sleep loss is an increasing phenomenon and has a major impact on our lives. For example, even a single night of sleep deprivation (SD) can severely affect hippocampal function. Modifications in synaptic strength directly influence neuronal communication, which is important for normal brain function as well as the processing and storage of information. Five hours of SD impairs hippocampus-dependent memory consolidation, and reduces spine density specifically in hippocampal area CA1, but not area CA3. Because the dentate gyrus (DG) is essential for hippocampus-dependent memory processing, we examined whether a brief period of SD also hampers the dendritic structure in the DG. For this purpose, male C57BL/6J mice were randomly assigned to the control or SD group. SD was performed for five hours using the gentle stimulation method. After sacrifice, brains were impregnated with Golgi stain. Subsequently, coronal sections were analyzed for spine density. We showed that five hours of SD decreases spine density in the DG. Furthermore, SD hampered spine density predominantly in the inferior blade of the DG, while the superior blade was largely unaffected. The reductions in spine numbers were most prominent in the first few branches of the dendritic tree and selective for thin and branched spines. In conclusion, the inferior blade of the DG remarkably seems to be more vulnerable to SD than the superior blade at the level of structural plasticity. The suggestive loss of connectivity in the DG may contribute to memory deficits observed after SD, as structural reorganization in this subregion is fundamental for cognitive processes, such as pattern separation. In addition to sleep loss, these structural impairments often accompany neurodegenerative diseases and, therefore, this study may contribute to the development of new therapeutic approaches to combat disorders that are associated with memory deficits.



Disclosures: **F. Raven:** None. **P. Meerlo:** None. **E.A. Van der Zee:** None. **T. Abel:** None. **R. Havekes:** None.

Poster

122. Structural Plasticity and Circuit Remodeling II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 122.16/C12

Topic: B.07. Synaptic Plasticity

Title: Plastic changes in dendritic spines of hippocampal pyramidal neurons after Pentylenetetrazole treatment

Authors: ***C. E. ROMERO-GUERRERO**[1], N. VÁZQUEZ-HERNÁNDEZ[2], M. FLORES-SOTO[2], F. L. MARTÍN-AMAYA-BARAJAS[2], A. TEJEDA-MARTÍNEZ[2], S. OROZCO-

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Abstract: Epilepsy is characterized by the synchronous discharge of specific neuronal groups in the brain, which is associated with imbalance of neuronal excitatory-inhibitory processes. However, the mechanisms that generate seizures derived from the loss of inhibitory control of neurons firing synchronously are unknown. Adult rats were injected with 65 mg/kg of Pentylenetetrazole, ip. Behavior was video-recorded, and at 30 minutes they were sacrificed for the subsequent study of pyramidal neurons from the hippocampal CA1 field, by the Golgi method. Six neurons per rat were studied, and spines were counted in a dendritic segment of 50 μm in length per neuron. All the experimental animals presented stereotyped activity, anterior limb clonus, and tonic-clonic seizures before 30 minutes. The experimental animals showed more spines than controls, whilst the proportional density of thin spines decreased concomitantly with an increase in mushroom, stubby and wide spines. The inhibition of the GABAergic activity induced by Pentilentetrazol could provoke disinhibition of the glutamatergic neurotransmission that occurs on dendritic spines. Accordingly, a likely synaptic potentiation (increase of mushroom spines) is suggested, as well as an over-excitation possibly mediated by the greater amount of stubby and wide spines.

Disclosures: C.E. Romero-Guerrero: None. **N. Vázquez-Hernández:** None. **M. Flores-Soto:** None. **F.L. Martín-Amaya-Barajas:** None. **A. Tejeda-Martínez:** None. **S. Orozco-Suarez:** None. **I. González-Burgos:** None.

Poster

122. Structural Plasticity and Circuit Remodeling II

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Topic: B.07. Synaptic Plasticity

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Title: Functional and anatomical specificity of spinogenesis in the motor cortex during motor learning

Authors: *N. G. HEDRICK[1,2,3,4], Z. LU[1,2,3,4], E. A. BUSHONG[3,5], P. YAO[3], Y. XUAN[6], B. LIM[1], M. H. ELLISMAN[3,5], T. KOMIYAMA[1,2,3,4];
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Abstract: Plasticity in the motor cortex is thought to underlie the flexible generation of novel skilled motor patterns. Motor learning accompanies the formation of new dendritic spines in the motor cortex, but how the new spines modify the existing circuit is unknown. Using longitudinal 2-photon imaging of either GCaMP6f or iGluSNFR, we monitored spinogenesis and how it relates to the activity of nearby dendritic spines on the apical dendrites of layer 2/3 excitatory neurons in the primary motor cortex while a mouse learned a cued lever-press task. We found that the presence of task-related synapses in early learning sessions predicted nearby spine additions on the same dendrite. The pairs of new spines and nearby task-related spines show higher activity correlation than distance-matched pairs, especially during movements, indicating that such spine pairs form "clusters" that are co-active during movements. Furthermore, co-activation of such clusters tended to coincide with movements that are similar in kinematics and successful at achieving a reward, suggesting that cluster co-activation represents the engagement of a learning-related circuit for the generation of more stereotyped behavior. In order to understand the local connectivity rules of new clustered spines that form during learning, we performed correlated light- and electron microscopy (CLEM) to investigate with EM the cellular micro-environment of dendrites imaged *in vivo* during learning. We found that new spines form structural synapses, supporting the notion that such observations acquired from in vivo imaging represent meaningful changes to the circuit. By tracing the presynaptic axon contacting the new spines, we found that a subset of these synapses connected with axons contacting another, nearby task-related spine, suggesting that some synaptic clusters reflect the duplication of axonal contacts on a single dendrite. Taken together, these data reveal a functional principle in which task-related spine activity during learning guides the formation of nearby new spines, some of which contact the same task-related axon, to generate local clusters of synapses with similar functional roles.

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Poster

122. Structural Plasticity and Circuit Remodeling II

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Program #/Poster #: 122.18/C14

Topic: B.07. Synaptic Plasticity

Support: NIH R01DC05640
NIH R01NS104911
NIH P41GM103412

Title: Toric spines on space-specific neurons: Location, expression and intracellular organization

Authors: D. SANCULI[1], P. HARRAST[1], K. PANNONI[2], T. SHAMLOO[1], E. A. BUSHONG[3], M. H. ELLISMAN[4], ***W. M. DEBELLO**[1];
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Abstract: Toric spines are novel dendritic protrusions found on a subset of space-specific neurons (SSNs) in the barn owl auditory localization pathway. Using serial blockface scanning electron microscopy (SBFSEM), we previously reported the unusual ultrastructure of toric spines and their unprecedented capacity for synaptic convergence - up to 11 different axons innervating a single spine. Here we investigate the precise location of toric SSNs within the auditory space map. This map is constructed from convergence in the axonal projections from the lateral shell of the central nucleus of the inferior colliculus (ICCls) onto SSNs in the external nucleus (ICX). We labeled toric and non-toric SSNs via injection of retrograde tracer in the deep layers of optic tectum (OT), which receives a monosynaptic connection from both SSN populations. All labeled cells were mapped relative to cytoarchitectonic markers calbindin, calretinin and CaMKII. Consistent with previous results, most SSNs were located within ICX with a minority found in ICCls; both toric and non-toric SSNs were found in each. Preliminary data indicate that the plasticity protein CaMKII is expressed in some, but not all, toric spines and is independent of CaMKII expression in the soma and dendrites. These results are consistent with previous studies implicating toric spines as a microstructural locus of learning. To further investigate their intracellular organization we partially reconstructed unlabeled neuropil in the SBFSEM volume. Dendrites studded with toric or typical spines could be clearly identified along with intracellular organelles including mitochondria and cytoskeletal elements. Quantitative comparison of the prevalence and volumetric density of these internal structures is underway. In total, our localization of toric spines with the ICCls-ICX microcircuit lays a foundation for identifying the sources of axonal input, and characterization of intracellular organization provides the first glimpse into the inner workings of a highly unusual synaptic integrator. Future work will be aimed towards determining whether the patterns convergence onto toric spines are adaptably adjusted during prism-induced plasticity, and whether this is accompanied by internal rearrangement.

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Poster

122. Structural Plasticity and Circuit Remodeling II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 122.19/C15

Topic: B.07. Synaptic Plasticity

Support: BBSRC-UK
MRC-UK

Title: Diazepam-induced breakdown of inhibitory synapses mediated by phospholipase C delta/calcium/calcineurin signalling pathway

Authors: M. W. NICHOLSON[1], A. B. ALI[1], M. R. DUCHEN[2], ***J. N. JOVANOVIC**[1]; [1]UCL Sch. of Pharmacy, Univ. Col. London, London, United Kingdom; [2]Biosci., Univ. Col. London, London, United Kingdom

Abstract: Benzodiazepines facilitate the inhibitory actions of GABA by binding to GABAA receptors (GABAARs), ligand-gated chloride/bicarbonate channels, which are the key mediators of transmission at GABAergic synapses in the brain. Here we report that prolonged exposure to diazepam, the most widely used benzodiazepine in clinic, leads to a gradual breakdown of GABAergic synapses. The loss of synapses and the preceding, time- and dose-dependent decrease in surface levels of GABAARs, mediated by dynamin-dependent internalisation, were blocked by Ro 15-1788, a competitive benzodiazepine antagonist, and bicuculline, a competitive GABA antagonist, indicating that prolonged enhancement of GABAAR activity by diazepam is integral to the underlying molecular mechanism. Characterisation of this mechanism has revealed a metabotropic-type signalling downstream of GABAARs, involving mobilisation of intracellular calcium and activation of the calcium/calmodulin-dependent phosphatase calcineurin, which promotes their endocytosis leading to disassembly of inhibitory synapses. Functional coupling between GABAARs and intracellular calcium stores was sensitive to phospholipase C (PLC) inhibition, and regulated by PLCδ, a PLC isoform found in direct association with GABAARs. Thus, a PLCδ/calcium/calcineurin signalling converts the initial enhancement of GABAARs by benzodiazepines to a long-term downregulation of GABAergic synapses, this potentially underpinning the development of pharmacological and behavioural tolerance to these widely prescribed drugs.

Disclosures: **M.W. Nicholson:** None. **A.B. Ali:** None. **M.R. Duchen:** None. **J.N. Jovanovic:** None.

Poster

122. Structural Plasticity and Circuit Remodeling II

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Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 122.20/C16

Topic: B.07. Synaptic Plasticity

Support: Mary Tucker Currie Grant to JWG

Title: Memories at the neuromuscular junction are resistant to extinction

Authors: *M. M. TARBET[1], K. HUDSON[3], E. LOUT[2], J. GRAU[1];
[1]Psychological and Brain Sci., [2]Biomed. Sci., Texas A&M Univ., College Station, TX; [3]Texas A&M Hlth. Sci. Ctr., College Station, TX

Abstract: Prior studies have shown that neurons within the spinal cord can support instrumental learning (Grau et al., 1998, *Behav. Neurosci., 112*, 1366). In a typical experiment, rats undergo a thoracic (T2) transection and are subsequently tested by applying electrical stimulation (shock) to one hind leg whenever the leg is extended. Over time, transected animals learn to maintain the stimulated leg in a flexed position that minimizes net shock exposure. Treatments that impair spinal cord function (e.g., i.t. lidocaine), or that disrupt communication between the periphery and the spinal cord (e.g., cutting the sciatic nerve), block learning. Interestingly, if communication with the spinal cord is cut after the response is acquired, animals given response-contingent shock continue to maintain the leg in a flexed position. This implies that a peripheral modification contributes to the maintenance of the learned response. The present study explores the circumstances under which the learned response is weakened (extinguished). In Experiment 1, rats underwent a spinal transection and were trained for 30 min with response contingent shock the next day. After 25 min of training, half the animals had the sciatic nerve cut. Performance was tested for an additional 30 min, with or without response-contingent shock. During testing, all animals continued to maintain a flexion response. Next (Experiment 2), we examined whether exposure to non-contingent stimulation would weaken the learned response. Spinally transected rats received 30 min of training followed by 6 min of variable intermittent shock given independent of leg position. Performance was then assessed for 30 min. Exposure to non-contingent shock had no effect on the maintenance of the learned response. Further work is being conducted to examine whether a longer period of non-contingent stimulation, applied to other dermatomes, will lead to the extinction of the learned response.

Disclosures: **M.M. Tarbet:** None. **K. Hudson:** None. **E. Lout:** None. **J. Grau:** None.

Poster

122. Structural Plasticity and Circuit Remodeling II

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Topic: B.07. Synaptic Plasticity

Support: NIH-RISE (R25-GM061838)
NIH-COBRE (2P20GM103642)

Title: Patterned activation of the temperature-gated TRPA1 channel promotes rapid morphological changes at the *Drosophila* neuromuscular junction

Authors: *C. MALDONADO-DIAZ, B. MARIE;
Anat. and Neurobio., Inst. of Neurobio. - UPR Med. Sci. Campus, San Juan, Puerto Rico

Abstract: Activity-dependent changes in synaptic structure and function are thought to be the basis of learning and memory. The *Drosophila* glutamatergic neuromuscular junction (NMJ) has been used as a model to study the molecular mechanisms underlying activity-dependent plasticity. By performing a stimulation paradigm consisting of patterned depolarizations, the NMJ undergoes rapid changes similar to those seen in dendritic spines in mammalian hippocampal cultures. A challenge remains to identify a stimulation paradigm able to promote activity-dependent plasticity in intact animals. Here, we study animals expressing the temperature-gated calcium channel TRPA1 in motor neurons and submitted to constant or patterned temperature shifts. We ask whether these transgenic flies can be used to study activity-dependent plasticity. We first exposed TRPA1 expressing larvae to constant 29°C temperature for 90 minutes. We found that this approach is not sufficient to promote a significant increase in structural changes when compared to control NMJs submitted to the same temperature shifts. We then submitted larvae to patterned stimulations (5 pulses of temperature shift from 21°C to 29°C within 90 minutes) and showed that this stimulation protocol was able to promote structural changes at the NMJ. Surprisingly, we noticed that prolonged rest after stimulation allowed for increased structural changes not only in TRPA1 expressing NMJs, but also in control NMJs. This suggests that acute temperature increase alone may have an effect on the neuronal activity driving structural changes. To limit this effect, we tested a paradigm using the smallest degree of temperature change capable of controlling TRPA1 activation (5 pulses from 23°C to 27°C within 90 minutes). We showed that this was sufficient to promote morphological changes. More importantly, prolonged rest showed no further increase in structural changes in controls or transgenic NMJs, showing that a high degree of temperature change alone can account for the structural modifications. In conclusion, we demonstrate that TRPA1 activation works as a tool to promote structural changes. However, we found that acute temperature shifts can alter the structure of the NMJ. Interestingly, evidence suggests that the mammalian brain is exposed to

temperature fluctuations due to blood flow, provoking functional consequences on synaptic efficacy. Similarly, work on hippocampal cell cultures showed that acute temperature shifts modulate short term synaptic plasticity properties. We now plan to use the *Drosophila* NMJ to further evaluate the effects of acute temperature shifts on neuronal activity.

Disclosures: C. Maldonado-Diaz: None. **B. Marie:** None.

Poster

122. Structural Plasticity and Circuit Remodeling II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 122.22/C18

Topic: B.07. Synaptic Plasticity

Support: NIH RISE R25-GM061838
NIH COBRE 2P20GM103642

Title: The role of the planar cell polarity pathway in regulating activity-dependent synaptic plasticity

Authors: *C. M. DOMINICCI-COTTO[1], B. MARIE[2];
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Abstract: Synapses are shaped by plastic events that promote or limit changes in synaptic strength. Modifications in synaptic strength due to electrical activity are often accompanied by structural changes in synapse shape and number. At the *Drosophila* neuromuscular junction (NMJ) activity-dependent plasticity is characterized by the apparition of new synaptic structures after repeated stimulation. Our lab recently showed that these activity-dependent modifications are regulated presynaptically by Cortactin, a cortical actin binding protein, involved in regulating cytoskeletal dynamics and controlled by the Wnt/wingless (wg) signaling. Although the wg canonical pathway has been implicated in activity-dependent synaptic plasticity, it is still unclear whether the wg planar cell polarity pathway (PCP) is necessary for this phenomenon. We asked whether core molecules of the wg PCP pathway, which regulate cytoskeletal dynamics, can be involved in this process. The wg PCP pathway is composed of a series of small GTPases (Rac, Rho and Cdc42) which are downstream of disheveled (dsh) and upstream of JNK. Here, we looked at the appearance of de novo boutons formation after repeated stimulation in mutants and animals expressing the dominant-negative or constitutively-active form of some members of the PCP pathway. We found that dsh^1 mutants, which contain a PCP-specific mutation, had hindered plasticity after stimulation, suggesting that the PCP pathway has a role in this plasticity. We also studied diablo (dbo), a molecule that interacts with dsh to promote the PCP pathway; dbo mutants showed a reduction in the formation of new boutons upon stimulation. We also found

that late expression of constitutively-active Rac1 and dominant-negative Rho1 resulted in over-plastic synapses. In contrast, altering Cdc42 function did not affect activity-dependent synaptic plasticity. We hypothesize that, like for growth cone formation, Rac1 promotes protrusions formation during activity-dependent plasticity, while Rho1 is able to mediate retractions. In addition, we present data suggesting that JNK activity can also affect activity-dependent plasticity. Taken together our data demonstrate that the PCP pathway is essential to the regulation of activity-dependent synaptic plasticity.

Disclosures: C.M. Dominicci-Cotto: None. **B. Marie:** None.

Poster

122. Structural Plasticity and Circuit Remodeling II

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Topic: B.07. Synaptic Plasticity

Support: DFG Grant, SFB1158

Title: Visual deprivation-induced plasticity of electrical coupling

Authors: D. E. KOSER, ***M. WEINREICH**, H. MONYER;
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Abstract: Numerous studies have linked physiological adaption and pathological maladaptation processes to plastic changes at chemical synapses. Cortical interneurons, communicate not only by chemical but also by electrical synapses. Plasticity mediated by electrical synapses can be induced in an *in vitro* setting. However, the question remains whether altered electrical coupling in cortical interneurons occurs in an *in vivo* behavioural paradigm. Thus, we set out to investigate plasticity of electrical synapses between cortical interneurons induced by physiological stimuli. We focused on the visual cortex comparing visual deprived mice and mice housed in a normal light-dark cycle. This stimulus was chosen as it is a physiological, benign and strong stimulus. We first performed paired patch-clamp recordings of interneurons in the visual cortex in control and 24h visual deprived animals. We observed significant changes in electrical coupling between interneurons that belong to defined subtypes. The strongest effect was found between fast-spiking, putative parvalbumin-expressing (PV^{+}) interneurons, where the coupling decreases more than 5-fold after visual deprivation. Additional experiments indicated that this effect reaches a plateau after 4h of visual deprivation and can be reversed by light exposure. To reveal the underlying mechanism, we used sparse labelling in PV^{Cre} x $Cx36^{EGFP}$ mice to define the density, size and distribution of electrical synapses within PV^{+} interneurons. Preliminary results indicate

that plasticity of electrical synapses can be induced by a physiological stimulus and involves structural alterations.

Disclosures: D.E. Koser: None. **M. Weinreich:** None. **H. Monyer:** None.

Poster

123. Epilepsy: Human Studies

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 123.01/C20

Topic: B.10. Epilepsy

Support: U01-NS090407
G0802012
MR/M00841X/1

Title: Brain morphometric alterations accompanying generalized tonic clonic seizures with hypoxemia

Authors: *L. A. ALLEN[1], R. M. HARPER[2], S. VOS[1], C. A. SCOTT[1], L. VIELLA[4], N. LACUEY[4], J. S. WINSTON[1], B. WHATLEY[1], R. KUMAR[5], J. A. OGREN[3], G. WINSTON[1], S. LHATOO[4], L. LEMIEUX[1], B. DIEHL[1];
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Abstract: Rationale:
Generalized tonic-clonic seizures (GTCS) are the leading risk factor of sudden unexpected death in epilepsy (SUDEP). People who succumb to SUDEP show neuronal injury in autonomic and respiratory regulatory brain sites, although the mechanisms underlying such injury remain unknown. A failure of oxygenation to cells undergoing high metabolic demands during ictal events, either from apnea of from reduced perfusion via profound blood pressure loss, establishes a scenario for excitotoxic injury and impaired ability of regulatory sites to recover.
Methods:
Peripheral oxygen saturation (sPO2) measurements were obtained during long-term epilepsy monitoring of 43 GTCS patients with continuously-collected pulse oximetry. Regional brain morphometric (gray and white matter volume) alterations, relative to extent and duration of peri-ictal reductions in sPO2 (hypoxemia) in patients with GTCS were assessed using voxel-based morphometry (VBM) and segmentation-derived measurements of regional volume. N=43 healthy controls were included for comparison. Age and sex were used as covariates, and p-values were family-wise-error-rate (FWER) corrected ($p<0.05$).
Results:

Compared to controls, GTCS with hypoxemia showed gray matter volume loss in the bilateral thalamus, hypothalamus, cerebellum, vermis, periaqueductal gray, temporal pole and cingulate. GTCS without hypoxemia showed lateral cerebellar gray matter loss only. Subjects with severe hypoxemia (<75% O2 saturation) showed the most extensive thalamic gray matter loss, and extent of peri-ictal O2 loss correlated negatively with left ($r = -0.429$, $p = 0.03$) and right ($r = -0.468$, $p = 0.02$) thalamic volume. White matter loss in the medulla and parabrachial pons appeared in GTCS with hypoxemia, compared with controls.
Conclusions:
GTCS with hypoxemia are accompanied by volume alterations to autonomic- and respiratory-regulatory brain regions. Loss was most extensive within the thalamus and declined with severity of hypoxemia. This study may provide insights into pathomechanisms underlying brain structural alterations in SUDEP, and may clarify the nature of strategies that might be incorporated to prevent neural injury during ictal events.

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Poster

123. Epilepsy: Human Studies

Location: Hall A

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Program #/Poster #: 123.02/C21

Topic: B.10. Epilepsy

Support: the Intramural Research Grants (28-4) of NCNP
Challenging Exploratory Research (25670486) of JSPS

Title: Expression of astrocyte related receptors and channels in epilepsy lesions

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Abstract: Epilepsy is one of the major neurologic diseases, and astrocytes play important roles in epileptogenesis. To investigate possible roles of astrocyte-related receptors in patients with intractable epilepsy associated with focal cortical dysplasia (FCD), hippocampal sclerosis (HS) and other conditions, we examined resected epileptic foci from 51 patients, including 23 FCD (including type I, IIa, and IIb), 20 HS, 5 tuberous sclerosis complex, and 3 low-grade astrocytoma. Control samples were from 21 autopsied brains of patients without epilepsy or neurologic deficits and 5 patients with pathologic gliosis without epilepsy. Immunohistochemical and immunoblot analyses with antibodies against purinergic receptor subtypes P2RY1, P2RY2,

P2RY4, potassium channels of Kv4.2 and Kir4.1, and metabotropic receptors of mGluR1 and mGluR5 were performed. Anti-glial fibrillary acidic protein (GFAP), anti-NeuN, and anti-CD68 immunostaining was used to identify astrocytes, neurons, and microglia, respectively. Most GFAP-immunopositive astrocyte cells in the brain samples from patients with epilepsy were P2RY1-, P2RY2-, P2RY4-, Kv4.2-, Kir4.1-, mGluR1-, and mGluR5-positive, whereas samples from controls and pathologic gliosis showed lower expression levels of these astrocyte-related receptors. The findings suggest that, although these receptors are necessary for astrocyte transmission, formation of the neuron-glia network, and other physiologic functions, overexpression in the brains of patients with intractable epilepsy may be associated with activation of intracellular and glio-neuronal signaling pathways that contribute to epileptogenesis.

Disclosures: M. Itoh: None.

Poster

123. Epilepsy: Human Studies

Location: Hall A

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Topic: B.10. Epilepsy

Support: KAKENHI 15K06751
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KAKENHI 19H03542

Title: Epileptogenesis of the subiculum associated with hippocampal sclerosisin patients with MTLE: *Ex vivo* optical imaging study

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Abstract: Introduction: Mesial temporal lobe epilepsy (MTLE) is the most frequent focal epileptic syndrome in adults, and the majority of seizures originate primarily from the hippocampus. The resected hippocampal tissueoften showssevere neuronal loss as that referred to hippocampal sclerosis (HS).Accordingly, there isa paradox between the clinical and pathological features: why should epilepsybe derived from such degeneratedtissue? Here we investigated epileptiform activities *ex vivo*using living hippocampal tissuetaken from patientswith MTLE. **Methods:** We prepared acute brain slices from patients with MTLE within 45 min after resection, and opticalimaging orlocal field potential recordings (LFP) was performed *ex vivo*. We also used a brain block corresponding to the mirror surface of each slice

and performed histopathological examination. **Results:** We revealed that epileptiform activities developed from the subiculum, regardless of the existence of HS. We found spontaneousrhythmic activitiesin the subiculum and detected discrete component of high frequency oscillations (HFO), a clinical biomarker of the ECoG suggesting the epileptogenic regions.Immunohistochemistry of the HS tissue revealed loss of inwardly rectifying K+channel 4.1 (Kir 4.1) in astrocytes in the subiculum, indicating failure of the extracellular K+buffering and possible association with neuronal hyperexcitability. **Conclusion:** These results indicate that pathophysiological alterations involving the subiculum could be responsible for epileptogenesis in patients with MTLE.

Disclosures: H. Kitaura: None. **H. Shirozu:** None. **H. Masuda:** None. **M. Fukuda:** None. **Y. Fujii:** None. **A. Kakita:** None.

Poster

123. Epilepsy: Human Studies

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Topic: B.10. Epilepsy

Support: 220365 of CONACyT (México)

Title: Evaluation of REST/NRSF expression and its transcriptional repression effect on the GRIA2 and GABRD genes in the hippocampus of patients with temporal lobe epilepsy

Authors: *V. NAVARRETE-MODESTO, Sr[1], S. OROZCO-SUAREZ[1], M. ALONSO-VANEGAS[2], I. FERIA-ROMERO[1], L. ROCHA[3];
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Abstract: Rational. REST/NRSF acts as a transcriptional repressor of neuronal genes when it binds to its consensus sequence (site RE1) in DNA. Some reports show that after seizures induction in animals models, there is an overexpression of REST/NRSF. *In vivo* and *in vitro* studies have shown that the GRIA2 and GABRD genes possess the RE1 sites in their promoter regions.**Aim.** Evaluate the expression of REST/NRSF in hippocampus of patients with Mesial Temporal Lobe Epilepsy (MTLE), its regulatory function on GluR2 and GABRD genes and investigate the correlation with clinical and psychiatric variables.**Methods:** Hippocampal tissue was obtained from patients with MTLE (n=28) and autopsy hippocampus as a control was used (n=13). The mRNA and protein expression of REST/NRSF, GRIA2 (GluR2) and GABRD ($GABA_{A\delta}$) was evaluated by real-time PCR and Western Blot respectively. The binding of

REST/NRSF to the GRIA2 and GABRD promoters was evaluated by chromatin immunoprecipitation (ChIP). Finally, the correlation with clinical and psychiatric variables was evaluated.
Results: The expression of REST/NRSF mRNA increasing 141.63% ($p<0.05$) and protein levels increasing 353% ($p<0.05$). The correlation analysis revealed that the higher the frequency of epileptic seizures, the higher the protein levels of REST/NRSF ($r=0.658$, $p<0.05$). No correlations was found with other clinicals variables. The patients with MTLE plus mood disorders exhibited an overexpression of REST/NRSF in mRNA (55% vs autopsies, $p<0.05$) and protein (148% vs autopsies, $p<0.05$). Fifteen patients had MTLE without psychiatric disorders. The hippocampus of these patients showed a more evident overexpression of REST/NRSF (mRNA, 171%, $p<0.001$ vs autopsies; protein 230%, $p<0,001$ vs autopsies). It was shown that REST/NRSF binds to the RE1 site in the promoters of GRIA1 (3.37% more than autopsies) and GABRD (4.37% more than autopsies), which correlated with a 41% ($p<0.05$) and 37% ($p<0.05$) of decrement in the transcriptional expression respectively. **Conclusion:** REST/NRSF is overexpressed in the hippocampus of patients with MTLE which depends on the frequency of seizures and underlies the development of psychiatric comorbidities. Overexpression of this repressor transcription factor is important in the regulation of GRIA2 and GABRD genes in the pathophysiology of MTLE.

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Poster

123. Epilepsy: Human Studies

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Fondation pour la recherche médicale (FDM20170839111)
Fondation Assistance Publique - Hôpitaux de Paris (EPIRES)

Title: Brain cholesterol metabolism as new pathway biomarkers of status epilepticus

Authors: *A. HANIN[1,2], P. BAUDIN[1], S. LECAS[3], D. ROUSSEL[1], E. TEYSSOU[4,1], M. DAMIANO[5], D. LUIS[1], V. LAMBRECQ[3,1,5], I. PLU[3,6], B. MATHON[3,7,1], D. BONNEFONT-ROUSSELOT[10,8], S. DEMERET[9], R. BITTAR[8], F. LAMARI[8], V. NAVARRO[3,5,1];
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Abstract: Status epilepticus (SE) is a life-threatening prolonged epileptic seizure that requires benzodiazepines and antiepileptic drugs. In around 25% of cases, SE is refractory to these drugs and requires anesthetic drugs. In refractory SE (RSE), the excessive and sustained activation of neurons may induce excitotoxic processes. As a result, patients with RSE may present focal or diffuse brain atrophy (MRI), especially in hippocampal structures, with subsequent severe neurological sequels. One previously unexplored mechanism participating in the excitotoxicity observed in SE may involve the homeostasis of cholesterol in the brain. Brain cholesterol is synthetized *de novo* by astrocytes and transported by apolipoprotein E (ApoE) to neurons, where it is metabolized in 24-hydroxycholesterol (24-OHc) by the cholesterol-24 hydroxylase enzyme (CYP46A1). We recently reported that a viral AAV vector that codes for a shRNA, blocking the expression of CYP46A1, resulted in progressive neuronal loss and epileptic abnormalities when injected in the mice hippocampus. We found that these cellular and EEG modifications were similar as those observed in humans and animal models of RSE. We speculated that the accumulation of cholesterol in the neuronal membranes during SE might increase their viscosity and decrease the mobility of the neurotransmitter receptors out of the synapses, thereby increasing excitotoxicity and focal neuronal death. To test this hypothesis, we have set up a LC/MS-MS method to screen cholesterol content and a wide panel of its precursors and metabolites in both mice and humans. We studied the homeostasis of cholesterol in mice brains through inducing SE by means of acute intrahippocampal injection of kainic acid (compared to PBS). We also collected blood samples, cerebrospinal fluid and post mortem brain tissue of patients suffering from SE and from a control population. Moreover, we performed immunohistochemically analysis reflecting brain cholesterol homeostasis. We identified changes in brain cholesterol metabolism in the mice model, namely a decrease of 24-OHc levels and an increase of desmosterol levels in the hippocampus. A decrease of 24-OHc levels in serum and an increase of the synthesis of the cholesterol were also found in patients compared to controls. Additionally, we noticed disturbances in lipid panels, notably the increase of ApoE in the blood of SE patients. Finally, immunohistochemically analysis showed neuronal death and gliosis in both mice and patients. To conclude, our results show the increase of the synthesis of brain cholesterol as a consequence of SE. We speculate that statin might provide a neuroprotective effect in SE.

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Poster

123. Epilepsy: Human Studies

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Support: National Natural Science Foundation of China (81871064)
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Title: Kininogen as a biomarker and a possible therapeutic target during epileptogenesis

Authors: *A. GHOSH[1], J. ZOU[2], Q. WANG[1], Q. LU[2], S. LI[1];
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Abstract: There is no known biomarker for predicting epilepsy before its onset (*i.e.* the *epileptogenesis* period). In this study, we aimed to explore the possible use of novel proteins as potential serum or cerebrospinal fluid (CSF) biomarkers during epileptogenesis. To do this, proteomic analysis was employed in the CSF from the LiCl-Pilocarpine-induced epileptic rats after 5 days of inducing status epilepticus (SE). As a result, 6 proteins, including Kininogen (KNG) 1 and 2, were found to be increased in the CSF of the LiCl-Pilo-treated rats compared to the controls. Higher level of KNG in the CSF was also confirmed by ELISA: LiCl-Pilocarpine (2.61 ± 0.41 µg/ml, $n = 10$), LiCl (0.98 ± 0.40 µg/ml, $n = 11$) ($p = 0.031$, one-way ANOVA) and naïve groups (0.21 ± 0.3 µg/ml, $n = 7$) ($p = 0.001$, one-way ANOVA). Western blot detection of KNG also revealed a higher expression of this protein in the hippocampus of epileptic rats: LiCl-Pilo (4.42 ± 0.84 fold, $n = 4$), LiCl (2.03 ± 0.76 fold, $n = 4$) ($p = 0.002$, one-way ANOVA), compared to naïve animals ($p < 0.001$, one-way ANOVA, $n = 4$). However, serum level of KNG was not significantly different between the groups. KNG is the precursor molecule of the kallikrein-kinin system that has been implicated in the pathophysiology of brain disorders such as Alzheimer's and brain cancer such as glioma. In order to investigate whether KNG in CSF could be a biomarker for epileptogenesis in human patients, we further enrolled 12 patients (9 male, 3 female, 17-77 yrs) with encephalitis and 5 control patients (1 male, 4 female, 3 with headache, 1 with depression, and 1 with schizophrenia, 19-39 yrs) in our study. ELISA study for KNG was done on the CSF and serum collected during the postacute phase of encephalitis, and a 2-year follow-up was done for any possible development of epilepsy in these patients. The results revealed a significantly higher expression of KNG in the CSF of encephalitis patients (1.89 ± 0.22 µg/ml, $n = 12$) compared to the controls (1.07 ± 0.09 µg/ml, $n = 5$) ($p < 0.01$, t-test). Interestingly, in the encephalitis patients, higher KNG levels in the postacute phase was detected in the CSF who developed epilepsy during the 2-year follow up than that of patients who did not

develop epilepsy. In contrast, serum level of KNG was not significantly different between the groups, a finding similar to that of in the animal study. Taken together, our data suggests KNG as a potential CSF biomarker for epileptogenesis and gives a possible insight that this protein might play an important role in the pathophysiology of epilepsy. Through overexpression or knockdown studies of KNG in the rat hippocampus, the involvement of this protein in epileptogenesis is our ongoing investigation.

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Poster

123. Epilepsy: Human Studies

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Topic: B.10. Epilepsy

Support: CNPq
Fapemig

Title: Altered intracelular signaling pathways in the PBMC of patients with temporal lobe epilepsy

Authors: *M. SILVA, F. AMARAL, A. GONÇALVES, A. TEIXEIRA, É. VIEIRA, A. DE OLIVEIRA;
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Abstract: INTRODUCTION: Epilepsy is a neuronal disorder with a high prevalence. The inflammatory process can induce neuronal damage and contribute to the etiopathogenesis and recurrence of seizures in epilepsy. Thephosphatidylinositol-3-kinase/mechanistic target of rapamycin (PI3K/*mTOR*) pathway regulates the production of inflammatory mediators by immune cells and may be involved in epilepsy.In this context, we have investigated if peripheral mononuclear cells of patients with Temporal Lobe Epilepsy (TLE) reveal deregulations in the PI3K/mTOR pathway, which may contribute to abnormal production and release of inflammatory mediators. **METHODS:** Blood samples were taken from patients with TLE and non epileptic subjects, aged between 18 and 65 years old, who agreed to participate by signing the Informed Consent. In addition to the peripheral blood, we collected the medical history and sociodemographic data, as well as we measured the weight and height of the patients. Peripheral blood was collected in vacuum tubes containing sodium heparin and was centrifuged.The cells were subjected to culture in the presence or absence of blocker of the enzymes PI3K and mTOR, and were subsequently stimulated byphytohaemagglutinin (PHA). Cytokines were evaluated by using Human Inflammatory CBA method. This project was approved by the Research Ethics Committee - CEP (Nº147543/2013). **RESULTS:** The basal levels of TNF were different in

patients with TLE. When the PBMC was stimuled by PHA, the levels of IL-10 and TNF was elevated just in TLE patients. In addition, inhibition of PI3K and mTOR resulted in the decrease of the levels of TNF and IL-10 in the PBMC epileptic patients cells. **CONCLUSION:** In the present study, we have demonstrated that both pro- and anti-inflammatory mediators can be altered in patients with epilepsy, and cytokine production by immune cells is controlled differently in cells from healthy controls. The alteration in the levels of these mediators could contribute for the pathogenesis of epilepsy and for the refractoriness to the treatment. However, further studies are necessary to better understand the role of these cytokines in the pathophysiology of TLE. **ACKNOWLEDGMENT:** Fapemig and CNPq

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Poster

123. Epilepsy: Human Studies

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Topic: B.10. Epilepsy

Support: Consejo Nacional de Ciencia y Tecnología Grant 261481
Consejo Nacional de Ciencia y Tecnología Scholarship 347414

Title: Cannabinoid receptors in microvessels of the blood-brain barrier of the temporal cortex and hippocampus of patients with drug-resistant temporal lobe epilepsy

Authors: M. NUÑEZ-LUMBRERAS[1], F. CARMONA-CRUZ[1], V. SANCHEZ-VALLE[1], M. A. ALONSO VANEGAS[2], R. GUEVARA-GUZMAN[3], *L. L. ROCHA[4];
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Abstract: In the blood brain barrier (BBB), the activation of CB1 and CB2 receptors (CB1R and CB2R) has been suggested to protect against damage. The breakdown of the BBB is a common feature of epilepsy. However, at present it is unknown the CB1R and CB2R expression and activation in the BBB of subjects with drug resistant epilepsy. The aim of this study was to characterize the expression and activation of CB1R and CB2R in BBB microvessels (MVs) of brain tissue obtained from patients (n=12) with drug-resistant temporal lobe epilepsy (DRTLE). Hippocampus and temporal neocortex samples were collected immediately after their surgical resection. The results obtained were compared with those from autopsies (n=12). MVs were isolated through the homogenization and centrifugation of the brain tissue. MVs were submitted to the following experiments: a) western blot to determine the protein expression of CB1R and

CB2R; and b) binding assay with [^{35}S] GTPγS to estimate CB1R and CB2R-induced G protein activation (Emax and pEC50). Western blot experiments revealed a decreased expression of CB1R and CB2R in hippocampus (61%, $p<0.001$; 74%, $p<0.01$, respectively). In contrast, temporal neocortex showed a high protein expression (CB1R, 86%, $p<0.01$; CB2R, 149%, $p<0.01$). The binding assay with [^{35}S]-GTPγS did not reveal significant differences between autopsies and DRTLE subjects, neither the maximum stimulation values (Emax), nor potency of stimulation (pEC50) in both, hippocampus (CB1, $p<0.1$; CB2, $p<0.1$) and temporal neocortex (CB1, $p<0.1$; CB2, $p<0.1$). The present study provides for the first time the estimation of expression and G protein activation of CBR1 and CBR2 in the MV of patients with DRTLE. The protein expression of CB1R and CB2R depended on the brain area evaluated. Nevertheless, the functionality of these receptors was maintained at autopsy values. Further in vitro experiments are necessary to determine the specific role of CBR1 and CBR2 in the integrity of BBB of patients with DRTLE.

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Poster

123. Epilepsy: Human Studies

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BONFOR

Title: CRISPR mediated manipulation of epilesy associated genes and its application in mouse and human organotypic hippocampal slice cultures

Authors: *D. TSORTOUKTZIDIS[1], K. M. VAN LOO[1], S. SCHOCH[1], H. KOCH[2], A. J. BECKER[1];
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Abstract: Precise genome editing in combination with viral delivery systems provides a valuable tool for neuroscience research. Traditionally, the role of genes in neuronal circuits has been addressed by overexpression or knock-out/knock-down systems. However, those techniques do not manipulate the endogenous loci excluding important information regarding the contribution

of the genomic nature in biological processes, especially important when studying the human brain. The CRISPR system is a powerful tool for specific manipulation of the endogenous genome and it can be used to i) switch On and Off endogenous genes; ii) label endogenous proteins with fluorescent markers and iii) introduce/revert specific mutations. Here, we constructed a CRISPR system for targeting the promoter of the epileptogenesis associated gene encoding the T-Type calcium channel Cav3.2 to switch On (CRISPRa-activator) and Off (CRISPRi-inhibitor) its expression in a mouse neuroblastoma cell line (NS20Y), in mouse dissociated cortical neurons and in organotypic mouse hippocampal slices. Transfection of NS20Y cells with a reporter construct (Cav3.2 promoter driving m-Ruby/venus expression) and the CRISPRa system showed a strong expression of the fluorescent protein in comparison to the basal activity of the Cav3.2 promoter whereas almost no detectable fluorescence was seen after transfection with CRISPRi. A combination of adeno-associated viral and lentiviral gene delivery of the CRISPRa components lead to the activation of the Cav3.2 promoter in mouse cortical neurons and organotypic slices. Quantitative RT-PCR revealed increased relative expression of Cav3.2 in NS20Y cells transfected with CRISPRa (2.64 fold change, *N=3*) in comparison to control cells. Consistently, endogenous expression of Cav3.2 was reduced using the CRISPRi system (1.5 fold reduction, *N=3*). In addition, we transduced human organotypic hippocampal slices resected from epileptic patients, with lentiviruses. These results reflect the possibility of manipulating the expression of the epilepsy-associated gene Cav3.2 *ex-vivo* and provide a valuable tool for specifically study the contribution of the calcium channel in epileptogenesis. Furthermore, combining gene manipulation by CRISPRa/i with lentiviral gene delivery we can confirm this process in a human scenario.

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Poster

123. Epilepsy: Human Studies

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The Lou Lou Foundation

Title: Neuronal and synaptic pathology in human CDKL5 deficiency disorder

Authors: *J. C. WACKER[1], K. A. SANSALONE[1], M. YENNAWAR[2], F. E. JENSEN[1], D. M. TALOS[1];
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Abstract: CDKL5 deficiency disorder (CDD) is caused by mutations of the cyclin-dependent kinase-like 5 (CDKL5) gene. Disease features include early-onset epilepsy and cognitive dysfunction. CDKL5 is highly expressed in the brain during synaptogenesis. Loss of CDKL5 function in mice has been associated with increased axonal connectivity, decreased dendritic arborization and downregulation of several post-synaptic proteins. We hypothesized that similar structural changes may arise from *CDKL5* mutation in humans, causing a shift in the excitation/inhibition balance likely contributing to the disease phenotype.
Postmortem hippocampal and temporal lobe cortical samples from two patients with CDD (5.7 and 30 years) and region-matched control specimens from patients with normal neurological history (3.5-29 years; n=6) were obtained from the NIH NeuroBioBank. CDD was confirmed by genetic testing and both cases presented with epilepsy and cognitive disability. Hippocampal and temporal lobe surgical specimens from treatment-resistant epilepsy cases from the University of Pennsylvania (21-28 years; n=4) were used for additional comparison. Samples were subjected to histological, immunohistochemical (IHC) and Western blot (WB) analysis. Statistical significance was assessed by one-way ANOVA.
Hematoxilyn and eosin revealed diffuse cortical thinning but no evidence of neuronal loss in CDD cases, except for a marked reduction of hippocampal granule cell densities. IHC for neuronal marker MAP-2 revealed a relative preservation of dendritic arbors in CDD cases, while immunostaining for phoshorylated neurofilament SMI 312 demonstrated longer and more elaborated axonal branches in CDD hippocampus and temporal lobe cortex, consistent with increased axonal growth. The hippocampal levels of SMI 312, the pre-synaptic marker synapsin and the post-synaptic scaffolding protein PSD-95 were further quantified by WB. SMI 312 was highly upregulated in CDD (212% of control), but not in epilepsy cases (38% of the control), suggesting a rather direct effect of CDKL5 mutation on axonal development, as opposed to a seizure-induced change ($p<0.01$). Synapsin levels were higher in both CDD (195%) and epilepsy (151%) patients compared to controls ($p<0.05$), while there were subtler differences in PSD-95 levels between groups (133% of the control in CDKL5 and 49% of the control in the epilepsy cases; $p<0.05$).
Our data is consistent with increased pre- and post-synaptic function in human CDD, which along with previously reported upregulation of GluA2-lacking AMPARs in the same patients (J Neurosci. 2041-18.2019), provides a basis for new therapeutic strategies for this disorder.

Disclosures: J.C. Wacker: None. **K.A. Sansalone:** None. **M. Yennawar:** None. **F.E. Jensen:** None. **D.M. Talos:** None.

Poster

123. Epilepsy: Human Studies

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 123.11/C30

Topic: B.10. Epilepsy

Title: Role of autoinflammation in acute seizure generation and epilepsy

Authors: *S. KUNDA[1], R. G. LAFRANCE-COREY[2], G. A. WORRELL[2], C. L. HOWE[1]; [1]Neurol., [2]Mayo Clin., Rochester, MN

Abstract: Epilepsy is a chronic neurologic disorder characterized by recurrent seizures and variable cognitive and neurodevelopmental issues. In many individuals the underlying pathogenesis is unknown, but burgeoning evidence indicates a link between neuroinflammation and seizure development. Therapeutically targeting inflammation in epilepsy is of particular interest for patients who do not respond to the current standard of care. Likewise, identifying changes in peripheral immune status may provide a unique biomarker for acute seizure prediction, irrespective of the nature of the initial insult. In our first study, using high-resolution repeated-measures temporal profiling of serum cytokine levels and flow cytometric analysis of the activation status of circulating neutrophils and monocytes, we established an immune profile for patients undergoing multi-day continuous video-EEG monitoring. We identified a temporal correlation between clinical seizure events and increased levels of serum monocyte chemoattractant, CCL2, as well as evidence of acute peri-ictal changes in the status of inflammatory monocytes. Furthermore, we also use innovative peri-electrode large molecule microdialysis at the site of intracranial EEG recording electrodes to analyze dynamic changes in the levels of CCL2, TNFa, IL1b, and IL6 in the hippocampus following viral infection in a viral encephalitis epilepsy mouse model. In our second study, we investigate an autoinflammatory and autoimmune seizure phenotype without any known genetic mutations and receptor autoantibodies. In a subset of severe drug- refractory pediatric patients, we were able to delineate innate cell and stimulus-dependent defects in the Toll-Like Receptor (TLR) and NFkB pathways and the subsequent production of chemokines including IL1b. We hope that the thorough characterization of these unique cases paired with the temporally-resolved data set of peri-ictal chemokine fluctuation in a general epileptic population as well as mouse models will help us understand the acute inflammatory cascade which underlies seizure generation.

Disclosures: S. Kunda: None. **R.G. Lafrance-Corey:** None. **G.A. Worrell:** None. **C.L. Howe:** None.

Poster

123. Epilepsy: Human Studies

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 123.12/C31

Topic: B.10. Epilepsy

Support: JAES Foundation
Personal grant, The University of Oulu Scholarship Foundation

Title: Respiratory related brain pulsations are disturbed in epilepsy

Authors: *J. KANANEN[1], T. TUOVINEN[1], V. KORHONEN[1], N. HUOTARI[1], H. HELAKARI[1], H. ANSAKORPI[1], P. LEVAN[2], V. KIVINIEMI[1];
[1]Univ. of Oulu, Oulu, Finland; [2]Univ. of Freiburg, Freiburg, Germany

Abstract: Introduction: Patients with epilepsy (PWE) have altered coefficient of signal noise variation (CV) in fast-fMRI[1,2]. In this study we estimate the physiological source of the altered noise CV from band passed physiological signals present in fast-fMRI.
Methods: After informed consent 102 healthy controls (HC, 37.5 ± 15.4 yr., 51F) and 33 PWE (34.6 ± 10.2 yr., 21F), were imaged using Siemens 3T MRI with fMRI as before[1,2]. Subjects were scanned in two sites (Oulu & Freiburg) for 5-min (TR=100ms). Standard FSL pre-processing[3] with FIX-ICA, was used[4]. The data was band pass filtered to very low frequency (VLF = 0.01-0.1 Hz), respiratory (RB=0.11-0.51 Hz) and cardiac (CB=0.8-1.76 Hz) for CV and FFT power calculations. Voxel-wise CV (σ / μ, μ = signal mean, σ = standard deviation) and sum of spectral power density (SPD) were calculated. FSL randomise (10 000 non-parametric perm., age covariate) was used to evaluate group differences. The standard score of SPD maps were calculated over HC population and compared it to PWE group.
Results: Respiratory band signal showed elevated CV ($p < 0.01$, Fig.1A) and even more distinctively SPD power ($p < 0.001$, Fig.1B, for signal example Fig.1C) in basal and temporal brain areas. VLF and CB had no statistical differences. PWE had SPD power > 10 σ over control mean in 25/33 cases, while HC had none, for examples and SPD histograms in both groups, c.f. Fig.1D, E respectively.
Conclusion: The respiratory signal power fMRI signal significantly increased in PWE group in brain areas that control respiration and arousal. Using standard score to threshold SPD maps, individual differences in PWE subjects could be detected. This indicates that physiological brain pulsation abnormality could be influencing epileptiform activity.
References: 1. Kananen J. et al., 2018, Brain Behav 2. Kiviniemi V. et al., 2016, JCBFM3. Jenkinson M. et al., 2012, NeuroImage 4. Griffanti, L. et al., 2014. Neuroimage

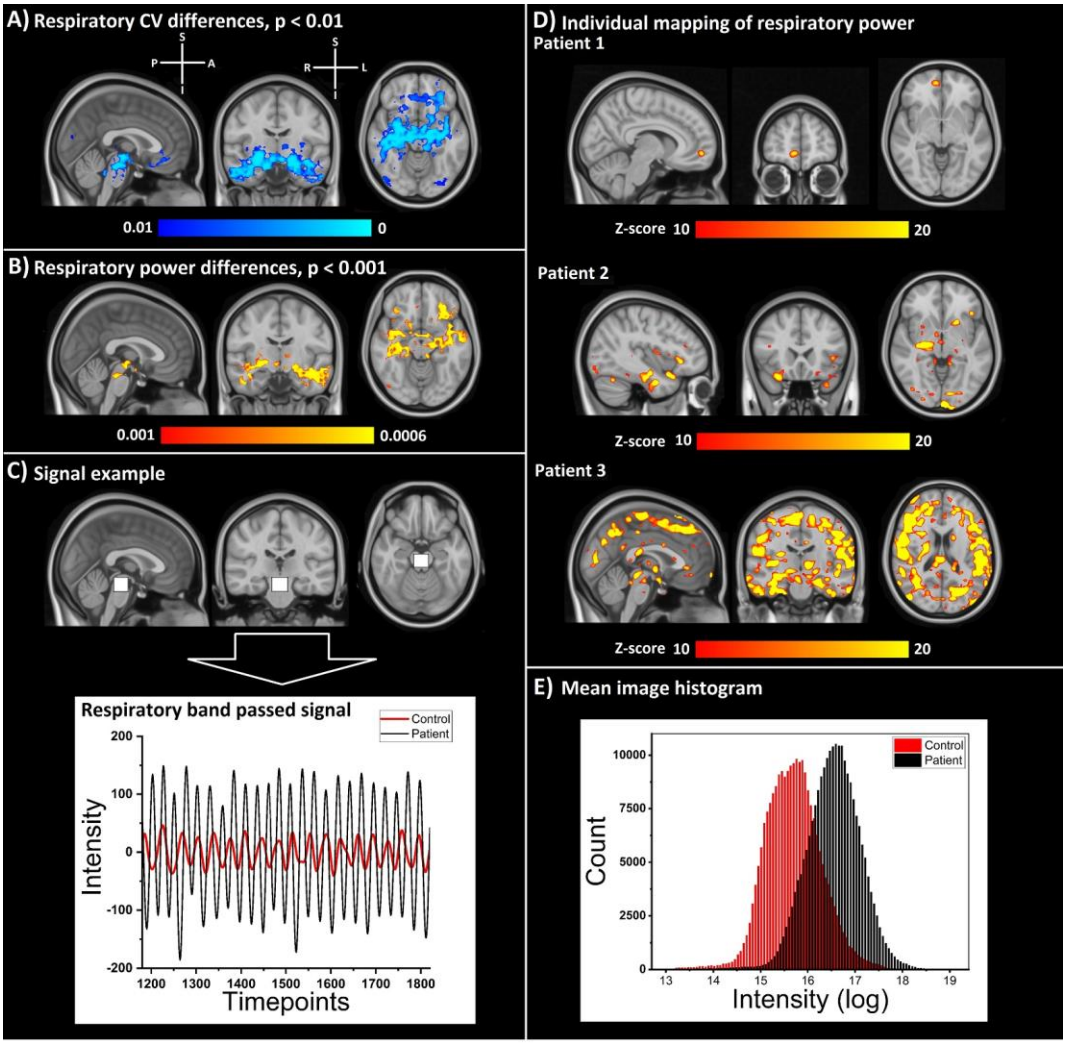

Figure 1. A) CV differences between groups in respiratory band, p-value < 0.01. **B)** Respiratory band spectral power density sum differences between groups, p-value < 0.001. **C)** Example respiratory band passed signal of one patient and one control from selected brain stem region of interest (white cubic). **D)** Example individual maps of periodogram with minimum value of Z-score 10 from three patients. **E)** Histograms from group (102 HC and 33 PWE) mean image.

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Poster

123. Epilepsy: Human Studies

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 123.13/C32

Topic: B.10. Epilepsy

Support: This work has been supported by the Brazilian agency CAPES

Title: The use of auditory steady-state responses to actively probe abnormal coupling associated with epilepsy

Authors: V. R. CARVALHO[1], S. S. CASH[3], E. M. A. M. MENDES[1], *M. F. D. MORAES[2]; [1]Programa de Pós-Graduação em Engenharia Elétrica, [2]Núcleo de Neurociências, Univ. Federal de Minas Gerais, Belo Horizonte, Brazil; [3]Dept Neurol, Mass Genl Hosp, Boston, MA

Abstract: Auditory Steady-State Responses (ASSR) are evoked potentials generated by sound stimuli presented at a relatively fast rate such that transient responses overlap. Amplitude-modulated (AM) tones are a particular case of ASSR stimuli that recruit neurons responding to the carrier frequency, in an oscillatory pattern which follows the modulating frequency. Aside from its use in evaluating auditory pathway integrity, AM-ASSR has been used as a diagnostic tool to quantify the degree of coupling and recruitment of brain structures that may be compromised in pathologies such as schizophrenia and bipolar disorder. This work aims to evaluate the use of ASSR to unveil features related to epilepsy. We hypothesized that putative epileptogenic areas would present abnormal amplitude responses and hyper-connectivity to surrounding networks that may be recruited by ASSR stimuli, thus significantly modifying the evoked response pattern. The experimental protocol involves the use of AM sound stimuli to elicit ASSRs in patients with refractory epilepsy implanted with deep electrodes. Stimulation consisted of 6 trials each 60 seconds in duration, with 30 s silence periods in between. In each trial, an 85 dB Amplitude Modulated (AM) tone is played with two speakers facing each side of the patient's head. Different Carrier frequencies (1 kHz, 2 kHz, pink noise, white noise, brown noise and frequency sweep) were modulated at 79.58 Hz for each trial. We evaluated the ASSR amplitude and Phase-Locking-Value (PLV) between channel-pairs for two patients; which showed enhanced responses for temporal and hippocampal electrodes and hemispheric asymmetry. An intriguing finding is that, for each electrode, response profiles show that middle contacts (as opposed to more superficial contacts), in regions of white matter, yielded higher energy responses surrounding the modulatory frequency band. Connectivity matrices were calculated before and during ASSRs, giving an idea of how active probing changes network synchrony and topology evaluation, with the most visible being increases in intra and inter hemisphere connectivity between temporal electrodes in the ASSR frequency band. Our results can be considered as a successful proof of principle for the use of ASSR in Epilepsy, although the low number of patients and the lack of well-defined seizure foci in the selected patients limit the strength of data interpretation. With the validation of the proposed hypothesis, the diagnostic value of the ASSR for unveiling abnormal coupling within and around epileptogenic regions would open a new venue for epilepsy diagnosis and presurgical evaluation in which passive EEG analysis is inconclusive.

Disclosures: V.R. Carvalho: None. **S.S. Cash:** None. **E.M.A.M. Mendes:** None. **M.F.D. Moraes:** None.

Poster

123. Epilepsy: Human Studies

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Support: NIH R01-NS094399 (W.S.)
Doris Duke Charitable Foundation Clinical Scientist Development Award #2015096 (W.S.)
NIH K01-ES026839 (S.G.)
NIH K08-NS069783 (S.G.)

Title: Temporal analysis of preictal high-frequency oscillation rates in refractory epilepsy

Authors: *J. SCOTT, S. REN, S. GLISKE, W. STACEY;
Univ. of Michigan, Ann Arbor, MI

Abstract: Purpose: High-frequency oscillations (HFOs) have become an accepted and important biomarker of epilepsy, as numerous studies have shown improved clinical outcomes for resection of tissue with high HFO rates. While most clinical literature focuses on the spatial context of HFOs in delineating the seizure onset zone for surgical planning, few studies have adequately addressed potential temporal relationships of HFO rates with preictal states and imminent seizure generation. Accordingly, here we have analyzed HFO rates as a continuous function of time until seizure onset to better understand their potential involvement in seizure generation, spread, and subsequent termination. Methods: We analyzed intracranial EEG data from 30 patients with refractory epilepsy undergoing long-term pre-surgical evaluation. Utilizing an automated HFO detector that removes muscle and electrode artifacts, we grouped HFOs into hour-long windows centered on each of a patient's recorded seizures. Using the non-parametric Nelson-Aalen hazard model, we then estimated HFO rates as a continuous function of time relative to the aligned seizure onsets, and compared the resulting data with seizure onset zone and resected volume. We also performed the same procedure for half-hour segments of baseline interictal data, and using Pearson's correlation, made statistical comparisons of potential preictal temporal trends against those of baseline. Results: We found in general that HFO rates do not increase preictally as onset is reached. A large increase in HFO rates was found during ictal activity for the majority of patients, followed by an accompanying decrease in HFO rates back to baseline postictally. Immediately following seizure offset, a number of patients showed significantly decreased HFO activity before baseline rates resumed. Channels in the seizure onset zone generally had the highest HFO rates for all compared epochs (baseline, preictal, ictal and postictal). This effect was also pronounced in the resected volume channels. Discussion: These results corroborate existing findings that HFO rates within epileptic tissue are higher overall, through all stages of

interical, preictal, ictal, and postictal. However, there was no significant increase in HFO rate in the preictal period. This finding suggests that HFO rate is not a reliable temporal biomarker of seizure onset time.

Disclosures: J. Scott: None. **S. Ren:** None. **S. Gliske:** None. **W. Stacey:** None.

Poster

123. Epilepsy: Human Studies

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Program #/Poster #: 123.15/C34

Topic: B.10. Epilepsy

Support: American Epilepsy Society Junior Investigator Award
Michigan Institute for Computational Discovery & Engineering Catalyst Grant

Title: Mechanisms of dynamic inhibitory neuronal control of human seizures

Authors: *O. J. AHMED[1], T. JOHN[1], S. SUDHAKAR[1], E. K. BRENNAN[2], A. J. LORENZO GONZALEZ[1], J. NAFTULIN[3], G. A. MASHOUR[1], L. R. HOCHBERG[4], S. S. CASH[5]; [2]Neurosci. Grad. Program, [1]Univ. of Michigan, Ann Arbor, MI; [3]Massachusetts Gen. Hosp., Boston, MA; [4]Brown Univ., Providence, RI; [5]Dept Neurol, Mass Genl Hosp, Boston, MA

Abstract: Inhibitory neuronal activity is critical for the normal functioning of the brain, but is thought to go awry during neurological disorders such as epilepsy. There exist at least three distinct theories for how inhibition may decrease during a seizure: excessive chloride accumulation inside neurons that turns inhibition into excitation; hyperpolarization of fast-spiking (FS) inhibitory neurons by other inhibitory neurons; and depolarization block of FS neurons due to excessive synaptic drive or extracellular potassium accumulation. Despite its fundamental importance, it is not known which, if any, of these theories explain how seizures invade the human neocortex. Here, using large-scale recordings of neocortical single neurons in patients with secondarily generalized tonic-clonic seizures, we show that FS activity first increases as a seizure spreads across the neocortex, clearly impeding and altering the spatial flow of fast epileptic traveling waves. Strikingly consistent and unexpected dynamics then follow: some FS cells pause their firing due to transient inhibition from other cells. However, these hyperpolarized FS cells always resume their firing after a pause. Cessation of FS cell firing then occurs due to massive depolarization of their membrane potential (decoded using a novel method), leading to depolarization block. RS cells increase their firing immediately after the cession of nearby FS cells. However, RS cells, too, subsequently enter depolarization block. Our results are only fully consistent with one theory of why inhibition fails in the middle of seizures: depolarization block of FS neurons. Our simple computational models of excessive depolarization reproduce all the biophysics of the in vivo human single unit data. These results

extend our understanding of the dynamically precise cellular interplay that occurs during secondarily generalized seizures in humans.

Disclosures: O.J. Ahmed: None. **T. John:** None. **S. Sudhakar:** None. **E.K. Brennan:** None. **S.S. Cash:** None.

Poster

123. Epilepsy: Human Studies

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 123.16/DP03/C35

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: B.10. Epilepsy

Support: NIH R01 NS084142
CRCNS R01 NS095368

Title: Action potential alterations and cell-type specific activity through ictal recruitment in humans

Authors: *E. M. MERRICKS[1], E. H. SMITH[3], G. M. MCKHANN, II[2], R. R. GOODMAN[4], S. A. SHETH[5], B. GREGER[6], P. A. HOUSE[3], A. J. TREVELYAN[7], C. A. SCHEVON[1]; [1]Neurol., [2]Neurosurg., Columbia Univ., New York, NY; [3]Univ. of Utah, Salt Lake City, UT; [4]Neurosurg., Icahn Sch. of Med., New York, NY; [5]Neurosurg., Baylor Col. of Med., Houston, TX; [6]Sch. of Biol. and Hlth. Systems Engin., Arizona State Univ., Tempe, AZ; [7]Newcastle Univ., Newcastle Upon Tyne, United Kingdom

Abstract: Much recent debate has focused on cell-type-specific patterns of activation at seizure onset. However, action potential waveform changes during seizures can confound detection sensitivity and classification of single units. We developed template matching-based methods to identify single neurons in ictal microelectrode recordings from 27 patients with intractable focal epilepsy. Neuronal identities were defined as action potentials occurring within the convex hull in the feature space of standard, cluster-sorted neurons in the period either side of the seizure, while discarding those with < 1% chance of occurring in a χ^2-distribution based on Mahalanobis distance. Action potential match likelihood was calculated using the Gaussian distribution of voltages in the original neuron from which probabilistic firing rates were calculated. Neurons were then subclassified by putative cell type using waveforms and firing patterns.
We identified two distinct neuronal activity patterns at seizure onset. Type 1, found in 8 patients, was characterized by tonic firing, reduced spike amplitude (91.4% ± 7.0%; $p < 0.05$, Mann-Whitney U test), and increased half-width (133.8% ± 19.0%; $p < 0.05$, Mann-Whitney U test). Type 2 showed burst firing with no change in waveform. All neurons returned to their pre-ictal

wave shapes after seizure termination. We interpret Type 1 as consistent with recruited tissue as defined in prior animal studies and Type 2 as a downstream effect of recruited tissue. Of the 413 neurons, 48 (11%) were identified as putative interneurons. Of these, 35 (73%) showed significantly increased firing during seizures; 1 (2%) showed a decrease. While interneuron firing was maintained throughout the seizure, there was out-of-phase firing and a prominent, transient increase in firing of interneurons just prior to the onset of Type 1 activity.
We conclude that the distinction between tissue that has been recruited versus penumbra is maintained at the level of single neurons. Strict, novel template matching methods enabled us to demonstrate continued firing of the putative inhibitory population following ictal invasion. The interneuron activity patterns during the ictal transition suggest that multiple mechanisms are involved, including altered inhibitory effects and depolarization block. However our data do not appear to corroborate an interneuronal triggering mechanism.

Disclosures: E.M. Merricks: None. **E.H. Smith:** None. **G.M. McKhann:** None. **R.R. Goodman:** None. **S.A. Sheth:** None. **B. Greger:** None. **P.A. House:** None. **A.J. Trevelyan:** None. **C.A. Schevon:** None.

Poster

123. Epilepsy: Human Studies

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 123.17/C36

Topic: B.10. Epilepsy

Support: NIH R01 - NS092882
NIH UH2/3 - NS95495

Title: Measuring DC dynamics at multiple spatial scales in human intracranial electrophysiology

Authors: *V. SLADKY[1,4], V. KREMEN[1,2,5], P. NEJEDLY[1,4], P. KLIMES[1,4], J. CIMBALNIK[1,4], J. VAN GOMPEL[3], M. STEAD[1,2], G. A. WORRELL[1,2];
[1]Neurol., [2]Physiol. and Biomed. Engin., [3]Neurosurg., Mayo Clin., Rochester, MN; [4]Intl. Clin. Res. Ctr., St. Anne's Univ. Hosp. Brno, Brno, Czech Republic; [5]Robotics, and Cybernetics, Czech Tech. Univ. in Prague, Prague, Czech Republic

Abstract: Direct current (DC) potentials and slow baseline EEG fluctuations can be measured using Platinum/Iridium electrodes implanted in the brain. Slow wave potential activity can be associated with multiple mechanisms, including neuronal, glia and slow pH substrate changes. There are several neurological diseases connected with slow wave and DC potentials, including spreading depression (SD), hypoxic-ischemic SD-like depolarization, and seizures. Changes in excitatory activity and overall sodium and potassium concentration and influence of glia cells on extracellular space are among proposed mechanisms of seizure-related DC shifts. Previous

studies using clinical, macro-scale intracranial electrodes (~10mm^2) have characterized seizure related DC fluctuations in focal human epilepsy.
We utilized wide-band recordings from hybrid depth electrodes containing Platinum/Iridium clinical macroelectrode contacts and microwires (40 μm) for electrophysiological DC recordings to quantify local and wide-spread DC fluctuations. Five patients with mesial temporal lobe epilepsy were implanted with hybrid depth electrodes in the hippocampus. Data were recorded and retrospectively quantified over different brain states, vigilance - awake, sleep, and pre-ictal, ictal, and postictal periods.
The primary finding was that brain regions generating spontaneous focal seizures had greater DC fluctuations at baseline, during seizures, and post-seizure then other brain regions. There was no clear difference between inter-ictal and pre-ictal DC fluctuations in either brain region. On an individual patient level this was true for 4 out of 5 patients. In contrast, only 1 patient had a higher DC shift measured at the microscale level during seizures. At the microdomain level we did not observe consistent changes prior to seizures.

Disclosures: **V. Sladky:** None. **V. Kremen:** None. **P. Nejedly:** None. **P. Klimes:** None. **J. Cimbalnik:** None. **J. Van Gompel:** None. **M. Stead:** None. **G.A. Worrell:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Financial interest related to technologies licensed to Cadence Neuroscience Inc., Financial interest related to technologies licensed to NeuroOne Inc..

Poster

123. Epilepsy: Human Studies

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 123.18/C37

Topic: B.10. Epilepsy

Support: R01 NS095368
R01 NS084142

Title: Correlates of hippocampal interictal epileptiform discharges on scalp EEG recordings

Authors: *S. LEE, N. ISSA, W. VAN DRONGELEN;
Univ. of Chicago, Chicago, IL

Abstract: Epileptiform activity limited to the hippocampus is thought to be largely undetectable by scalp EEG recording methods, possibly due to the structure’s curved shape and deep location in the brain. Due to the difficulties in capturing hippocampal activity on the scalp, patients with suspected temporal lobe seizures may remain undiagnosed or require invasive intracranial monitoring to confirm a diagnosis.Clinical observations have noted that some hippocampal interictal epileptiform discharges (HC-IED) are temporally associated with scalp patterns such as

small sharp spikes that are visible on a single-trial basis. In this study, we utilized an array of signal processing methods to identify and characterize manifestations of HC-IEDs in scalp recordings. Using a data set of simultaneous intracranial and extracranial recordings obtained from epilepsy patients undergoing preoperative monitoring, we classified hippocampal IEDs by their waveform morphology. Signal averaging of scalp signals associated with different classification groups showed an emergence of clearly identifiable scalp spikes associated with HC-IEDs. Cross-correlation analysis showed that a subset of IEDs show tight temporal locking between scalp and intracranial signals, suggesting signal propagation via volume conduction. These IEDs also showed in scalp topographic maps, a bilaterally symmetric dipole, in line with a deep dipole source. In contrast, other groups of hippocampal IEDs showed delayed correlations between scalp and intracranial signals, suggesting that the signal seen on the scalp comes from a secondary source. These IEDs showed a more complex non-symmetric rotating scalp topography, suggesting a larger role for physiological propagation that underlies the scalp signals observed on the scalp. Finally, using signal-to-noise ratio analysis of single trials, detectability of these scalp correlates on a single-trial basis all patients analyzed had at least 1 group of HC-IEDs that showed a significant SNR on a single-trial basis in over 25% of HC-IEDs, suggesting that these scalp signals may be useful in identifying HC-IEDs using non-invasive methods.

Disclosures: S. Lee: None. **N. Issa:** None. **W. van Drongelen:** None.

Poster

123. Epilepsy: Human Studies

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 123.19/C38

Topic: B.10. Epilepsy

Support: National Institute of Health
Brain Canada

Title: Decreases in cellular firing accompany saccade onsets in human mesial temporal lobe structures and neocortex

Authors: *A. SCHJETNAN[1], C. KATZ[2], K. PATEL[2], V. A. BARKLEY[1], A. NADERIAN[1], H. BABU[3], S. KALIA[1], T. VALIANTE[1,4];
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Abstract: Background: In the primate brain, saccadic eye movements are strongly correlated to mnemonic processing. With invasive local field potential (LFP) recordings in humans, we have shown that in mesial temporal lobe (MTL) structures, saccade-associated potentials demonstrate

phase clustering in the theta and alpha bands without associated power increases. This specific electrophysiological signature suggests that neuronal firing rates are modulated in time, without increases in spike rates.
Methods: To explore this hypothesis, we performed single unit recordings in epilepsy patients undergoing intracranial EEG monitoring with macro-micro depth electrodes. Depth electrode placement was determined clinically. Subjects performed a visual search task with continuous eye tracking to ascertain saccade onset timing. Offline spike sorting and peri-saccadic time histograms were constructed for well-separated units.
Results: From 245 neurons recorded from the MTL and occipital lobe, we examined saccade-related modulation. We found a firing rate decrease in 20% of the neurons recorded in the MTL and in 90% from the occipital cortex. Additionally, occipital units demonstrated a 'rebound' increase in spiking following the inhibitory period, whereas the MTL units did not. Furthermore, the decrease in firing rate appears to be modulated by the directionality of the saccades, which suggests that a corollary may mediate the firing changes we observed.
Conclusions: Single unit recordings in humans suggest that inhibition dominates the peri-saccadic interval in MTL structures and neocortical regions. These results are consistent with the lack of power increases observed in the LFP in MTL sites.

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Poster

123. Epilepsy: Human Studies

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 123.20/C39

Topic: B.10. Epilepsy

Title: Investigating intrinsic electrophysiological diversity of human pyramidal cells across cortical layers and between individuals

Authors: H. MORADI CHAMEH[1], L. WANG[1], L. ZHANG[1], P. CARLEN[1], S. TRIPATHY[2], *T. A. VALIANTE[1];
[1]Krembil Res. Inst., Toronto, ON, Canada; [2]Krembil Ctr. for Neuroinformatics, Ctr. for Addiction and Mental Health, Dept. of Psychiatry, Toronto, ON, Canada

Abstract: The hyperpolarization-activated non-specific cation current, I_h, greatly shapes a neuron's subthreshold and active integrative properties. Here, we wanted to understand how I_h and other intrinsic features contribute to the electrophysiological differences between superficial (L2/3) and deep layer (L5) pyramidal neurons in human neocortex.We performed whole-cell recordings *in vitro* from 214 pyramidal neurons from unaffected cortical tissue resected from 51 patients undergoing neurosurgery for temporal lobe epilepsy or tumor resection at Toronto

Western Hospital. We validated our results in a second dataset of 413 neurons recorded from 42 patients collected by the Allen Institute for Brain Sciences.We found that human L5 pyramidal neurons had more prominent sag and larger I_h currents relative to L2/3 neurons. In addition, L5 neurons were more excitable, with rebound bursting following hyperpolarizing input, higher input resistances, and more depolarized resting potentials. Pharmacologically blocking I_h produced a larger change in membrane properties in L5 compared to L2/3 neurons and markedly reduced the observed cell-to-cell variability across cells. Though we did not find evidence for a specific resonant frequency in either L5 or L2/3, we found that L5 neurons are better able to track inputs at the delta and theta frequencies. Lastly, by correlating neuronal physiology with patient-level demographic features, we found that sag amplitudes are larger in neurons recorded from older patients and those with a tumor diagnosis. Sag is a dominant feature of the human cortical microcircuit and is prominently expressed in L5 pyramidal cells. In addition, neuronal I_h is not a fixed feature of a cell type but instead dynamically changes due to a subject's disease state and over the lifetime, providing a potential correlate for age-related cognitive decline.

Disclosures: H. Moradi Chameh: None. **L. Wang:** None. **L. Zhang:** None. **P. Carlen:** None. **S. Tripathy:** None. **T.A. Valiante:** None.

Poster

123. Epilepsy: Human Studies

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Program #/Poster #: 123.21/C40

Topic: B.10. Epilepsy

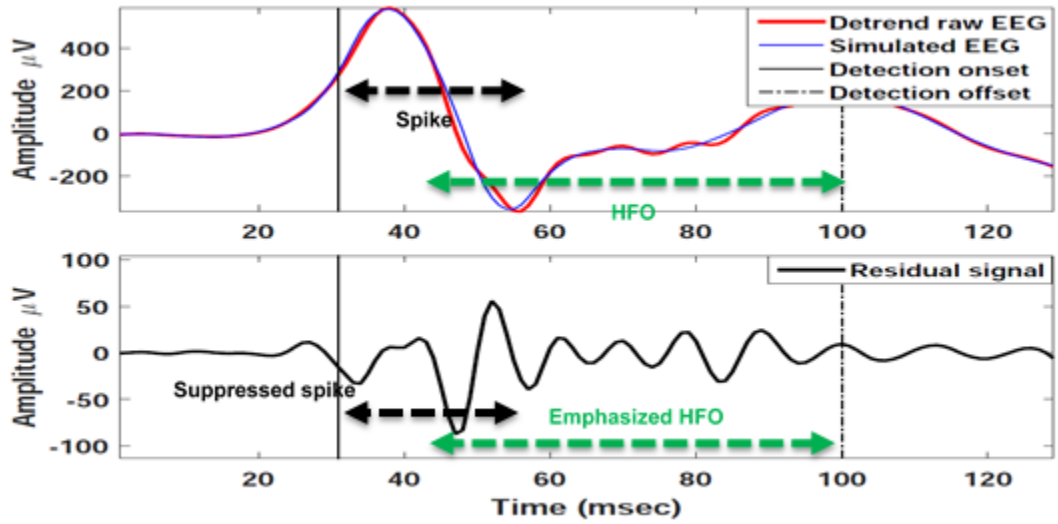
Support: Scholarship support from University of Babylon in Iraq to AA
NSF Grant 1539068

Title: Discrimination of high frequency oscillations from sharp transients in the electrocorticogram of patients with refractory epilepsy

Authors: *A. F. AL-BAKRI[1], C. HADDIX[1], M. BENSALEM-OWEN[2], P. MODUR[4], S. SUNDERAM[3];
[1]Biomed. Engin., [3]Dept. of Biomed. Engin., [2]Univ. of Kentucky, Lexington, KY; [4]Univ. of Texas, Austin, TX

Abstract: High frequency oscillations (HFOs) are potentially useful biomarkers of epileptogenic brain tissue. In diagnostic use for surgical treatment, true HFOs first need to be identified and their region of activity demarcated to predict the location and extent of cortex to be resected for a patient to become seizure-free. HFOs co-occurring with spikes are potentially stronger markers of epileptogenic cortex than HFOs alone. But HFOs masked by spikes may be rejected as artifacts (false negatives). The purpose of this study is to develop an algorithm that retains such

events while rejecting genuine artifacts based on rhythmicity criteria. With IRB approval, eight epilepsy patients admitted for invasive presurgical evaluation were monitored using intracranial EEG (iEEG) with 1000 Hz sampling rate. HFO candidates were first identified using a slightly modified version of a well-known algorithm (Staba et al., 2002), which is highly sensitive to HFOs but admits spikes and other artifacts which, when filtered, look like genuine HFOs. This deficiency is addressed here by modeling the transient baseline (see figure) of the iEEG around a detection. When a spike fitted thus is subtracted from the signal the residual does not produce a false ripple when sent through a highpass filter. An HFO riding on the spike remains in the residual after the spike is eliminated. If the baseline does not contain a spike, it is unaffected. This approach decouples HFOs from spikes and other artifacts in the iEEG. A superset of 2500 detections made by the standard algorithm were selected at random from 1-3 channels in each recording and further screened using our two-step algorithm. True HFOs were distinguished from spikes with a sensitivity of 80%, specificity of 80%, positive prediction value of 94%, and Cohen's kappa of 50%. This procedure mostly rejected spikes while retaining highly rhythmic HFOs and HFOs co-occurring with spikes whose site of origin is strongly correlated with the seizure onset zone (SOZ) demarcated by the physician.



Disclosures: **A.F. Al-Bakri:** None. **C. Haddix:** None. **M. Bensalem-Owen:** None. **P. Modur:** None. **S. Sunderam:** None.

Poster

123. Epilepsy: Human Studies

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 123.22/C41

Topic: B.10. Epilepsy

Support: NSF GRFP DGE-1840990

Title: Characterizing the relationship between functional connectivity and neurocognitive deficits in benign epilepsy with centrotemporal spikes

Authors: *E. SPENCER[1], D. CHINAPPEN[3], L. OSTROWSKI[3], D. SONG[3], S. STOYELL[3], C. CHU[3], M. A. KRAMER[2];
[1]Dept. of Neurosci., [2]Dept. of Mathematics and Statistics, Boston Univ., Boston, MA; [3]Dept. of Neurol., Massachusetts Gen. Hosp., Boston, MA

Abstract: Benign epilepsy with centrotemporal spikes (BECTS) is the most common childhood focal epilepsy. While all patients spontaneously enter into remission by adolescence, BECTS is linked to the development of various sensorimotor deficits that in some cases follow patients into adulthood. There is evidence in other studies of focal epilepsies that the way the brain transiently coordinates the flow of information between cortical regions, i.e. functional connectivity, is disrupted. However, there is limited understanding of the specific differences in functional connectivity between BECTS patients at time of diagnosis, patients in remission, and healthy individuals, and whether these functional connectivity changes correlate with behavioral deficits. We hypothesize that the impact of BECTS during a critical period in cognitive development has long-lasting effects on the functional connections, and that the differences in functional connectivity provide the neurological basis for deficits present later in life. We propose a data analysis pipeline to address this hypothesis. We analyze high-density electroencephalography recordings sourced to the brain surface to map out the functional connections at different stages of BECTS, and compare these functional connections with age-matched controls to characterize how signaling between cortical areas is disrupted. Then, we determine which differences are predictive of task performance on language and motor tasks. By understanding the differences in brain network organization, we may understand why neurological impairments develop in certain individuals with BECTS and establish a direct relationship between functional connectivity and cognitive processes.

Disclosures: E. Spencer: None. **D. Chinappen:** None. **L. Ostrowski:** None. **D. Song:** None. **S. Stoyell:** None. **C. Chu:** None. **M.A. Kramer:** None.

Poster

123. Epilepsy: Human Studies

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 123.23/C42

Topic: B.10. Epilepsy

Title: Impaired thalamocortical functional connectivity of different thalamic nuclei in temporal lobe epilepsy

Authors: L. ZHANG[1], D. LIU[2], L. WANG[1], B. XIAO[3], H. CHEN[1], L. FENG[3], *R. LI[1];
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Abstract: Temporal lobe epilepsy (TLE) is the most common adulthood form of focal epilepsy, which is characterized by seizures that often originate from limbic structures, including the hippocampus. Previous studies suggested that the thalamus, as a key relay or processing hub of neuronal information flow between cortical-subcortical structures, have been implicated in seizures propagation, awareness maintenance and seizure related cognitive deficits. However, the specific functional alterations between different thalamic subregions and cortical systems in patients with TLE are still unknown. In the present study, we choose the anterior (ANT), ventral posterior medial (VPM) and central lateral (CL) nuclei of the thalamus as seeds to investigate the divergent thalamocortical connectivity patterns in patients with TLE. Functional connectivity (FC) was adopted to explore the role of different thalamic nuclei in TLE. Pearson's correlation analysis was further used to assess the association between FC alterations and disease duration, seizure frequency and the immediate and delayed memory scores. Our results revealed typical differential spatial FC patterns of different thalamic seeds in both healthy controls (HC) and TLE patients ($P < 0.05$, FDR corrected). Furthermore, relative to HC, significantly abnormal FC between different thalamic seeds and distinct cortical networks in TLE were observed ($P < 0.05$, GRF corrected). For the ANT, TLE showed decreased FC in the limbic system and medial prefrontal cortex, while increased FC in the dorsal lateral prefrontal cortex. For the VPM, we observed decreased FC in the sensorimotor network and cerebellum. We also found decreased FC between the cluster of midbrain and the CL. In addition, the decreased FC between the left ANT and the left hippocampus was positively correlated with the immediate ($r = 0.56$, $p = 0.0071$) and delayed ($r = 0.50$, $p = 0.0168$) memory scores. These findings suggest different thalamic nuclei owns divergent thalamocortical connectivity patterns and may help explain why focal temporal lobe seizures often disrupt widespread cortical networks and play a negative role in cognition sustainence in patients. Further understanding of different thalamocortical circuits for different thalamic nuclei in temporal lobe epilepsy may lead to improved treatments directly targeting different modes of impaired function.

Disclosures: R. Li: None. **L. Zhang:** None. **D. Liu:** None. **L. Wang:** None. **B. Xiao:** None. **H. Chen:** None. **L. Feng:** None.

Poster

123. Epilepsy: Human Studies

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 123.24/C43

Topic: B.10. Epilepsy

Support: ERC starting grant [716321]

Title: Selective network dynamic abnormalities in juvenile myoclonic epilepsy revealed by MEG energy landscape

Authors: *D. KRZEMINSKI[1], K. SINGH[1], K. HAMANDI[1], N. MASUDA[2], J. ZHANG[1]; [1]CUBRIC, Cardiff Univ., Cardiff, United Kingdom; [2]Dept. of Engin. Mathematics, Univ. of Bristol, Bristol, United Kingdom

Abstract: Juvenile myoclonic epilepsy (JME) is the most common syndrome of the idiopathic generalized epilepsy. Previous studies suggested that JME might be a network disorder affecting resting-state brain activity. However, it is unknown whether JME patients had widespread or selective abnormalities in the network dynamics across different resting-state networks (RSN). Here, we used a novel pairwise maximum entropy model (pMEM) and energy landscape analysis to characterize network dynamics and its abnormalities in MEG (magnetoencephalography) resting-state data.

26 JME patients and 26 age- and gender-matched controls underwent a MEG recording session, in which 5-minutes eyes-open resting-state MEG data were collected from a 275-channel CTF system. Pre-processed MEG data were co-registered to individual participant's MRI structural scan and source localized to the 90 AAL atlas regions using LCMV beamformer. We fitted the pMEM to the oscillatory power envelopes in theta (4-7 Hz), alpha (8-13 Hz), beta (15-25 Hz) and gamma (30-60 Hz) bands in three RSNs: default mode network (DMN), frontoparietal network (FPN) and sensorimotor network (SMN). The pMEM allows estimating the occurrence probability of each network state, with its regional activity and pairwise regional correlations to be constrained by empirical data.

The pMEM provided an accurate fit to the oscillatory activity data (JME: R^2=0.92 Controls: R^2= 0.94). We used energy values derived from the pMEM to depict an energy landscape of the network, with a higher energy state corresponding to a lower occurrence probability. When comparing the energy landscape between groups, JME patients showed a lower number of local energy minima ($F(1, 50)=5.27$; $p=0.03$) and had higher energy values than controls in the theta, beta and gamma-band of FPN oscillatory activity and the beta-band of DMN activity, but not in the SMN. Furthermore, a leave-one-out classification of individual participants using support vector machines and the energy values in the t-band FPN showed that the pMEM measures provided high predictive power in discriminating JME patients from controls. These findings suggest that JME patients had impaired multi-stability in selective networks and frequency bands in the frontoparietal networks.

Disclosures: D. Krzeminski: None. **J. Zhang:** None. **K. Singh:** None. **K. Hamandi:** None. **N. Masuda:** None.

Poster

123. Epilepsy: Human Studies

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 123.25/C44

Topic: B.10. Epilepsy

Support: NSERC RGPIN-2016-06182

Title: Development of a multi-compartment model of a human L5 pyramidal neuron suggests inter-species h-channel kinetic differences

Authors: *S. RICH, H. CHAMEH, V. SEKULIC, F. SKINNER, T. VALIANTE;
Krembil Res. Inst., Toronto, ON, Canada

Abstract: Most existing multi-compartment neuron models are constrained by non-human data. However, the correlation between neurons in animal models and humans is not one-to-one, leaving the use of these models in a human setting inherently fraught with caveats. Moreover, these models are typically created utilizing an averaged snapshot of the activity of a given cell type, ignoring the cell-to-cell variability known to cause major differences between similarly classified neurons.

Here we present a model of a human L5 cortical pyramidal neuron with the morphology and primary constraining electrophysiological data obtained from the same cell. *In vitro* whole-cell recordings were obtained from neurons from patients undergoing neurosurgery for temporal lobe epilepsy or tumor resection. The recordings focused on the dynamics of the h-current, which were identified with hyperpolarizing current clamp experiments in the presence of tetrodotoxin. Morphology of this neuron was obtained from biocytin fills and was reconstructed via IMARIS software for model development.

The modeling process utilized tools in the NEURON simulation environment and a “cycling” approach to closely fit the results of the hyperpolarizing current clamp experiments while maintaining reasonable spiking behavior relative to that seen in other human L5 pyramidal neurons. The final model parameters faithfully replicated h-channel mediated voltage responses, as well as general human L5 repetitive spiking and post-inhibitory rebound (PIR) characteristics driven by other channel types. Importantly, this model reproduced resonance and frequency-dependent gain features observed in these cells experimentally that were not used in model creation, lending further credibility to the model capturing an essence of this cell type’s dynamics.

Comparing the features of the h-channel in this human model to analogous rodent models suggests key differences between the dynamics of this channel which provide testable hypotheses in the exploration of crucial inter-species h-channel differences. Such insights could provide important translational information for building computational models of human circuits.

Indeed, it has recently been demonstrated that h-channel densities differ between mouse and human L2/3 pyramidal cells; our findings suggest that in addition to these inter-species differences in h-channel density, differences in channel kinetics may also contribute to the uniqueness of human L5 pyramidal cells.
This model is not only one of the first human pyramidal cell models, but the only of which the authors are aware that takes into account potential cell-to-cell variability.

Disclosures: **S. Rich:** None. **H. Chameh:** None. **V. Sekulic:** None. **F. Skinner:** None. **T. Valiante:** None.

Poster

123. Epilepsy: Human Studies

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 123.26/C45

Topic: B.10. Epilepsy

Support: Joint US (NSF) & German (DRG) Collaborative Research in Computational Neuroscience, IIS-1515168
NSF Career Award, 1351112
NIH P41, GM103545, “Center for Integrative Biomedical Computing”

Title: Patient-specific computational models of cortical activation for seizure improvement

Authors: *C. M. CHARLEBOIS[1], B. J. PHILIP[2], D. N. ANDERSON[1], A. D. DORVAL[2], C. R. BUTSON[3];
[1]Biomed. Engineering, Scientific Computing & Imaging Inst., [2]Biomed. Engin., [3]Scientific Computing & Imaging Institute, Biomed. Engineering, Neurology, Neurosurgery,Psychiatry, Univ. of Utah, Salt Lake City, UT

Abstract: Electrical stimulation can be an effective therapy for patients with epilepsy. However, clinical outcomes are variable; some patients achieve complete freedom from seizures while others experience little to no effect. Patient-specific computational models can be used to investigate the neurophysiological effects of stimulation, but it is unclear how fiber orientation and cortical geometry impact model predictions. The objective of this study was to use computational models to predict effective and ineffective stimulation parameters for seizure arrest and investigate the effects of cerebral spinal fluid (CSF) thickness on activation profiles. We created both theoretical and patient-specific finite element meshes and solved the bioelectric field problem for multiple stimulation parameters for 2.3 mm diameter subdural electrodes. We observed considerable variability in electrode locations relative to the cortical surface in a single subject (Fig. 1A). We estimated neural activation using the Hessian matrix of the second spatial derivative of the extracellular voltage. We found cathodic stimulation preferentially activated

fibers tangential to the electrode surface whereas anodic stimulation preferentially activated fibers orthogonal to the electrode surface. For a simple theoretical case of an electrode on flat cortex, anodic stimulation activated fibers deeper within cortex compared to cathodic stimulation at the same magnitude (Fig. 1B). As the CSF thickness increased under the electrode the maximum depth of activation decreased (Fig. 1B). These results have the potential to inform selective and optimal cortical stimulation parameters to modulate pathogenic epileptic circuits and improve seizure arrest. Additionally, the observed variability in electrode locations highlights the importance of using patient-specific computational models to study the effects of cortical stimulation. Future work will include correlating patient-specific activation with seizure outcomes in a cohort of patients implanted with the Responsive Neurostimulation System.

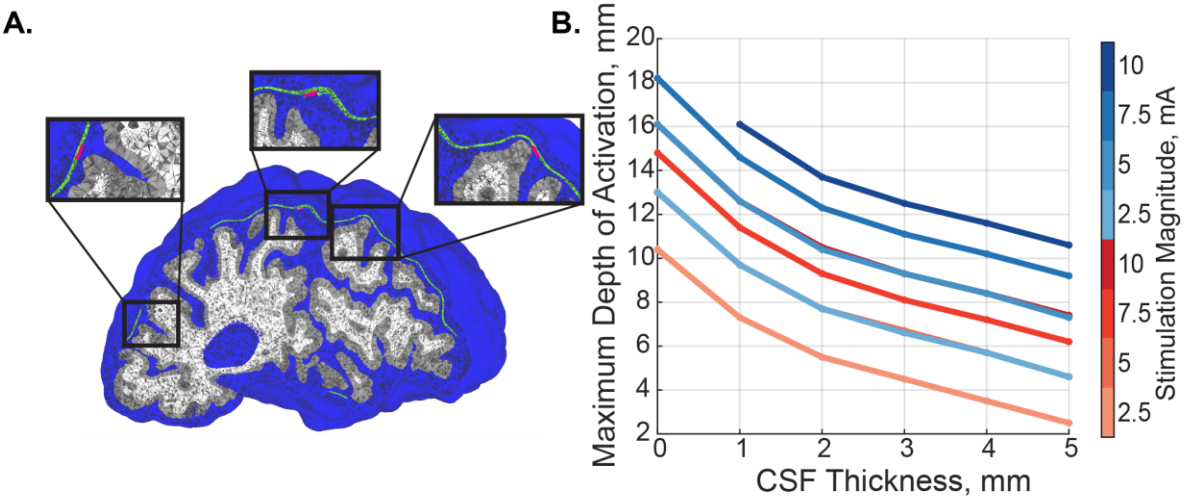

Figure 1. A. Patient-specific finite element mesh. The three insets show how variable the electrode (pink) location can be relative to the complex cortical geometry. **B.** Depth of activation for a theoretical flat cortical model. We varied the CSF thickness and magnitude of cathodic (red traces) and anodic (blue traces) stimulation.

Disclosures: C.M. Charlebois: None. **B.J. Philip:** None. **D.N. Anderson:** None. **A.D. Dorval:** None. **C.R. Butson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IntellectMedical. F. Consulting Fees (e.g., advisory boards); NeuroPace, IntellectMedical, Advanced Bionics, Abbott, Boston Scientific, Functional Neuromodulation.

Poster

123. Epilepsy: Human Studies

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 123.27/C46

Topic: B.10. Epilepsy

Support: NSF DGE-1840990

Title: Utilizing convolutional neural networks and bayesian modeling to predict patient outcomes

Authors: *J. NADALIN[1], L.-E. MARTINET[4], U. EDEN[2], C. CHU[5], M. A. KRAMER[3];
[2]Mathematics and Statistics, [3]Dept. of Mathematics and Statistics, [1]Boston Univ., Boston, MA;
[4]Dept. of Neurol., [5]Neurol., Massachusetts Gen. Hosp., Boston, MA

Abstract: Spike ripples in human neural voltage recordings have been proposed as a biomarker of epilepsy and recently associated with the most common type of childhood epilepsy syndrome: benign epilepsy with centrotemporal spikes (BECTS). To date, finding and identifying spike ripple events has been a time-intensive and laborious process involving hours of manual visual inspection and validation by clinical experts. A semi-automated process identifies candidate spike ripple events, saving considerable time and effort, but still requires expert validation. We propose a new framework to fully automate this process using techniques from machine learning and statistical analysis. We utilize a convolutional neural network and Bayesian statistical model on spectrogram data from candidate spike ripple events to predict patient outcomes. Preliminary results show high accuracy in classification of individual spike ripple events, and strong evidence for the ability of this method to accurately predict subject status (healthy, active disease, or in remission).

Disclosures: J. Nadalin: None. **L. Martinet:** None. **U. Eden:** None. **C. Chu:** None. **M.A. Kramer:** None.

Poster

123. Epilepsy: Human Studies

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 123.28/C47

Topic: B.10. Epilepsy

Support: Mass Life Sciences Center
Neuroelectrics Corporation

Title: Targeted non-invasive neurostimulation reduces seizures in epilepsy patients with prior surgical interventions

Authors: *G. RUFFINI, **N. A. SMITH**;
Neuroelectrics, Cambridge, MA

Abstract: Objective: To determine whether epilepsy patients with prior surgeries deemed ineffective can see meaningful seizure reductions with non-invasive neurostimulation,

specifically using transcranial current stimulation (tCS). **Background**: A significant number of medication resistant epilepsy patients treated with surgery do not see a significant reduction in seizure count. These patients have no other non-pharmacological options. **Design/Methods**: The authors are involved in a clinical trial under an open FDA IDE to evaluate the effectiveness of targeted transcranial current stimulation (tCS) to reduce seizures. We previously reported that patients resistant to medication treated saw a median seizure reduction of 47% from baseline eight weeks after completing the treatment. After the start of the trial, the FDA agreed to expand the inclusion criteria to allow patients with prior surgical interventions. For each of these patients a personalized stimulation montage was determined by using an MRI of the patients head to build a finite-element model to model current flows in the brain. A genetic algorithm was then used to determine placement and current levels of between six and eight scalp electrodes to deliver currents preferentially to the seizure focus. These patients then followed the standard treatment protocol of 10X 20 minute stimulation sessions over two weeks, with eight weeks of follow-up. No changes in any medications were made. **Results**: Three patients - one with prior resective surgery, one with burr holes from a prior EEG, one with a VR shunt - were treated with this protocol. These patients reported seizure reductions in the eight weeks following treatment of 75%, 44%, and 90% respectively. No adverse events were reported for any of these patients. **Conclusion**: Targeted non-invasive neurostimulation using transcranial current stimulation may be an effective therapy for controlling seizures in surgical non-responders.

Disclosures: G. Ruffini: A. Employment/Salary (full or part-time):; Neuroelectrics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuroelectrics. **N.A. Smith:** None.

Poster

124. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 124.01/C48

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AMED 19bk0104077h0003
KAKENHI 17H03557
KAKENHI 19K08010

Title: Alzheimer's disease model mouse analysis focusing on Drebrin

Authors: *N. KOGANEZAWA[1], Y. KAJITA[2], H. YAMAZAKI[1], T. SAITO[3], Y. SEKINO[4], T. C. SAIDO[3], T. SHIRAO[1];
[1]Gunma Univ. Grad. Sch. of Med., Maebashi, Japan; [2]Dept. of Physiol., Tohoku Univ. Sch. of Med., Sendai, Japan; [3]RIKEN Ctr. for Brain Sci., Wako, Japan; [4]Endowed Lab. of Human Cell-Based Drug Discovery, Grad. Sch. of Pharmaceut. Sciences, The Univ. of Tokyo, Tokyo, Japan

Abstract: Alzheimer's disease (AD) is one of neurodegenerative diseases and the most common cause of dementia. Pathology of AD includes the extracellular accumulation of amyloid beta peptide, the intracellular accumulation of hyper-phosphorylated tau, the loss of drebrin from dendritic spines, and the loss of neuronal cells mainly in the cerebral cortex and hippocampus. Among them synaptic dysfunction has most correlation with cognitive dysfunction in the AD brains. Drebrin is an actin binding protein and stabilizes actin filaments. Drebrin-decorated stable actin filaments accumulate in dendritic spines and are thought to be crucial for synaptic plasticity. Drebrin has been decreased at onset of dementia in AD. We therefore hypothesized that loss of drebrin, that is, loss of stable actin filaments from dendritic spines elicits synaptic dysfunction and causes dementia in AD. Here we used the *App* knock-in (KI) mouse model of AD ($App^{NL\text{-}G\text{-}F}$ KI mouse), to analyze the details of abnormal synapse in AD. First we performed immunohistochemical analysis using $App^{NL\text{-}G\text{-}F}$ KI mice brains and wild-type mice brains. We focused on the cortex and found no drebrin immunoreactivity around amyloid plaques in the $App^{NL\text{-}G\text{-}F}$ KI mice brains, however, no clear difference of drebrin immunoreactivity in other regions than amyloid plaques between two types of mice brains. This result indicates that endogenous amyloid beta reduces drebrin *in vivo* and this is consistent with our previous data showing drebrin clusters reduction by exogenous amyloid beta *in vitro*. We further used primary hippocampal cultured neurons derived from the $App^{NL\text{-}G\text{-}F}$ KI mice ($App^{NL\text{-}G\text{-}F}$ KI neurons) and evaluated synaptic status based on drebrin cluster number using high-content imaging analysis. Our data showed $App^{NL\text{-}G\text{-}F}$ KI neurons had less drebrin clusters indicating low functionality of synapse. These data suggest that the loss of drebrin from the dendritic spine in AD brains causes synaptic dysfunction.

Disclosures: N. Koganezawa: None. **Y. Kajita:** None. **H. Yamazaki:** None. **T. Saito:** None. **Y. Sekino:** None. **T.C. Saido:** None. **T. Shirao:** None.

Poster

124. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 124.02/C49

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The dendritic improvement by histone deacetylase 2 specific inhibition in amyloid beta precursor protein overexpression mice

Authors: *K. OGAWA, **D. NAKATSUKA**, T. IZUMI, T. TSUKAMOTO, M. OYAMA, K. NIIDOME, H. YAMAKAWA, H. ITO;
Shionogi & Co., LTD., Osaka, Japan

Abstract: Current drug for Alzheimer's disease (AD) is symptomatic treatments that only ameliorate the symptoms. Therefore, disease-modifying therapy, such as neuroprotective and

neurorestorative interventions, is strongly desired. However, there is no approved treatment with a proven disease-modifying effect. Recently, epigenetics has gained particular attention for its role in memory. It was shown that specific inhibition of histone deacetylase 2 (HDAC2) restore memory impairment in neurodegenerative model mice. Moreover, in wild type mice, HDAC2 specific inhibition induces dendritic and spine growth. Meanwhile, it was known that dysmorphology of dendrites and spines was seen in amyloid β precursor protein (APP) over expression mice. However, in the APP overexpressed condition, whether specific inhibition of HDAC2 restore dendritic and spine damages is still unknown. In this study, we aimed to evaluate if HDAC2 specific inhibition can restore dendritic and spine impairment in the APP overexpressed condition using PS/APP mice (mutant presenilin 1 and APP transgenic mice). We specifically inhibited HDAC2 in the CA1 region of hippocampus using adeno associated virus coding HDAC2 RNAi. Neural morphology was analyzed using golgi staining method. In the PS/APP mice, dendrites and mushroom-like spines in the CA1 region of hippocampus were impaired as previously reported. We demonstrated that HDAC2 specific inhibition restored dysmorphology in dendrites and mushroom-like spines, as well as the impairment of long-term potentiation and episodic memory in PS/APP mice. These results indicate that HDAC2 specific inhibition can recover damaged neuron under a state in which APP was overexpressed. Furthermore, it is expected that HDAC2 specific inhibition has the potential to be disease-modifying therapy in the treatment for AD.

Disclosures: **K. Ogawa:** A. Employment/Salary (full or part-time):; SHIONOGI & CO., LTD. **D. Nakatsuka:** A. Employment/Salary (full or part-time):; SHIONOGI & CO., LTD. **T. Izumi:** A. Employment/Salary (full or part-time):; SHIONOGI & CO., LTD. **T. Tsukamoto:** A. Employment/Salary (full or part-time):; SHIONOGI & CO., LTD. **M. Oyama:** A. Employment/Salary (full or part-time):; SHIONOGI & CO., LTD. **K. Niidome:** A. Employment/Salary (full or part-time):; SHIONOGI & CO., LTD. **H. Yamakawa:** A. Employment/Salary (full or part-time):; SHIONOGI & CO., LTD. **H. Ito:** A. Employment/Salary (full or part-time):; SHIONOGI & CO., LTD..

Poster

124. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 124.03/C50

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: France Alzheimer
IUF

Title: Alteration of area CA2 is linked to social memory deficit in the Tg2576 model of Alzheimer's disease

Authors: *C. C. REY[1], V. ROBERT[2], M. LOISY[2], V. CATTAUD[1], C. LEJARDS[1], R. A. PISKOROWSKI[3], C. RAMPON[1], V. CHEVALEYRE[2], L. VERRET[1];
[1]CRCA, CNRS Toulouse Univ., Toulouse, France; [2]INSERM U894, Paris, France; [3]Team Synaptic Plasticity and Neural Networks, Inserm U894, Paris, France

Abstract: Among the cognitive impairments observed in Alzheimer's disease (AD) patients, one of the most unbearable is their inability to recognize or remember other persons. However, little is known about the neural substrates of social memory deficits in AD. Recently, it was shown that inactivation of pyramidal neurons in area CA2 of the hippocampus induces specific social memory impairments. Area CA2 also contains a high density of parvalbumin (PV)-expressing inhibitory interneurons with unique properties, such as particularly high presence of their specific extracellular matrix, the perineuronal net (PNN). PNNs are forming with an activity-dependent manner around PV cells during their maturation and are ensuring the stabilization of synapses. Furthermore, PV interneurons in area CA2 express a unique long-term depression mediated by Delta opioid receptor (DOR-iLTD) which may contribute to social memory formation. We previously showed that transgenic mice resembling key features of AD display impaired PV cell function linked to their memory deficits. Thus, we wondered whether enhancing PV cell function in area CA2 of AD mice would restore social memory. First, a stereological analysis of PV/PNN staining in the area CA2 revealed that the absolute number of PV+ and PV+/PNN+ cells are dramatically decreased in Tg2576 compared to non-transgenic (NTg) mice. Using voltage-clamp recordings of acute hippocampal slices, we observed a decreased of DOR-iLTD associated with an increase of the excitability in area CA2 of Tg2576 compared to NTg mice. We then addressed the behavioral consequences of the reduced inhibition in this region, and found that Tg2576 mice exhibit intact sociability, but display impaired social memory. Neuregulin-1 (NRG1) is involved in experience-dependent maturation of PV cells and promotes the formation of PNN. Furthermore, NRG1 and its receptor ErbB4 are playing active roles in regulating inhibitory transmission and plasticity in area CA2. Hence, we proposed that NRG1 local injection in area CA2 of AD mice may be beneficial. We indeed found that NRG1 injection in area CA2 of Tg2576 mice was able to locally increase the number of PV+ and PV+/PNN+ cells and to improve social memory, without affecting other types of hippocampal-dependent memory. We conclude that the alteration of PV cells and their PNN in area CA2 disrupt the establishment of social memory in Tg2576 mice.

Disclosures: C.C. Rey: None. **V. Robert:** None. **M. Loisy:** None. **V. Cattaud:** None. **C. Lejards:** None. **R.A. Piskorowski:** None. **C. Rampon:** None. **V. Chevaleyre:** None. **L. Verret:** None.

Poster

124. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 124.04/C51

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSFC Grant 81571038
NSFC Grant 81771145
the National Key Research and Development Program of China 2016YFC1306300

Title: Chronic administration of GABAA receptor agonist tunes hippocampal network in a mouse model of Alzheimer's disease

Authors: *Y. LI[1], K. ZHU[2], L. LI[1], N. LI[1], X. XIAO[2], L. LI[2], X. WANG[1], Y. ZHENG[2];
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Abstract: Hippocampus is responsible for computing contextual and spatiotemporal information and an indispensable brain region for processing learning and memory. The imbalance of inhibitory and excitatory input results in disrupted action potential output mainly from pyramidal neurons in hippocampus. Thus, the derangement of the neural circuit and subsequent epileptiform activity in hippocampus was considered a critical event involved in cognitive disorders, e.g. Alzheimer's disease (AD). However, the mechanism of neuronal hyperactivity involved in cognitive circuit dysfunction and AD pathology is still enigmatic. We have found that CA1 pyramidal neurons in hippocampus of a mouse model of AD, 5XFAD mouse presented hyper discharging due to postsynaptic dislocation of γ-aminobutyric acid A ($GABA_A$) receptors which caused an imbalance of excitation/inhibition in the neural circuit. Here, we sought to investigate whether slow and persistent activation of $GABA_A$ receptors could restore hippocampal network and prevent cognitive dysfunction in early stage of 5XFAD mouse to estimate the value of $GABA_A$ receptors as a target for AD treatment. We treated 5XFAD male mice at 3.5 months old with chronic intracerebroventricular infusion of $GABA_A$ receptor agonist, Gaboxadol (GBX) by mini-osmotic pumps (5 μM,0.25 μl/hr, n=4), and observed an obvious improvement in hippocampus-dependent behaviors after 28 days of continuous administration, compared with 5XFAD mice pumped with saline (n=3). More interestingly, Aβ accumulation not only in hippocampus but also in cortex near to lateral ventricles of the mouse model treated with GBX was significantly cleared. As expected, the $GABA_A$ receptor subunits, α1 and γ2 expression and synaptic location were restored. These data imply that CA1 neuronal hyperactivity due to inhibitory postsynaptic disruption may be a trigger for Aβ production and aggregation, most importantly, for cognitive disorder occurrence in early stage of AD.

Disclosures: Y. Li: None. **K. Zhu:** None. **L. Li:** None. **N. Li:** None. **X. Xiao:** None. **L. Li:** None. **X. Wang:** None. **Y. Zheng:** None.

Poster

124. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 124.05/C52

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: European Research Council (724866)
Legacy Heritage Biomedical Program of the Israel Science Foundation (1849/17)
Israel Science Foundation (1663/18)
Israel Ministry of Science, Technology and Space

Title: State-dependent regulation of CA1 ensembles *in vivo* in Alzheimer models

Authors: *D. ZARHIN[1], N. GEVA[3], Y. ZIV[3], I. SLUTSKY[2];
[1]Dept. of Physiol. and Pharmacol., [2]Tel Aviv Univ., Tel Aviv, Israel; [3]Dept. of Neurobio., Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Pure memory impairments constitute the earliest clinical sign of Alzheimer's disease (AD), the most frequent form of late-life dementia. Understanding the neural correlates of AD-associated memory failures requires measurements of hippocampal activity in awake, freely behaving mice. While previous 2-photon studies have been instrumental in identifying aberrant activity in CA1 circuits in mouse AD models, they were performed only under anesthesia and at single time points. To explore AD-associated hippocampal pathophysiology, we combined large-scale Ca^{2+} imaging over extended time periods by utilizing miniature microscopy and extracellular electrophysiology in CA1 hippocampal circuits. We used chronic, longitudinal recordings of the same CA1 pyramidal neurons using GCaMP6f-based Ca^{2+} imaging in behaving wild-type (WT) mice *vs.* APP/PS1 model of familial AD. Unexpectedly, we did not observe hyperactivity in CA1 network of APP/PS1 mice during exploration. Average level of CA1 activity, reflected by the mean Ca^{2+} rates per neuron and the number of active neurons, was similar between WT and APP/PS1 mice. However, a profound difference was observed in response of CA1 network activity to general anesthesia, associated with unconsciousness and amnesia. While WT mice displayed a significant reduction in the number of active CA1 neurons and their activity in response to anesthesia, the opposite reaction was detected in APP/PS1 mice. Notably, neuronal populations were largely non-overlapping in awake and anesthetized states. Augmented and synchronized somatic CA1 Ca^{2+} activity was accompanied by pathological spike waveforms in the *stratum radiatum* of anesthetized APP/PS1 mice. Pathological CA1 spikes were robustly induced in several AD models by distinct anesthetics. Based on these results, we propose that dysregulation of hippocampal activity set points in anesthetized, amnestic brain states may present the neurobiological basis underlying accelerated progression of AD pathophysiology by anesthesia proposed by earlier studies.

Disclosures: **D. Zarhin:** None. **N. Geva:** None. **Y. Ziv:** None. **I. Slutsky:** None.

Poster

124. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 124.06/C53

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: National Academy of Sciences of Armenia

Title: Electrophysiological and morphological study of the synaptic processes in hippocampus, amygdala and basal meynert nucleus neurons in dynamics of development of Alzheimer's disease

Authors: *V. SARGSYAN, N. BEHNAM, J. SARKISSIAN;
Orbeli Inst. Physiol, Yerevan, Armenia

Abstract: Objective: One of the main forms of synaptic plasticity found at the excitatory synapses is the long-term potentiation (LTP), which is altered in neurodegenerative diseases. Compensatory mechanisms are responsible for the clinical signs of suppression of neurodegeneration. Intervention into their mechanisms on an example of the ratio of excitatory and depressor synaptic responses will contribute to the development of therapeutic strategies.
Methods: After 12-28 weeks (w) of experiment on the model of Alzheimer's disease (AD), an activity of single neurons of hippocampus (H), Amygdala (Am) and, nucleus basalis of Meynert (NBM) to high frequency stimulation (HFS) of entorhinal cortex (EC) was recorded. The high frequency stimulation of H resulted in an activity of single neurons of the Am and NBM. By means of on-line selection and special mathematical analysis, tetanic potentiation (TP) and depression (TD) further combined with posttetanic uni- and multidirectional sequences (PTP and PTD), were revealed. In morpho- and histochemical study, the method of revelation of Ca2+-dependent phosphorylation was used.
Results: On the 12th weeks of experiment a strong TD of NBM and Am neurons to HFS of H, as well as a weak TD in H and NBM neurons to HFS of EC were found. TP occurred by the activation of EC in the H (TP PTP) and in Am (TP PTD) neurons, equal to and above the norm in neurons of Am to HFS of H. In the NBM neurons of to HFS of EC, the weakest excitation to HFS of H was detected. In the H neurons to HFS of EC and in Am and NBM neurons to HFS of H, after 13-28 weeks, TD and tetanic excitation in all cases were low, which indicating on depletion of compensatory opportunities. Morpho- and histo-chemical changes of H, Am and NBM neurons on the model of AD were characterized by total tendency to structural-metabolic dysfunctions, with distortion of forms, as well as central chromatolysis.
Conclusion: The absence of expressed depression, presupposed by us as a protector in the present study, makes it necessary to involve pharmacological interventions with a view to its

strengthening, and therefore is the subject of the next explorations. Electrophysiological data have been confirmed morphologically.
Keywords: *Alzheimer's disease; Hippocampus; Rostral amygdalopiriform area; Basal nucleus of meynert; Single spike activity; Acid phosphatase*

Disclosures: **V. Sargsyan:** None. **N. Behnam:** None. **J. Sarkissian:** None.

Poster

124. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 124.07/C54

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: FAPESP 2016/04297-6
FAPESP 2017/10404-2
CAPES - Finance Code 001

Title: Laminin and aβ oligomers compete for PrP^C binding and their availability are altered by sleep deprivation

Authors: *M. H. M. DA LUZ[1], J. M. V. PINO[1], T. G. DOS SANTOS[3], H. K. M. ANTUNES[2], B. D. ANTONIO[1], M. C. LANDEMBERGER[3], K. S. LEE[1];
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Abstract: Laminin (LN) can interact with PrP^C to promote neuritogenesis via Erk, while biding of amyloid-beta oligomers (Aβo) to PrP^C generates synaptic dysfunction via Fyn kinase. In both pathways, mGluR1 is recruited as co-receptor. The involvement of PrP^C/mGluR1 in these opposite functions suggests that this complex acts as a key element in the regulation of synaptic activity, which oscillates along the sleep-wake cycle. Thus, we investigated how sleep deprivation can interfere these molecular pathways. We also verified the interference of Aβo on LN binding to PrP^C and on neuritogenesis. C57BL/6 mice (3-month males) were subjected to sleep deprivation for 3 days (SD) and hippocampi were collected at two time points of circadian period (13h and 21h). Immunoenzymatic Assay (ELISA) and Western Blotting were performed for quantification of Aβ40 and Aβ42 peptides and protein levels. Competition between oAβ and LN was evaluated by in vitro binding assay. Neurite outgrowth assay was carried out using neurons expressing PrPC (Prnp+/+) or not (Prnp0/0). Images obtained were analyzed with NeuronJ of Image J version 1.50i. We observed that SD reduced PrP^C levels in active state (21h), but didn't change mGluR1 levels. Moreover, SD tended to increase the levels of Aβ peptides and Fyn kinase phosphorylation in rest period (13h). Laminin levels did not change between groups, but the phosphorylation of ERK tended to increase in SD13h group. Through an in vitro binding

assay, we observed that Aβo can compete with LN for PrP^C binding. The influence of Aβo was also observed in neuritogenesis. LN promoted longer neurite outgrowth than not treated cells in both control and *Prpn* knockout cells. Aβ alone did not show any effects. When both ligands were added together, a discrete reduction was observed compared with LN group only in control cells. These indicate that LN binding to PrP^C can be affected by the presence of Aβo attenuating neuritogenesis.

Disclosures: M.H.M. da Luz: None. **J.M.V. Pino:** None. **T.G. dos Santos:** None. **H.K.M. Antunes:** None. **B.D. Antonio:** None. **M.C. Landemberger:** None. **K.S. Lee:** None.

Poster

124. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 124.08/C55

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant K23 AG038357
John Douglas French Alzheimer's Foundation

Title: Depletion of voltage-gated potassium channel Kv1.1 contributes to behavioral deficits in transgenic mouse model of Alzheimer's disease

Authors: *J. M. CHOQUETTE[1], S. T. PETERS[1], V. FOMENKO[2], J. C. XU[3], R. J. CRAFT[4], K. GIMLIN[5], *K. A. VOSSEL[1];
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Abstract: Patients with Alzheimer's disease (AD) are at risk for seizures and accelerated cognitive decline. Mechanisms of seizures and related synaptic dysfunction in AD are active areas of investigation. Alterations in ion channels have been identified in transgenic mouse models of AD, but levels of voltage-gated potassium channels have not been fully explored. In particular, little is known about the Kv1.1 channel in AD. Mutations in Kv1.1 or autoantibodies against Kv1.1 cause neuronal overexcitation in several human diseases, including episodic ataxia type 1, epilepsy, myokymia, and limbic encephalitis, indicating that Kv1.1 is critical for regulating neuronal excitability. To explore voltage-gated potassium channels in a model of AD, we used hAPP-J20 mice ages 4-6 months, and determined mRNA levels of four highly expressed voltage-gated potassium channels - Kv1.1, Kv1.2, Kv1.4, and Kv4.2 - in dentate gyrus, entorhinal cortex, motor cortex, and somatosensory cortex. Using RT-qPCR, we identified reductions in Kv1.1 mRNA in the somatosensory cortex of hAPP-J20 mice. Western blotting revealed reductions in the Kv1.1 channel but not the Kvbeta2 subunit in the somatosensory cortex of hAPP-J20 mice. Immunolabeling revealed that the reductions in Kv1.1 channels in the

somatosensory cortex occurred in both pyramidal cells and GABAergic interneurons. Kv1.1 levels in the somatosensory cortex were lower in hAPP-J20 mice, which overexpress mutant hAPP and have high Abeta1-42 levels, than in the wild-type hAPP line I5, which overexpresses hAPP at levels similar to J20, suggesting some dependence on Abeta1-42 levels. Levetiracetam treatment (75 mg/kg/day) for 28 days did not affect Kv1.1 levels in hAPP-J20 mice indicating that reduction of Kv1.1 is not a consequence of hyperexcitability, but rather could contribute to hyperexcitability. To assess the functional relevance of Kv1.1 depletion in the context of an AD model, we crossed Kv1.1 heterozygous mice (Kv1.1 +/-) with hAPP-J9 mice, which have lower expression of mutant hAPP than the J20 line. We performed behavioral tests on mice ages 4-9 months. While the Kv1.1 +/- mice and hAPP-J9 mice had normal survival, the double mutant (Kv1.1 +/- hAPP-J9) mice had premature mortality, as well as impairments in elevated plus maze (2 independent cohorts) and light-dark box tests (n=16-25 mice/genotype) of anxiety. Together, these data indicate that elevated Abeta1-42 levels may act synergistically with Kv1.1 depletion to exacerbate cognitive deterioration in AD. Ongoing investigation will further characterize the relationships between Kv1.1 loss and AD-related hyperexcitability.

Disclosures: **J.M. Choquette:** None. **S.T. Peters:** None. **V. Fomenko:** None. **J.C. Xu:** None. **R.J. Craft:** None. **K. Gimlin:** None. **K.A. Vossel:** None.

Poster

124. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 124.09/C56

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 NS094597

Title: Dysregulation of synaptic neurotransmitter systems in human Alzheimer's disease brain cortex revealed by integration of proteomics and neuropeptidomics analyses

Authors: *V. Y. HOOK, S. PODVIN, C. LIETZ, R. RISSMAN;
Univ. of Calif., San Diego, La Jolla, CA

Abstract: Synaptic dysfunction is hypothesized to participate in the neurodegeneration of Alzheimer's disease. Synapses are the cell-cell junctions of neurotransmission required for chemical neurotransmitter communication among neural circuits for mediating behaviors. It is logical to hypothesize that synapses of affected human Alzheimer's disease (AD) brain cortex displays alterations in synaptic protein systems responsible for neurotransmission. To evaluate this hypothesis, human brain cortex tissue from AD and age-matched controls were analyzed by isolation of synaptosomes (by density gradient centrifugation) representing nerve terminals associated with post-synaptic components. The synaptic proteomes of these synaptosome

samples were analyzed by global proteomics mass spectrometry, as well as by peptidomics for analyses of endogenous peptide neurotransmitters. Integration of peptidomics analyses of endogenous peptide neurotransmitters provides an innovative 'neuropeptidomics' dimension to 'proteomics' of synaptic protein systems. Proteomics data indicated identification of more than 5,500 proteins among AD and control synaptosomes (4 subjects per group). Proteins were categorized as those (a) present only in AD (158 proteins), (b) present only in controls (281 proteins), and (c) present in both AD and control (5193 proteins) with quantitative comparisons for up-regulation and down-regulation. For the majority of proteins shared in AD and controls, quantitation revealed distinct patterns of up-regulated and down-regulated proteins in AD compared to controls, with significance values of $p < 0.05$. GO (gene ontology) analyses indicated up-regulation in AD of cellular localization and signaling functions, including vesicle mediated transport, and down-regulation in AD of biological quality and phosphotidyl inositol signaling. Peptidomics data revealed the differential presence of peptide fragments of Aβ, tau, chromogranin A, VGF, enkephalin, dynorphin, and other neuropeptides in AD compared to controls. Specific peptides were present in only AD, in only control, or in both AD and control. This rich dataset of endogenous peptidomics and proteomics of synaptosomes reveals dysregulation of synaptic neurotransmission systems in human AD brain cortex.

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Poster

124. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 124.10/C57

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Busch Biomedical Grant (Rutgers University)
Aresty Foundation funding for Undergraduate Research
Support from the School of Arts and Sciences

Title: Disruptions of learning, memory and experience-induced auditory cortical plasticity in a next-generation rat model of Alzheimer's disease

Authors: *A. SHANG[1], S. TSAUR[1], S. BYLIPUDI[1], D. SULLIVAN[1], M. D. TAMBINI[2], L. D'ADAMIO[2], K. M. BIESZCZAD[1];
[1]Psychology Dept., Rutgers Univ., New Brunswick, NJ; [2]NJMS Dept. of Pharmacology, Physiol. & Neuroscience, Brain Hlth. Inst., Rutgers Univ., Newark, NJ

Abstract: There is a strong link between auditory function and cognitive decline. Hearing loss in midlife has been identified as a risk factor for progression to Alzheimer’s disease (AD) (Livingston et al., 2017), and central auditory processing disorders can precede AD onset by 5-

10 years (Iliadou et al., 2003), which posits auditory function as a possible early biomarker for later-life progression to dementia. While the primary auditory cortex (A1) is a key site for learning-dependent sensory neuroplasticity that contributes to long-term memory formation, the relationship between auditory system function and cognition remains largely understudied in animal models of AD. Here, we used a next-generation model (NexGenMo) of AD developed by Luciano D'Adamio to examine auditory cognitive function and cortical plasticity in a CRISPR-mediated point mutation knock-in (KI) rat. KI introduces one ($App^{S/H}$; n = 6) or two copies ($App^{S/S}$; n = 6) of a familial Swedish mutation to the amyloid precursor protein (APPswe), vs. controls with the humanized sequence knocked-in ($App^{H/H}$; n=6). Animals were trained on a 2-tone associative/auditory discrimination (2TAD) task, which requires them to respond (by barpress; BP) to one tone (5.0 kHz) for reward, and to inhibit BPs to another tone (11.5 kHz). Successful auditory learning and cue-selective memory formation has been shown to depend on learning-induced A1 plasticity that sharpens and strengthens acoustic representation of learned cues in long-term memory. We hypothesized that APPswe would produce disrupted learning and memory due to failures of normal learning-induced cortical plasticity. 2TAD learning and performance levels were delayed and blunted in animals with APPSwe. Memory Tests immediately after reaching performance level criterion also revealed that the memory formed for the rewarded tone was less precise with APPswe. Another Memory Test 5 weeks later showed that time-dependent consolidation processes that sharpen auditory memories were also disrupted with APPSwe. Electrophysiology revealed normal auditory brainstem responses (ABRs) and cortical physiology to support that learning deficits were not due to hearing loss or atypical A1 organization. However, learning-induced A1 tuning bandwidth changes were different between genotypes, and paralleled the learning and memory delays with APPswe. This is the first report on the behavioral and neurophysiological consequences of this NexGenMo of AD on auditory associative learning, and the first of few studies examining memory-related sensory cortical function in AD.

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Poster

124. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 124.11/C58

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CAPES
CNPq
FAPERJ
Alzheimer's Association

Title: mTORC1 signaling in abeta-oligomer-based and transgenic murine models of Alzheimer's disease

Authors: *D. COZACHENCO[1], F. C. RIBEIRO[1], A. AGUILAR-VALLES[2], F. G. DE FELICE[3], N. SONENBERG[4], S. T. FERREIRA[1];
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Abstract: The mammalian target of rapamycin (mTOR) plays a fundamental role in cell proliferation, survival and autophagy. In particular, mTOR complex 1 (mTORC1) has a key role in the regulation of protein synthesis activated by stimuli such as amino acids, insulin and other growth factors. Both insulin signaling and protein synthesis are impaired in Alzheimer's disease (AD), the most common cause of elderly dementia. Because of this, the mTORC1 signaling pathway has received much attention in recent AD research. However, results from such studies remain controversial. While most groups have shown an upregulation of the mTORC1 pathway in experimental models of AD, others have reported an opposite effect. We are currently studying the mTORC1 pathway in two different models of AD: wild-type Swiss mice receiving intracerebroventricular (icv) infusions of amyloid-β oligomers (AβO), toxins that build up in the AD brain and are thought to cause synapse failure and memory loss, and transgenic APP/PS1 mice. Our results show that mTORC1 signaling is significantly reduced in the mouse hippocampus 7 days after icv infusion of AβOs. Surprisingly, we do not find any changes in mTORC1 signaling in the hippocampi of APP/PS1mice, a transgenic model engineered to overproduce the amyloid-β peptide, at either 4 or 8 months of age, when AD-related neuropathology and memory impairment are detected. Results may point to potentially critical differences between acute and chronic models of AD, and to disease stage-dependent regulation of mTORC1 signaling. Further, results may be connected to recently reported differences in mTORC1 subcellular localization (plasma membrane versus lysosomes), a possibility we are currently exploring. Resolving these apparently conflicting results may be an important step towards proper understanding of mTORC1 signaling in AD, and to the development of therapies focused on reestablishing proteostasis.

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Poster

124. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 124.12/C59

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: FAPERJ

INNT
CNPq
NIH Grant 2R01NS047384-13

Title: Targeting defective protein synthesis to correct memory impairment in Alzheimer's disease models

Authors: *M. M. OLIVEIRA[1], M. V. LOURENCO[3], F. LONGO[1], N. KASICA[4], W. YANG[5], T. MA[6], F. G. DE FELICE[3], E. KLANN[2], S. T. FERREIRA[7];
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Abstract: De novo protein synthesis is a core requirement for long-term memory consolidation. Amongst several regulators of translation, eIF2α phosphorylation (eIF2α-P) impairs synaptic plasticity and cognitive performance by inhibition of eIF2B, which is necessary for formation of the ternary translation initiation complex. In recent years, disrupted protein synthesis has been implicated in a number of neurodegenerative disorders, including Alzheimer's disease (AD). eIF2α phosphorylation plays a major role in the disruption of proteostasis in AD, mediating disease-related impairment of synaptic plasticity and cognitive deficits. However, whether other components of translation initiation exhibit aberrant activity or levels in AD remains unknown. We found that frontal cortical tissue from AD patients exhibits marked reductions in eIF2Bα and eIF2Bε. We thus hypothesized that boosting eIF2B activity might restore translation and rescue memory impairments in AD models. To test this hypothesis, we used ISRIB, a small-molecule compound that binds to eIF2B and enhances its catalytic activity even in the presence of inhibitory eIF2α phosphorylation, thus normalizing protein synthesis. Intraperitoneal administration of ISRIB prevented memory impairment induced by intracerebroventricular (i.c.v.) infusion of AD-linked amyloid-β oligomers (AβO) in mice. We further found that ISRIB rescued memory impairments displayed by APPswePS1ΔE9 AD model mice. Mechanistically, ISRIB rescued proteostasis, prevented ATF4 translation, and reduced dendritic spine loss in the hippocampi of AβO-infused mice. Our results suggest that counteracting eIF2α-phosphorylation-mediated downregulation of brain protein synthesis using ISRIB may be an attractive and yet unexplored therapeutic approach in AD.

Disclosures: **M.M. Oliveira:** None. **E. Klann:** None. **S.T. Ferreira:** None. **F.G. De Felice:** None. **M.V. Lourenco:** None. **F. Longo:** None. **T. Ma:** None. **N. Kasica:** None. **W. Yang:** None.

Poster

124. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 124.13/C60

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer’s Drug Discovery Foundation grant 20160803

Title: Mobilizing hippocampal noradrenergic fibers to produce dopamine improves contextual fear memory in Tg2576 (Alzheimer's model) mice

Authors: N. A. OTMAKHOVA, ***S. J. BIRREN**, J. LISMAN;
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Abstract: Hippocampal dopamine (DA) is required for long-term memory[1] and is supplied by co-release from noradrenergic (NE) fibers to a greater extent than from other DA sources[2]. The locus coeruleus, the source of NE, undergoes significant degeneration early in the course of Alzheimer’s disease (AD). However, the effect of this degeneration on the progress of AD remains unclear[3]. We proposed that loss of DA co-release from NE fibers in the hippocampus, rather than a decrease in NE content or release, could result in memory declines in AD. We tested this hypothesis in a long-term study of Tg2576 mice, a useful animal model of AD. Vehicle-treated wildtype littermate controls (1) were compared to Tg2576 mice (3-3.5 months old) treated for 5 months with (2) Vehicle; (3) N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP-4) to damage the NE system; (4) DSP-4 and Nepicastat to inhibit Dopamine-beta-hydroxylase (DBH) and increase DA levels; and (5) DSP-4 and Nepicastat and Droxidopa/Benserazide to replenish NE in the brain via a DA-independent pathway and block the peripheral action of Droxidopa. At the end of the five-month trial mice underwent behavioral testing and were sacrificed for brain tissue collection. We found that hippocampal-dependent contextual fear memory was decreased close to 50% for Tg2576 mice compared to WT littermates. This improved up to WT levels when NE fibers in the hippocampus were mobilized to produce more DA. Contextual fear memory indicators did not appear to depend on hippocampal NE content or DBH fluorescence but co-varied with hippocampal DA content. These data support a model in which increased DA, coming from NE pathways, contributes to behavioral improvements in an Azheimer’s model. This work was funded by the Alzheimer’s Drug Discovery Foundation, grant 20160803.[1]Lisman, et al, Trends in Neuroscience 34:536-547, 2011.[2]Duszkiewicz, et al, Trends in Neuroscience 42:102-114, 2019.[3]Gannon, et al, Brain Res. 1702:12-16, 2019.

Disclosures: **N.A. Otmakhova:** None. **S.J. Birren:** None. **J. Lisman:** None.

Poster

124. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 124.14/C61

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Tata grant

Title: An animal model of vascular dementia shows synaptic dysfunction and memory impairment

Authors: *L. DIWAKAR[1], K. CHITHANATHAN[1], D. TOMAR[1], R. D. GOWAIKAR[1], V. RAVINDRANATH[1,2];
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Abstract: Micro-infracts caused by ischemia, micro bleeds and hemorrhage are major contributors to vascular and mixed dementia in elderly population. Because vascular insults contributes significantly to the burden of disease pathology there is need to develop vascular dementia model with milder insult leading to memory impairment. In present study we used ET-1 (Endothelin 1) a 21 amino acid vasoconstricting peptide to produce transient ischemia. ET-1 was injected stereotactically into lateral ventricles of C57 mice. Expression of CD31, a marker of endothelial cells was measured to confirm vasoconstriction by immunohistochemistry. To assess the associative learning and spatial memory deficits, contextual fear conditioning, object recognition and Morris water maze task was performed. Activity dependent protein translation was measured in synaptoneurosomes prepared from hippocampus, which is crucial for synaptic plasticity. ET-1 injection resulted in considerable decrease in CD31 expression in blood vessels represented as number and length in hippocampal region after 3 days. Impaired blood flow following ET-1 injection leads to deficits in associative learning and spatial memory after 7 days reflected as loss of activity dependent protein translation in synaptoneurosomes prepared from hippocampal tissue. Further there was decreased Akt phosphorylation and down regulation of mTOR signaling cascade in hippocampus indicating potential dysfunction in synaptic plasticity. However the transient memory deficits could be inversed by 30 days. Further, when we gave repeated vascular insults once every three weeks there was sustained associative learning and spatial memory deficits. These results suggested that repeated minor vascular insult gradually worsen the pathology leading to learning and memory impairment similar to Alzheimer’s disease.

Disclosures: L. Diwakar: None. **K. Chithanathan:** None. **D. Tomar:** None. **R.D. Gowaikar:** None. **V. Ravindranath:** None.

Poster

124. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 124.15/C62

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Hippocampal and cerebellar abnormalities in a mouse model of Alzheimer's disease

Authors: L. RIS[1], M. FASSIN[1], A. LEROY[2], J. MARQUEZ-RUIZ[4], ***G. CHERON**[3]; [1]Neurosci., Univ. of Mons, Mons, Belgium; [2]Lab. of Neurophysiol. & Movement Biomechanics, [3]Fsm-Université Libre De Bruxelles, Brussels, Belgium; [4]División de Neurociencias. Univ. Pablo de Olavide, Seville, Spain

Abstract: Recent data support an integrative hypothesis that the cerebellum participates to the cognitive and neuropsychiatric deficits in Alzheimer disease (AD). Beside its well-known role in sensori-motor control and learning, the cerebellum has a role in cognitive processes and in spatial navigation requiring the formation of a hippocampal spatial map and the induction of goal-directed behaviour. Reciprocity in this relation was also showed in eye-blink conditioning during which a tone is associated to corneal air puff and therefore to eyelid closure. This task is an associative learning dependent on LTD between parallel fibers and Purkinje cells but the trace version of this task is hippocampus dependent. The importance of cerebello-hippocampal network in navigation was also demonstrated by fMRI and virtual reality in humans. Here, we have investigated the spatial learning and memory (Morris Water Maze), the synaptic plasticity (LTP) in the hippocampus and the local field potential (LFP) related to the Purkinje cell firing in the cerebellum of a mouse model of AD, the APPswe/PSEN1dE9 mice. These mice begin to develop Aβ deposits by six months of age, with abundant plaques in the hippocampus and cortex by nine months. We demonstrated by stimulating Schaffer’s collaterals in transverse hippocampal slices that LTP was impaired in 7-month old APP/PS1 mice. By using Ethovision in Morris Water Maze, maximum swim time and the spatial reference memory were analysed. The time to reach the platform and the time spend in the quadrant were both significantly altered in AD mice with respect to littermate control confirming the important role played by the hippocampus in navigation. Electrophysiological exploration of the cerebellum of these AD mice demonstrated the emergence of spontaneous LFP oscillations occurring as dual-frequency oscillation peaking at 26.2 Hz +/- 10.1 Hz (range from 13 Hz to 46 Hz) and at 247 Hz +/- 50.9 Hz (range from 183 Hz to 459 Hz) throughout the cerebellum of all of the AD mice explored (n=5). These abnormal oscillations were absent in wild-type controls. Interestingly, such type of pathological LFP dual-frequency oscillations have been reported in ataxic knockout mice lacking calcium binding proteins and in mouse model of myotonic dystrophy for the fast oscillation. These results are discussed in line of the functional relationship between the cerebellum and the hippocampus.

Disclosures: **L. Ris:** None. **M. Fassin:** None. **A. Leroy:** None. **J. Marquez-Ruiz:** None. **G. Cheron:** None.

Poster

124. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 124.16/C63

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: FRENCH ALZHEIMER FOUNDATION

Title: Impact of D-serine depletion in the ß-amyloid cascade related to Alzheimer's disease

Authors: ***T. FRERET**[1], E. PLOUX[1], L. GORISSE[2], I. RADZISHEVSKY[3], H. WOLOSKER[4], J.-M. BILLARD[1];
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Abstract: D-serine, as a co-agonist of N-methyl-D-aspartate subtype of glutamate receptors (NMDAR), is a key regulator of their activation and hence involves in functional brain plasticity and memory process. The homeostasis of these receptors is affected by soluble oligomers of the beta-amyloid peptide (Aß) in Alzheimer´s disease (AD). In the course of AD, early functional dysregulations of NMDAR are well known, even though contribution of D-serine remains so far to be determined. In 3-4 month-old transgenic mice model of amyloïdogenesis (5xFAD) showing marked increase in Aß rates and apparent unaffected D-serine levels, extracellular electrophysiological recordings reveal impaired NMDAR-dependent long-term potentiation at CA1/CA3 hippocampal synapses, without significant changes in basal synaptic transmission. This deficit persists at 12 month of age when amyloid deposits are present with concomitant disabilities in cognitive functions. Generating 5xFAD mice with depletion of D-serine (through invalidation of the synthesis enzyme: Serine Racemase), we observed that these functional alterations and the long-term behavioral impairment were prevented whereas Aßo rates remain significantly elevated and comparable to 5xAFD mice. Therefore, these results provide convincing evidence for a critical and transient involvement of D-serine in hippocampal network dysfunctions and related cognitive disabilities driven by increased amyloidogenesis.

Disclosures: **T. Freret:** None. **E. Ploux:** None. **L. Gorisse:** None. **I. Radzishevsky:** None. **H. Wolosker:** None. **J. Billard:** None.

Poster

124. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 124.17/C64

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: Advanced European Research Council grants 232835
EU Seventh Framework Program HEALTH-2011 279017
Israel Science Foundation (ISF)-research grant no. 991/16
ISF-Legacy Heritage Biomedical Science Partnership-research grant no. 1354/15
Ministry of Science & Technology - Shamir fellowship for postdoctoral fellows no. 3-14251

Title: Silencing interferon gamma receptor expression at the choroid plexus abrogates the therapeutic capacity of PD-L1 blockade in a mouse model of Alzheimer's disease

Authors: *M. ARAD, H. BEN-YEHUDA, J. M. P. RAMOS, G. CASTELLANI, M. SCHWARTZ;
Neurobio., Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Emerging results from our laboratory have suggested that transient blockade of the Programmed cell death protein 1 (PD-1)/PD-L1 immune checkpoint pathway, results in improved cognitive performance and other pathological hallmarks in the amyloid-driven 5XFAD mouse model of Alzheimer´s disease (AD). This approach is based on the contention that the treatment induces the release of effector memory T cells, and thereby allows elevation of Interferon gamma (IFNγ) at the choroid plexus (CP). In this regard, we have proposed that this IFNγ signal activates the CP tissue to express leukocyte trafficking molecules that support the entry of monocyte-derived macrophages to the AD brain. Here, we assess the role of this pathway using 5XFAD-(IFNγ receptor 1)$^{loxP/loxP}$ mice. The mice were intracerebroventricularly (i.c.v.) injected with TAT-CRE recombinase or PBS, and following a 2 weeks recovery period, we administered a single intraperitoneally (i.p.) injection of anti-PD-L1 antibody or IgG. Four weeks later, cognitive performance of all mice was assessed. Our results show that the improvement in cognitive performance induced by anti-PD-L1 treatment was hindered following TAT-CRE injection, relative to controls. Overall, these results support our suggested mechanism that the effect of blocking PD-1/PD-L1 immune checkpoint pathway in AD modification involves IFN-γ-dependent signaling at the brain’s CP epithelium.

Disclosures: M. Arad: None. **H. Ben-Yehuda:** None. **J.M.P. Ramos:** None. **G. Castellani:** None. **M. Schwartz:** None.

Poster

124. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 124.18/C65

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Advanced European Research Council grants (232835)
EU Seventh Framework Program HEALTH-2011 (279017)
ISF 991/16
ISF-Legacy Heritage Biomedical Science Partnership-research grant no. 1354/15

Title: Monocytes as disease-modifying players driving the therapeutic effect of PD-L1 blockade in murine models of dementia

Authors: *H. BEN-YEHUDA[1], M. ARAD[1], J. M. PERALTA RAMOS[1], T. CROESE[3], G. CASTELLANI[1], J. C. L. SIPOS[1], S. FERRERA[1], T. M. SALAME[2], M. SCHWARTZ[1];
[1]Dept. of Neurobio., [2]Flow Cytometry Unit, Life Sci. Core Facilities,, Weizmann Inst. of Sci., Rehovot, Israel; [3]Clin. Neuroimmunology Unit, Inst. of Exptl. Neurology, Div. of Neurosci., San Raffaele Scientific Inst., Milan, Italy

Abstract: Alzheimer’s disease (AD) is a neurodegenerative pathology, and the most frequent cause of senile dementia. Our group has demonstrated that boosting the peripheral immune response by transiently blocking the immune checkpoint Programmed Death protein-1 (PD-1)/PD-L1 pathway, resulted in improved cognitive performance and reduction in pathological symptoms. Here, we characterized the immunological landscape in the periphery and in the brain following a single injection of anti-PD-L1 antibody, and assessed the role of monocyte-derived macrophages in the healing process. Using cytometry by time-of-flight (CyTOF), we examined the immune profile of blood mononuclear cells and brain immune cells isolated at different time points following PD-L1 blockade, in both 5XFAD and DM-hTAU murine models of AD and tau pathology, respectively. Specifically, we simultaneously assessed more than 30 parameters and analysed the multidimensional datasets through R/Bioconductor t-SNE maps displaying Phenograph-guided clustering of immune cell populations. Our findings show that targeting PD-L1 modifies the immunological profile of the blood and brain in a time-dependent manner. To assess the role of macrophages in the disease-modification process induced by the treatment, we neutralized C-C chemokine receptor type 2 (CCR2), which is involved in the migration of monocytes from the bone marrow to the blood and in their homing to inflamed tissues. Injection of anti-CCR2 antibody to DM-hTAU mice abolished the beneficial effect of anti-PD-L1 on disease pathology as assessed behaviorally using short-term and working memory tasks, as well as on cortical aggregated tau, measured by FRET-based immuno-assay.
Overall, this study describes the immune profile of murine blood and brain along disease

progression after immunotherapy, and demonstrates a key role for monocyte-derived macrophages during immune checkpoint blockade for dementia/AD treatment.

Disclosures: H. Ben-Yehuda: None. **M. Arad:** None. **J.M. Peralta Ramos:** None. **T. Croese:** None. **G. Castellani:** None. **J.C.L. Sipos:** None. **S. Ferrera:** None. **T.M. Salame:** None. **M. Schwartz:** None.

Poster

124. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 124.19/C66

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Advanced European Research Council
the Israel Science Foundation Legacy Heritage Biomedical Science Partnership-Research
Israel Science Foundation
the Consolidated Anti-Aging Foundation Chicago
the Adelis Foundation

Title: Immune-dependent regulation of cholesterol metabolism at the choroid plexus in Alzheimer's disease

Authors: *A. TSITSOU-KAMPELI, S. SUZZI, S. MEDINA, M. SCHWARTZ;
Weizmann Inst. of Sci., Rehovot, Israel

Abstract: The barrier systems of the brain regulate the bidirectional communication between brain and periphery, and are thereby fundamental for brain homeostasis. Among these barriers, the choroid plexus (CP) controls peripheral immune cell trafficking to the brain as well as molecular exchange, including lipid trafficking, between the blood and the cerebrospinal fluid. In chronic neurodegenerative conditions, such as Alzheimer’s disease (AD), alterations of the physiological properties of the CP impair its function. For example in animal model of Alzheimer’s driven by amylod plaques, the 5xFAD, immune cell trafficking through the CP is impaired and it was linked to deficiency in systemic interferon gamma (IFNγ) signaling. Consistently, antibody-mediated systemic blockade of anti-PD-1 (programmed death-1) or its ligand (programmed death-ligand 1, PD-L1) elevates IFNγ-expressing systemic effector memory T cells and leads to reduction in disease pathology, at least partially due to increased immune cell trafficking through the CP. The implications to lipid-trafficking-properties of the CP induced following such AD immunotherapy and how these changes could potentially alter the brain lipid state and contribute to disease pathology amelioration have not been elucidated. We found that anti-PD-L1 systemic blockade in 5xFAD mice induces transient changes in the lipid state in both

the periphery and the cerebrospinal fluid (CSF), particularly in the total cholesterol levels. Additionally, we found that anti-PD-L1 induces temporal changes of cholesterol metabolism-related genes at the CP. Among those, the expression of the key regulator of brain cholesterol homeostasis, cholesterol hydroxylase CYP46A1, was restored to wild-type control levels in treated 5xFAD mice. Addionally, CYP46A1 downstream target, apolipoprotein E (APOE), involved in cholesterol and Aβ clearance within the brain, was also induced following treatment. Interestengly, both CYP46A1 and APOE are reduced in primary CP cultures exposed to pro-inflammatory mediators that characterize brain inflammation in AD. Overall, our results suggest that systemic anti-PD-L1-mediated blockade in AD might rescue lipid trafficking at the CP and thereby contribute to the overall alleviation of disease pathology.

Disclosures: **A. Tsitsou-Kampeli:** None. **S. Suzzi:** None. **S. Medina:** None. **M. Schwartz:** None.

Poster

125. Alzheimer's Disease and Other Dementias: Imaging Studies II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 125.01/C67

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Rates of cerebral protein synthesis measured *in vivo* in a rat model of Alzheimer's disease

Authors: *C. J. MCARDLE, N. LOOMBA, I. LOUTAEV, L. KRYCH, C. SMITH;
Natl. Inst. of Mental Hlth., NIH, Bethesda, MD

Abstract: Alzheimer’s disease (AD) is a degenerative disorder of the central nervous system resulting in memory loss. The process of protein synthesis is essential for growth and maintenance of cells and is thought to be a critical process involved in long term memory formation. Protein synthesis also acts locally at the synapse to maintain stable neuronal connections. We studied a transgenic model of AD in the Fisher 344 rat that has all the hallmarks of the human disease including amyloid plaques, tau pathology, gliosis and neuronal loss (Cohen et al., 2013, *J Neurosci* 33:6245). Our preliminary study included six groups of female rats (Fisher 344 and AD) at three ages (6, 12, and 20 months). The number of rats of each genotype were 3, 5, and 3 for each age, respectively. We measured *in vivo* protein synthesis rates by means of the autoradiographic L-[1-^{14}C]-leucine method (Smith et al., 1988, *Proc Nat Acad Sci* 85:9341) in 16 regions, primarily cortical and limbic areas. Results were analyzed by a mixed model ANOVA with age and genotype as between subjects and region as within subjects variables. The genotype x region x age interaction was statistically significant (p=0.02). *Post-hoc* comparisons indicate that in 6 regions (retrosplenial cortex, frontal cortex, parietal cortex, septal nuclei, hippocampus, and hippocampal CA1 pyramidal cell layer), rCPS were statistically significantly lower (20-26%) in AD rats at 6 months of age. However, at 12 and 20 months of

age, most genotype differences were not statistically significant. In the AD groups, rCPS were relatively constant across the three ages. In contrast, in the control rats, rCPS decreased with age, particularly between 6 and 12 months. These decreases (24-27%) were statistically significant in retrosplenial cortex, frontal cortex, and the CA1 pyramidal cell layer. Our results indicate that decreased rCPS in AD rats was already present at 6 months of age before the appearance of neuropathological changes. Our study suggests that measurement of rCPS could be an early marker of disease progression and could be useful to identify subjects who might benefit from early treatment. Moreover, the measurement of rCPS in human subjects is now possible with positron emission tomography and L-[1-^{11}C]leucine (Schmidt et al., 2005, *J Cer Bl Flow& Metab* 25:617).

Disclosures: C.J. McArdle: None. **N. Loomba:** None. **I. Loutaev:** None. **L. Krych:** None. **C. Smith:** None.

Poster

125. Alzheimer's Disease and Other Dementias: Imaging Studies II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 125.02/C68

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01 AG054102
NIH Grant R01 AG053500
NIH Grant R01 AG053242
NIH Grant R21 AG050804

Title: Reduced fiber density in ArcAbeta but not in PSAPP or E22dABeta transgenic mice

Authors: M. AL-AMIN[1,2], J. GRANJEAN[3], *J. KIM[1];
[1]Indiana Univ. Sch. of Med., Indianapolis, IN; [2]The Univ. of Queensland, Brisbane, Australia; [3]Radbound Univ. Med. Ctr., Nijmegen, Netherlands

Abstract: Background
Alzheimer’s disease (AD) is characterized by the aggregation of amyloid-β (Aβ, a product of amyloid precursor protein or APP) and hyperphosphorylated tau proteins. In addition, contemporary evidence strongly argued that the impairment of structural connectomes and functional connectivity in many brain regions. In humans, AD patients showed fiber-specific white matter reduction. To date, little evidence has been found associating abnormal aggregation of Aβ with fiber-specific white matter properties in an animal model. The main aim of this study was to determine whether the measures of fiber density (FD), fiber bundle cross-section (FC), and fiber density and bundle cross-section (FDC) is linked with APP mutations in mice.
Methods

Diffusion-weighted image (DWI) (36 directions, b=1000 s/mm2) of three mouse strains, the PSAPP (n = 12), the E22ΔAβ (n = 12), and ArcAβ (n = 12) were included in this analysis. Previous study showed that APP mutations impaired white matter properties in ArcAβ and PSAPP mice. However, Aβ aggregations are varied in these mice strains, including predominant extracellular deposits in PSAPP, intracellular deposits in E22ΔAβ, and intra-, extra- cellular with vascular deposits in ArcAβ. Our analysis includes three strains with an equal number (n = 6) of transgenic and wild type mice. We used FSL and MRtrix3 programs to preprocess DWI data. We then performed novel fixel-based analysis technique available in MRtrix3, to measure the FD, FC and FDC measures in mouse brain.

Results

Our whole-brain analysis showed a significant reduction of fiber-specific white matter properties including FD, FC, and FDC in the ArcAβ mice in several regions. These measures were significantly reduced in the anterior commissure, corpus callosum and hippocampal commissure. However, there was no significant change in FD, FC, and FDC values in the whole brain of E22ΔAβ and PSAPP mice.

Conclusions

The findings of this fixel-based analysis study showed compelling evidence of reduced FD, FC and FDC in ArcAβ transgenic mice. Our results are consistent with previous studies those also showed a reduced white matter properties in both humans and animals. The observed reductions might indicate a decrement of axonal diameter or axon count and can explain the basis of structural and functional connectivity deficits in AD.

Keywords: Alzheimer Disease; white matter; fiber density; fiber cross-section

Disclosures: J. Kim: None. **M. Al-Amin:** None. **J. Granjean:** None.

Poster

125. Alzheimer's Disease and Other Dementias: Imaging Studies II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 125.03/C69

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: RF1AG047666

Title: Optogenetic fMRI of the basal forebrain in Alzheimer's disease and aging

Authors: *M. ASAAD[1], R. W. CHAN[2], H. LEE[2], E. DADGAR-KIANI[3], J. LEE[2,3,4,5];
[1]Mol. and Cell. Physiol., [2]Neurol. and Neurolog. Sci., [3]Bioengineering, [4]Neurosurg., [5]Electrical Engin., Stanford Univ., Stanford, CA

Abstract: Alzheimer’s disease (AD) is a growing health problem for which we still lack a clear mechanistic understanding or reliable treatment options. In this work, we sought to better

understand the neurological changes that occur as a consequence of disease progression. We focused on the cholinergic basal forebrain system, which projects widely throughout the brain and is known to be affected in Alzheimer's disease pathology. By using precise optogenetic activation of these cells during fMRI, we examined the functional consequences of cholinergic basal forebrain activity in normal mice and an animal model of Alzheimer's disease. In order to specifically target cholinergic cells, ChAT-cre transgenic mice were injected with a viral vector expressing ChR2 under the DIO promoter. These mice were crossed with the APP/PS1 transgenic model of Alzheimer's disease enabling us to compare the basal forebrain cholinergic system in mice that are young (2-5 months old, n=13), aged (7-16 months old, n=11) and aged Alzheimer's disease model (7-16 months old, n=14). We investigated the spatial extent and magnitude of activation throughout the brain in these three groups. In all cases, we found that cholinergic basal forebrain stimulation led to robust activation in basal forebrain, cortex, and thalamus. However, the magnitude of activation was weaker in the aged and Alzheimer's disease model groups. Interestingly, we observed one significant inhibitory response ($p < 0.05$, FWE-corrected), which was located in the somatosensory cortex of the young mice. Surprisingly, this response was absent in the aged mice but remained in the Alzheimer's disease model group. Taken together, these results reveal multiple key findings for better understanding Alzheimer's disease pathology. First, optogenetic fMRI allowed us to measure the brain-wide functional response to activity in the cholinergic basal forebrain. This is in contrast to much of the work on the basal forebrain cholinergic system to date, which has focused more on anatomical projections. Unlike optogenetic fMRI, these techniques do not convey information about the magnitude or sign of the functional response, especially for brain regions that are more than one synapse away from the basal forebrain. Second, we observed significant changes in the somatosensory cortex function of aged mice without AD. Finally, we found that this deficit does not occur in the AD model. These results imply that changes in the excitation-inhibition balance of the basal forebrain projections occur during aging, but in fact this deficit is protected against in the Alzheimer's disease model.

Disclosures: **M. Asaad:** None. **R.W. Chan:** None. **H. Lee:** None. **E. Dadgar-Kiani:** None. **J. Lee:** None.

Poster

125. Alzheimer's Disease and Other Dementias: Imaging Studies II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 125.04/C70

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: EU-FP7 TargetBrain and BrainPath (to M.H.)
DZNE
Cure Alzheimer's Fund (to E.M.)

MPG

Title: Tau protein impairs functional networks independently of aggregation propensity, but impairment can be reversed by suppressing Tau expression

Authors: C. GREEN[1], A. SYDOW[2], S. VOGEL[1], M. ANGLADA-HUGUET[2], D. WIEDERMANN[1], M. HOEHN[1], E. MANDELKOW[2,3], ***E.-M. MANDELKOW**[2,3];
[1]MPI for Metabolism Research, in vivo NMR Lab., Cologne, Germany; [2]German Ctr. For Neurodegenerative Dis. (DZNE), Bonn, Germany; [3]Ctr. of Advanced European Studies and Res. (CAESAR), Bonn, Germany

Abstract: Background: Abnormal aggregation of Tau is a hallmark of Alzheimer Disease (AD) and other tauopathies. Here we report the effects of expressing human Tau repeat domain (TauRD) with pro- or anti-aggregant mutations in regulatable transgenic mouse models of AD on functional neuronal networks and structural connectivity. Methods: Mice were generated to express the Tau repeat domain with the pro-aggregant mutation ΔK280, or with the additional aggregation-disabling mutations I277P+I308P ("anti-aggregant"), in a regulatable fashion (doxycyclin-dependent) (Mocanu et al., JNeurosci 2008; Sydow et al., JNeurosci 2011). Both mice were studied when mutant TauRD was switched ON for 12 months to reach the stage where pro-aggregant mice show cognitive impairment, whereas anti-aggregant mice remained cognitively normal. Then, mutant TauRD was switched OFF for 2 months so that soluble transgenic tau disappeared and cognition recovered in pro-aggregant mice. At these two time points (12 months TauRD ON, 2 more months TauRD OFF), resting state fMRI and diffusion MRI were used to determine changes in functional neuronal networks and fiber connectivities. Results: Functional connectivity was strongly reduced in animals during mutant TauRD expression. Unexpectedly, mice with the anti-aggregant tau mutant showed identical functional deficits as pro-aggregant mice, even though there was no cognitive decline by behavioral testing. Upon 2 months Tau switch-OFF, functional connectivity in both transgenic groups presented complete normalization of functional connectivity strength, similar to WT littermates. By contrast, structural connectivity was only marginally sensitive to mutant TauRD expression and doxycycline treatment. Conclusions: Our studies revealed a strong reduction of functional neuronal networks by increased TauRD expression , independent of its aggregation, which is reversible by switching Tau OFF. By contrast, behavioral, biochemical, and immunohistochemical assays point to a strong dependence on aggregation (i.e. only pro-aggregant mice become demented). Our results present evidence for early tauopathy biomarkers or a potential early stage drug target by functional networks analysis (Green et al., Mol. Neurodeg 2019).

Disclosures: **C. Green:** A. Employment/Salary (full or part-time):; MPI for Metabolism Research, Cologne, Germany. **A. Sydow:** A. Employment/Salary (full or part-time):; German Ctr. For Neurodegenerative Dis. (DZNE). **S. Vogel:** A. Employment/Salary (full or part-time):; MPI for Metabolism Research, Cologne, Germany. **M. Anglada-Huguet:** A. Employment/Salary (full or part-time):; German Ctr. For Neurodegenerative Dis. (DZNE). **D. Wiedermann:** A. Employment/Salary (full or part-time):; MPI for Metabolism Research, Cologne, Germany. **M. Hoehn:** A. Employment/Salary (full or part-time):; MPI for Metabolism

Research, in vivo NMR Lab, Dept. Radiology, Leiden Univ. Med. Center, Leiden, The Netherlands. **E. Mandelkow:** A. Employment/Salary (full or part-time):; German Ctr. For Neurodegenerative Dis. (DZNE), Center of Advanced European Studies and Research (CAESAR), Bonn, Germany. **E. Mandelkow:** A. Employment/Salary (full or part-time):; German Ctr. For Neurodegenerative Dis. (DZNE), Center of Advanced European Studies and Research (CAESAR), Bonn, Germany.

Poster

125. Alzheimer's Disease and Other Dementias: Imaging Studies II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 125.05/C71

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA R01AG054180-02S1
NIH/NIA P50AG047266
AMRIS

Title: Genetic diversity differentially impacts diffusion MRI measures in cortex and hippocampus of wildtype and 5xFAD mice

Authors: *M. M. GRUDNY[1], S. NEUNER[2], M. POMPILUS[1], A. DUNN[2], M. FEBO[1], C. C. KACZOROWSKI[2];
[1]Psychiatry, Univ. of Florida, Gainesville, FL; [2]The Jackson Lab., Bar Harbor, ME

Abstract: Genetic mutations in amyloid precursor protein (APP) and presenilin-1/2, each of which lead to an increase in toxic beta-amyloid, are linked to a high risk of Alzheimer’s disease (AD). However, the extent to which individual genetic variation affects neurobiological factors to regulate resilience/vulnerability to cognitive and non-cognitive symptoms of AD is not well understood. To begin to address this gap, the present study investigated brain-wide microstructural characteristics of genetically diverse mice expressing the 5xFAD transgene, each of which show differential susceptibility to AD-related symptoms. Specifically, we used high angular resolution diffusion MRI (HARDI) and quantified well-known diffusion tensor imaging (DTI) metrics such as the fraction anisotropy (FA) and mean diffusivity (MD), along with intracellular volume fraction (neurite density, NDI) and orientation dispersion (ODI) to investigate detailed morphological differences in hippocampal and cortical tissue. Young (6-8 m.o.) and aged (>12 m.o.) male and female 5xFAD mice on C57BL/6 (B6), F1-B6/DBA/2J (D2), or various BXD backgrounds, and their sex- and age-matched wildtype counterparts, were imaged at 11.1 Tesla. Two-way ANOVA indicated a significant strain x mutation effect in the primary motor cortex and dorsal hippocampal commissure of the D2 strain for FA, and in left and right entorhinal cortex and subiculum of BXD strains (Bonferroni $p < 0.05$). These results suggest that strain-specific variation in cognitive outcomes previously demonstrated to exist in

this panel (Neuner et al 2019) may perhaps be due to strain-specific variation in 5XFAD-induced microstructural alterations. Background strain was also seen to effect measures including MD, FA, NDI, and ODI in a broad range of brain regions implicated in learning and memory. We are currently processing functional magnetic resonance (fMRI) data sets in order to determine the effect of background strain on brain networks and testing a broad range of behaviorally-phenotyped and genetically-characterized F1-B6/BXD recombinant lines. Results that highlight brain regions involved in resilience to high-risk AD mutations may improve biomarkers for susceptibility and provide clues as to the nature of resilience to AD.

Disclosures: **M.M. Grudny:** None. **S. Neuner:** None. **M. Pompilus:** None. **A. Dunn:** None. **M. Febo:** None. **C.C. Kaczorowski:** None.

Poster

125. Alzheimer's Disease and Other Dementias: Imaging Studies II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 125.06/C72

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R56 AG050492-01A1
NIH Grant NUCATS UL1TR001422

Title: Non-invasive detection of soluble amyloid-beta oligomers using magnetic resonance imaging in a non-transgenic mammalian species

Authors: *N. B. ROZEMA[1], K. L. VIOLA[1], J. F. DISTERHOFT[2], W. L. KLEIN[1], C. WEISS[2]; [1]Dept. of Neurobio., Northwestern Univ., Evanston, IL; [2]Dept. of Physiol., Northwestern Univ., Chicago, IL

Abstract: The amyloid-β oligomer (AβO) hypothesis of Alzheimer’s disease (AD) is one of the central foci in Alzheimer’s research. The AβO hypothesis posits that synapse loss and nerve cell death leading to AD is instigated by soluble oligomeric species of the amyloid-β peptide. This hypothesis is based on the initial discovery that fibril-free synthetic preparations of the amyloid-β peptide were sufficient to inhibit long-term potentiation and cause selective neuronal death (Lambert et. al., 1998). Additionally, AβOs accumulate in an AD-dependent fashion and, in subsequent animal models, have been shown to impair learning and memory and elicit hallmark pathophysiological features of AD such as tau hyperphosphorylation, neuroinflammation, oxidative damage, and synapse deterioration. Finally, AβOs are among the first biomarkers of AD to accumulate. They therefore present the earliest pathology for diagnosis and therapeutic intervention. We therefore have developed a magnetic resonance imaging (MRI) probe (ACUMNS) by coupling the AβO-selective humanized monoclonal antibody ACU-193 (gift of Acumen Pharmaceuticals) to a magnetic nanostructure for *in-vivo* detection. In the present study,

New Zealand White rabbits, which have the same amino acid sequence for amyloid-β as humans, received injections of stabilized, covalently-crosslinked AβOs. AβOs accumulation was assessed by MRI following a single injection of ACUMNS for MRI visualization of injected AβOs. Validation of probe specificity was performed using immunohistochemical (IHC) staining and a comparison of confocal images to MRI. The movement of ACUMNS and subsequent binding to injected AβOs was determined by comparison of signal intensity in regions of the brain known to be vulnerable to amyloid-beta accumulation. The hippocampus exhibited a difference in mean intensity in AβO-injected rabbits relative to images slices from vehicle-injected rabbits. IHC analysis of these regions using the AβO-specific monoclonal antibody NU-2 showed that sections from AβO-injected rabbits display diffuse puncta-like staining associated with the presence of AβOs; sections from control group rabbits did not. Experiments are ongoing with additional animals to examine AβO-induced impairments in learning and memory using trace-eyeblink conditioning, a hippocampal-dependent associative learning task, as well as novel object and novel location recognition tasks.

Disclosures: **N.B. Rozema:** None. **K.L. Viola:** None. **J.F. Disterhoft:** None. **W.L. Klein:** None. **C. Weiss:** None.

Poster

125. Alzheimer's Disease and Other Dementias: Imaging Studies II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 125.07/C73

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG-019100
NIH Grant AG-024978
NIH Grant AG-036670
NIH Grant AG-055378
NIH Grant AG-062220
NIH Grant OD-011092

Title: Brain volumetrics in aged rhesus monkeys: A progress report

Authors: *S. C. DASH[1], Z. LIU[2], C. D. KROENKE[2], H. F. URBANSKI[1], M. L. VOYTKO[3], S. G. KOHAMA[1];
[1]Div. Neurosci., [2]Advanced Imaging Res. Ctr., Oregon Natl. Primate Res. Ctr., Beaverton, OR; [3]Dept. of Neurobio. & Anat., Wake Forest Univ. Sch. Med., Winston Salem, NC

Abstract: Studies of the effect of hormone therapy (HT) on cognitive function in post-menopausal women have produced inconsistent results. Although the exact reason is unclear, there is evidence that HT may be less effective if it is initiated several years after menopause has

occurred. This has led to the hypothesis that there is a “window of opportunity” for HT effectiveness, with initiation close to the onset of menopause being crucial. Previously we tested a group of aged female rhesus macaques (N=28, baseline age 18-26 years) on a spatial working memory and a visuospatial attention task, after ovariectomy (ovx) and HT (Kohama et al., 2016, J. Neuroscience). On both tests, estradiol (E) replaced animals performed better than the ovary-intact and ovx controls as well as the E-group supplemented with progesterone, thus supporting the “window of opportunity” hypothesis. In the present study the same animals continued on treatment (no further cognitive testing), anatomical MRI scans of the brain were collected repeatedly for several years on a 3T Siemens Magnatom system. T1-weighted MPRAGEs were collected both before, during and after ovx, HT and cognitive testing and comparison of WM, GM and ventricular space were performed over time and correlated to behavior for appropriate time points. T1-weighted MPRAGEs on treatment years 3, 4 and 5 were taken in quadruplicate at each scan time-point to increase the signal-to-noise ratio. Using a volumetric analysis pipeline, scans were averaged for each animal at each time-point, then masked and skull-stripped. For years 3, 4 and 5 found no change in total brain volume, as a factor of treatment, age or the interaction. Ventricular volume and white matter, displayed a slight albeit non-significant age-related increase, while gray matter volume showed an age-related decrease, which also fell short of significance.

Disclosures: **S.C. Dash:** None. **Z. Liu:** None. **C.D. Kroenke:** None. **H.F. Urbanski:** None. **M.L. Voytko:** None. **S.G. Kohama:** None.

Poster

125. Alzheimer's Disease and Other Dementias: Imaging Studies II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 125.08/C74

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH NIA F32AG054116
NIH NIA R01AG034570

Title: Regional distribution of longitudinal amyloid accumulation in cognitively normal older adults

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[1]Helen Wills Neurosci. Inst., Univ. of California, Berkeley, Berkeley, CA; [2]Psychological Sci., Rice Univ., Houston, TX

Abstract: Previous studies have found evidence for a quadratic relationship between global baseline amyloid and rate of change in amyloid over time. As baseline amyloid increases, amyloid accumulation also increases up to a certain point and then begins to decelerate. We

recently found this relationship in cognitively normal older adults as well, suggesting that high global amyloid levels even in asymptomatic people may reflect a relatively late stage of preclinical Alzheimer's disease (AD). Current studies have focused on global amyloid measures, however, the distribution of amyloid deposition may not be uniform across cortical regions. Understanding the progression of regional amyloid accumulation may be beneficial in detecting the earliest signs of AD pathology. Here, we examined the relationship between regional baseline amyloid and the regional rate of change in amyloid to determine which regions may be slowing their rate of amyloid accumulation versus other regions that may still be accumulating amyloid. Cognitively normal older adults (N = 68) underwent longitudinal amyloid PET imaging and structural MRI. We examined baseline amyloid and rate of change in amyloid within cortical lobes. We found a quadratic relationship between amyloid measures in the parietal and frontal lobes, but a linear relationship in the temporal lobes. Amyloid accumulation may be decelerating in parietal and frontal lobes, but is still increasing in temporal lobes, suggesting a distinct hierarchical pattern of amyloid spread. In the parietal lobe, we found that the precuneus was the biggest driver of the quadratic relationship between amyloid measures. The superior parietal cortex still exhibited a linear relationship, suggesting it is actively accumulating amyloid. In the frontal lobe, the rostral middle frontal, superior frontal, and the opercular gyri showed a quadratic relationship between amyloid measures, however, medial and lateral orbitofrontal cortex showed a strong linear relationship. In the temporal lobe, we found that the hippocampus and amygdala showed no relationships between amyloid measures, but a linear relationship was found in fusiform and inferior temporal gyrus. In more lateral regions, the middle and superior temporal lobes showed a quadratic relationship. This gradient in the temporal lobe suggests that amyloid deposition in neocortical regions decelerates, but is still spreading in the MTL. This suggests that amyloid accumulation rates are not uniform across the brain. Thus, it may be advantageous to examine the earliest regions of accumulation to slow disease progression.

Disclosures: L.A. Ferguson: None. **S.L. Leal:** None. **W.J. Jagust:** None.

Poster

125. Alzheimer's Disease and Other Dementias: Imaging Studies II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 125.09/C75

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR MOP-143311
CIHR MOP-126003

Title: Hippocampal and medial temporal lobe regions are differentially associated with BOLD signal variability in older adults with and without risk for cognitive decline

Authors: *J. W. VILLAFUERTE[1,2], T. J. GOOD[1,2], M. D. BARENSE[1,2], J. D. RYAN[1,2], C. L. GRADY[1,2];
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Abstract: Due to the progressive nature of Alzheimer's disease (AD), AD patients may benefit from its early detection. AD is characterized by cognitive decline and age has been identified as its strongest risk factor. Local brain signal variability (operationalized here as the voxelwise standard deviation of the blood oxygen level-dependent signal [SD_{BOLD}]) is sensitive to both age and cognitive performance, and has recently been used to differentiate individuals with AD from healthy controls. However, to be a useful biomarker of AD, SD_{BOLD} must be sensitive to the pathological changes that occur before AD diagnosis, and ideally, even before a diagnosis of Mild Cognitive Impairment (MCI). In a previous study of ours, we compared a group of ostensibly healthy older adults (n=20) who scored below the recommended threshold on the Montreal Cognitive Assessment (MoCA) and who had been shown to have reduced MTL volume in a previous study ('at-risk'), with healthy older adults (n=20) who scored within the normal range on the MoCA ('controls'). We utilized multivariate PLS analysis to compare the relationship between SD_{BOLD} and several potentially related variables (age, scores on the MoCA, global fractional anisotropy (FA), global mean diffusivity (MD), and scores on four cognitive factors) in both groups. We found that increased SD_{BOLD} in the MTL and occipital cortex was positively correlated with performance on cognitive control tasks but negatively correlated with memory scores in the control group. These relations were weaker in the at-risk group. We sought to extend these findings by exploring the relationship between our healthy and at-risk groups' volumetry data, specifically within MTL regions where group volumetric differences were previously found, and SD_{BOLD}. Both groups showed a negative correlation between SD_{BOLD} in the hippocampus and MTL volumes, particularly in entorhinal and parahippocampal cortex. These novel observations, in conjunction with the results from our previous study, suggest that volume loss in the MTL is associated with increased BOLD variability in older adults regardless of cognitive status, but that the link between this increased variability and memory performance is disrupted in the at-risk group. We propose this as proof of concept that the relationship between SD_{BOLD}, particularly in the MTL regions where AD pathology first occurs, and cognition, may be a useful early indicator of changes preceding MCI.

Disclosures: J.W. Villafuerte: None. **T.J. Good:** None. **M.D. Barense:** None. **J.D. Ryan:** None. **C.L. Grady:** None.

Poster

125. Alzheimer's Disease and Other Dementias: Imaging Studies II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 125.10/C76

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: T32AG058507
RF1AG041915
R01AG059874
R56AG058854
U54 EB020403
U01AG024904

Title: Detection of aging effect on white matter microstructure: A comparison of diffusion MRI preprocessing pipelines

Authors: *S. I. THOMOPOULOS[1], T. M. NIR[1], J. E. VILLALON-REINA[1], E. HADDAD[1], N. JAHANSHAD[1], R. I. REID[2], M. A. BERNSTEIN[3], B. BOROWSKI[4], C. R. JACK, Jr.[3], M. W. WEINER[5], P. M. THOMPSON[1];
[1]Imaging Genet. Center, Mark and Mary Stevens Neuroimaging and Informatics Inst., USC, Marina del Rey, CA; [2]Dept. of Information Technol., [3]Dept. of Radiology, [4]Mayo Clin. and Fndn., Rochester, MN; [5]Dept. of Radiology, Univ. of California San Francisco Sch. of Med., San Francisco, CA

Abstract: Diffusion MRI (dMRI) provides insight into microstructural brain changes with aging, especially in the white matter (WM), not obtainable with standard MRI. It is important to preprocess dMRI to improve model fit and boost sensitivity to effects of aging. Here we assessed LPCA denoising (designed for original image resolution; Manjon et al. 2013) and Gibbs deringing (designed for full *k*-space coverage; Kellner et al. 2018) tools on derived diffusion tensor imaging (DTI) scalar metrics in one of seven available ADNI3 protocols that was acquired with partial Fourier sampling and with scanner-altered voxel size (i.e., zero padded).
We evaluated 3T dMRI scans from 67 ADNI3 control subjects (M/F: 27/40; age 74.3±8.1 y; 10 sites) acquired with the basic_GE_25x protocol (6 b_0 + 48 b=1000 s/mm^2 volumes). In the most comprehensive processing pipeline, interpolated dMRI (256 x 256 matrix) were (1) denoised with LPCA by resizing images back to the original acquisition matrix (116 x 116), and (2) corrected for Gibbs ringing. Fig 1a outlines six variations of these steps, in addition to subsequent standardized processing steps. Fractional anisotropy (FA) and mean diffusivity (MD) maps were computed from corrected dMRI and warped to a template. Within the WM, voxel-wise DTI model error (RMSE) was compared between pipelines with paired T-tests. Random-effects regressions tested associations between age and DTI metrics, covarying for sex and age*sex, and grouping by acquisition site.
The most widespread age associations (FDR $q<0.05$) were detected with pipelines that included denoising (after image resizing) and Gibbs deringing (Fig 1b). These pipelines also had lower model fit RMSE (Fig 1c). Our preliminary analyses indicate that dMRI protocols that do not closely adhere to LPCA and Gibbs correction tool specifications may still benefit from these corrections. Ongoing studies are comparing multi-shell dMRI and multicompartment models to disentangle physiological components of these age effects.

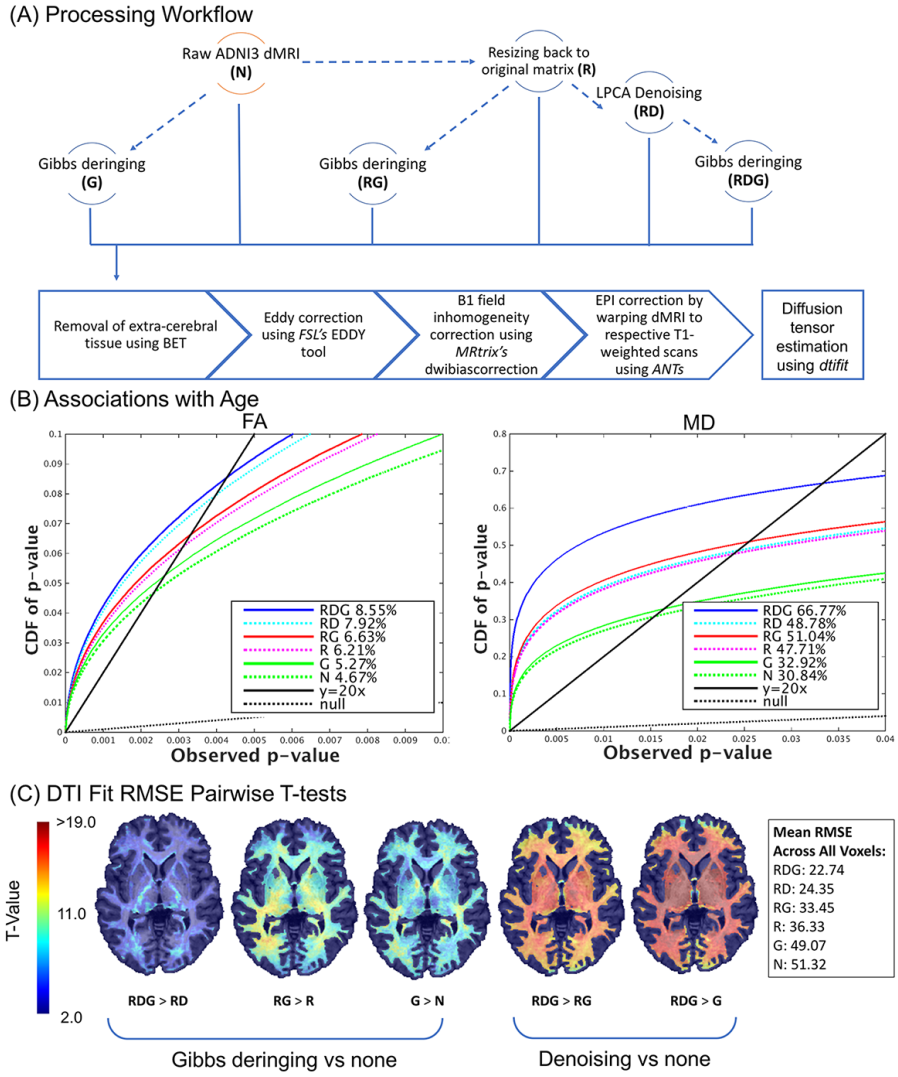


Figure 1. (A) Flow chart outlining the 6 dMRI preprocessing pipeline variations. **(B)** Cumulative distribution function (CDF) plot of *p*-values for tests of age effects on FA and MD in voxel-wise analysis of data from healthy controls. Curves that rise at a steeper rate than the black FDR significance threshold line represent significant voxels and the larger deviations represent more widespread associations. The percent of significant voxels for each test are noted in the legend. **(C)** *T*-values in regions that show significant differences in the RMSE of DTI model fit between pairs of processing pipelines consistently show lower error (positive *T*-value) for pipelines that include Gibbs correction over those that did not and lower error for pipelines that include denoising, despite necessitating data resampling.

Disclosures: S.I. Thomopoulos: None. **T.M. Nir:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Biogen, Inc. **J.E. Villalon-Reina:** None. **E. Haddad:** None. **N. Jahanshad:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Biogen, Inc. **R.I. Reid:** None. **M.A. Bernstein:** None. **B. Borowski:** None. **C.R. Jack:** None. **M.W. Weiner:** None. **P.M. Thompson:** B. Contracted Research/Research Grant (principal investigator for a drug

study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Biogen, Inc.

Poster

125. Alzheimer's Disease and Other Dementias: Imaging Studies II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 125.11/C77

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 AG055449

Title: Women show higher cerebral blood flow than men in older age

Authors: *E. R. SEAGO, V. ZACHARIOU, C. BAUER, B. T. GOLD;
Neurosci., Univ. of Kentucky, Lexington, KY

Abstract: Adequate cerebral blood flow (CBF) is essential for the delivery of oxygen and nutrients to brain tissue and for proper brain functioning. Previous studies have linked age-related declines in CBF with neuropsychological deficits and dementia, lower brain volume, and larger volume of white matter hyperintensities (WMH). WMHs are signal irregularities found on fluid attenuated inversion recovery (FLAIR) images that are associated with vascular disease and risk of Alzheimer's and other dementias. In this study we tested for sex differences in vascular health in older adults using the following three imaging metrics of brain health: CBF, WMH burden, and normalized brain volume. Forty-one cognitively normal older adults (ages 67-85) were recruited from the UK Sanders Brown Center on Aging. CBF was quantified using MRI pseudo-continuous arterial spin labeling (PCASL) scans and WMH volume was identified using the University of California, Davis WMH script and FLAIR images. Then, using individually defined FreeSurfer masks, we extracted lobar specific CBF values as well as gray matter and ventricular structure volume (in mm3) for each participant. Additionally, we classified WMHs as periventricular or deep using a pre-established ventricular template. Subsequently, using a multivariate ANOVA, we tested for a main effect of sex on CBF, structure volume, and volume of WMHs. Sex had a significant main effect on CBF ($F(4,33)=3.044$, $p= .031$), with women showing higher overall CBF.
This effect of sex on CBF was driven by occipital and temporal lobes. Sex also had a significant main effect on measures of brain volume ($F(2,37)=7.600$, $p=.002$) with women having both smaller normalized ventricular volume and larger normalized grey matter volume. However, sex did not have a significant main effect on WMH volume. These results suggest that CBF may be a more sensitive measure of vascular health than WMHs. However, future research with a larger sample sizes will be required to confirm this conclusion.

Disclosures: E.R. Seago: None. **V. Zachariou:** None. **C. Bauer:** None. **B.T. Gold:** None.

Poster

125. Alzheimer's Disease and Other Dementias: Imaging Studies II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 125.12/C78

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Cerebral type 1 metabotropic glutamate receptor availability in early Alzheimer's disease

Authors: *K. ISHII, **K. ISHIBASHI**, T. TAGO, M. SAKATA, K. WAGATSUMA, J. TOYOHARA;
Tokyo Metropolitan Inst. of Gerontology, Tokyo, Japan

Abstract: Objectives: Imaging of type 1 metabotropic glutamate receptor (mGluR1) in living human brains became possible in the early 2010s using positron emission tomography (PET). Cerebral mGluR1 can be involved in the regulation of neuronal excitability and synaptic plasticity, which are affected in Alzheimer's disease (AD). However, little evidence exists on the role of cerebral mGluR1 in the pathophysiology of AD. Two studies have investigated the relationship between mGluR1 and AD; one study found increased expression of cerebral mGluR1 in a mouse model of AD and the other found decreased expression of cerebral mGluR1 in postmortem brains accompanied by AD related-pathology. To advance the understanding of the exact role of mGluR1 in AD, we examined cerebral mGluR1 availability in living patients with early AD. **Methods:** Ten patients with early AD (78.9 ± 5.9 years) and 12 age-matched controls (74.6 ± 2.6 years) underwent PET using an mGluR1 radiotracer. All patients were anti-dementia drug-naive. Volumes-of-interest were placed on the frontal, parietal, and temporal cortices. The binding potential (BP_{ND}) was calculated to estimate cerebral mGluR1 availability using the simplified reference tissue model with the white matter as a reference region. Additionally, BP_{ND} maps were generated. **Results:** No significant difference was observed in BP_{ND} values between the AD and control groups in the frontal cortex ($p = 0.61$), parietal cortex ($p = 0.59$), or temporal cortex ($p = 0.27$). BP_{ND} maps were averaged and displayed in Figure1, showing that the distribution of BP_{ND} values was similar between the two groups across the cortical areas. **Conclusions:** This study suggests that cerebral mGluR1 availability is unchanged in early AD. However, because cerebral mGluR1 availability may change with the progression of AD, further longitudinal follow-up is necessary.

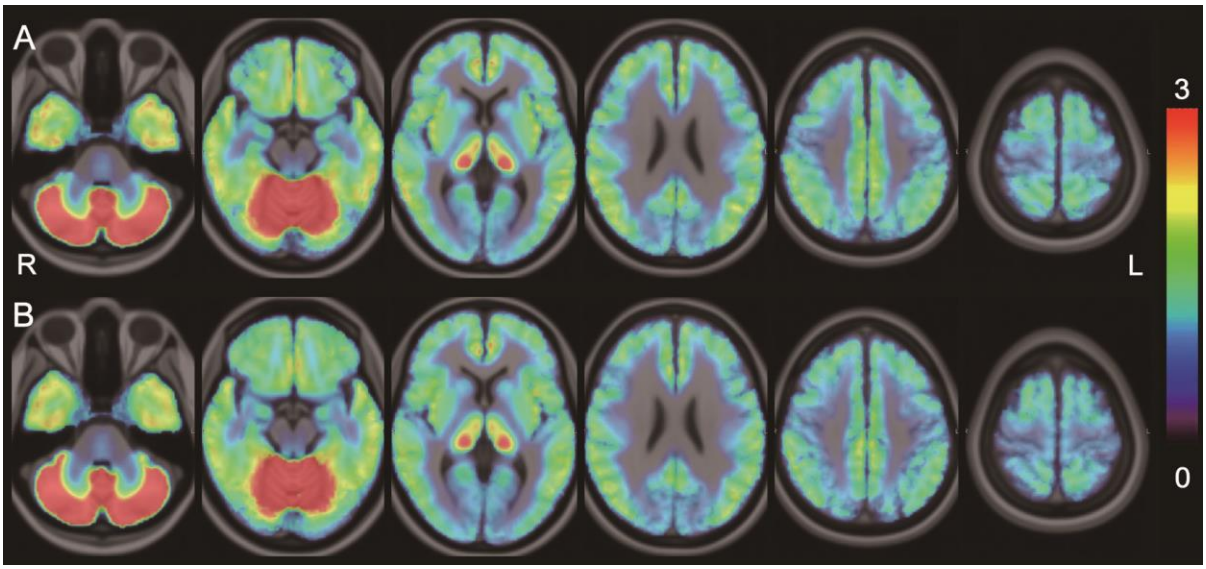


Figure 1: BP_{ND} maps of 10 patients with AD (A) and 12 controls (B) are displayed on the standard brain in the axial view. The rainbow-colored scale represents the magnitude of BP_{ND} values.

Disclosures: K. Ishii: None. **K. Ishibashi:** None. **T. Tago:** None. **M. Sakata:** None. **K. Wagatsuma:** None. **J. Toyohara:** None.

Poster

125. Alzheimer's Disease and Other Dementias: Imaging Studies II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 125.13/C79

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alfred und Anneliese Sutter-Stöttner Stiftung

Title: Manual correction of grey/white matter voxel misclassifications does not alter brain-behavioral correlations in subjects with very early Alzheimer's disease

Authors: F. HARTMANN[1], J. REINHARDT[2,3], R. W. KRESSIG[1,4], ***S. KRUMM**[1];
[1]Univ. Dept. of Geriatric Med. Felix Platter, Memory Clin. Felix Platter, Basel, Switzerland; [2]Div. of Diagnos. and Interventional Neuroradiology Univ. Hosp. Basel, Univ. Hosp. Basel, Basel, Switzerland; [3]Dept. of Neuroradiology, Univ. Hosp. Zurich, Zurich, Switzerland; [4]Fac. of Med., Univ. of Basel, Basel, Switzerland

Abstract: Voxel-based morphometry (VBM) is widely used in research and diagnosis of neurodegenerative diseases, such as Alzheimer's disease (AD). High quality magnetic resonance

imaging (MRI) and conscientious data preprocessing is necessary for reliable results. However, while automated preprocessing steps can be run simultaneously for several individuals, manual steps such as thorough by-hand correction of misclassifications of grey and white matter voxels are inordinately time- and resource-consuming. In search of an optimized VBM preprocessing protocol for MRI scans of AD patients, we investigated the direct impact of manual misclassification corrections on brain-behavioral VBM results. Sixty-three individuals with neurocognitive disorder due to very early AD [27 male; age = 75.67 ± 7.92 years; education = 12.90 ± 3.04 years; Mini-Mental State Examination score (MMSE) = 27.51 ± 2.04] and 64 demographically matched cognitively healthy control participants (38 male; age = 74.30 ± 6.76 years; education = 12.78 ± 2.57 years; MMSE = 29.23 ± 0.92) received neuropsychological testing [e.g. California Verbal Learning Task (CVLT)] and 3-Tesla MRI. T1 MPRAGE images were preprocessed by conducting all automated and manual steps [manually corrected dataset (MCD)]. Identical second time preprocessing was performed with the exception that manual correction of voxel misclassifications was skipped [manually uncorrected dataset (MUD)]. We performed VBM analyses over all participants using age, sex, and education corrected z-scores of CVLT measures [i.e. sum of words recalled in learning trials one to five (LTFR1-5) and number of recalled words during long delayed free recall (LDFR)]. Total intracranial volume was included as a covariate. Multiple regressions were performed twice, using either MCD or MUD. As expected, significant clusters were located in regions involved in episodic memory performance. Peak voxels were identical between MCD and MUD in all but two clusters (LTFR1-5 analyses, one mm difference each). Cluster sizes differed between MCD and MUD (no specific pattern). While significances at cluster-level were identical in MCD and MUD for LDFR, the LTFR1-5 analyses revealed two additional clusters in the MCD and one additional cluster in the MUD. To summarize, results of VBM analyses using either MCD or MUD differed mainly at the boarders of identified clusters. Thus, manual correction did not have an explicit effect on brain-behavioral correlations. We conclude that, although performing all preprocessing steps obviously remains the gold standard, skipping manual correction of voxel misclassifications is legitimate when investigating AD subjects.

Disclosures: **F. Hartmann:** None. **J. Reinhardt:** None. **R.W. Kressig:** None. **S. Krumm:** None.

Poster

125. Alzheimer's Disease and Other Dementias: Imaging Studies II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 125.14/C80

Topic: C.02. Alzheimer's Disease and Other Dementias

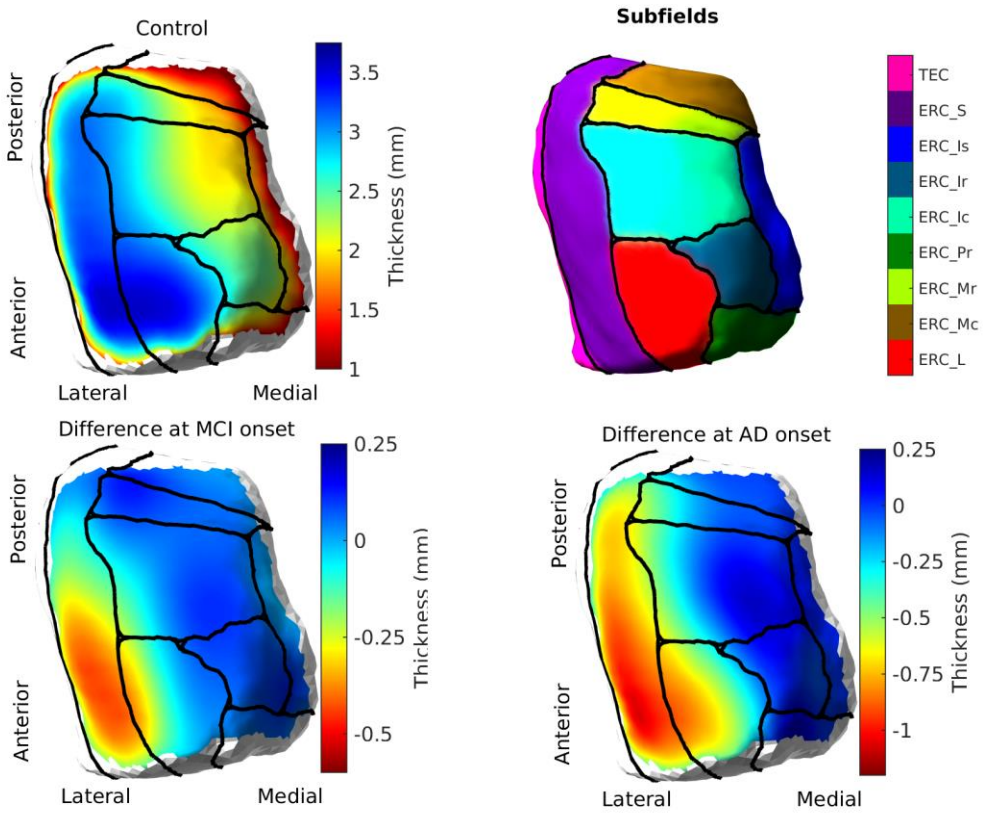
Support: ACI-1548562
P41-EB015909

R01-AG048349
U19-AG033655
Kavli Neuroscience Discovery Institute

Title: Longitudinal MRI study on the progression of transentorhinal cortical thinning during early stages of Alzheimer's disease: BIOCARD and ADNI cohorts

Authors: *S. KULASON[1], D. J. TWARD[1], T. BROWN[2], M. S. ALBERT[3], M. MILLER[1]; [1]Biomed. Engin., [3]Div. Cognitive Neurosci., [2]Johns Hopkins Univ., Baltimore, MD

Abstract: Histological evidence supports the idea that neurofibrillary tangles form in the transentorhinal cortex (TEC) before spreading to other regions during Alzheimer's disease. We previously showed that an increased atrophy rate can be detected in the TEC from longitudinal MRIs of subjects with mild cognitive impairment (MCI) when compared to controls. In this study, we present our first results demonstrating how to synchronize data across studies. We examined differences in TEC thinning across three groups: (1) subjects who progressed from normal cognition to MCI (n=27) from the BIOCARD cohort, (2) MCI subjects who progressed to Alzheimer's dementia (AD) (n=18) from ADNI, and (3) controls from both databases (n=17 BIOCARD, n=14 ADNI). The TEC and entorhinal cortex (ERC) were manually segmented in Seg3D and corrected for within subject variability through an automated approach called longitudinal diffeomorphometry. Cortical thickness was calculated by another automated approach that measures distance along a diffeomorphic mapping from the pial to gray-white surface. Subjects were fit to linear models of cortical thickness, adjusting for age, sex, batch effect, and subject random effect. The full model compared the atrophy rate in the three groups, covarying for the same measures. Permutation testing was performed on model residuals to determine pairwise group differences. The results were then mapped to an 11T atlas with Krimer's 9 subregions of the TEC and ERC to localize differences. We corrected for multiple comparisons using a family-wise error rate of 5% and rejected the null hypothesis of no group differences ($p < 0.0001$). The distribution of statistically significant regions show cortical thinning localized to the anterior TEC in subjects who progressed from control to MCI and cortical thinning throughout the TEC in subjects who progressed from MCI to AD. The figure shows cortical thickness and group differences projected onto the high-field atlas.



Disclosures: **S. Kulason:** None. **D.J. Tward:** None. **T. Brown:** None. **M.S. Albert:** None. **M. Miller:** None.

Poster

125. Alzheimer's Disease and Other Dementias: Imaging Studies II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 125.15/C81

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R56 AG058732
NIH Grant R01 AG055005
NIH Grant P01 AG010124
NACC New Investigator Award

Title: Cerebrospinal fluid evidence for accelerated brain age in Alzheimer's disease

Authors: ***N. G. KINNEY**[1], J. S. PHILLIPS[1], D. G. WAKEMAN[1], L. M. SHAW[2], M. GROSSMAN[1], D. A. WOLK[1], C. T. MCMILLAN[1];

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Abstract: Alzheimer's disease (AD) is an age-related neurodegenerative condition characterized by progressive atrophy in the medial temporal lobes that extends to frontal cortex in later disease stages. However, the relative contributions of atrophy related to "normal" aging or related to the accumulation of misfolded tau protein is not well understood. In this study we attempt to identify a "brain age" signature of neurodegeneration in AD that is independent of tau accumulation. To achieve this goal, we developed a multivariate algorithm by training a linear regression model to predict the chronological age (CA) of 431 healthy controls (Mean Age=49.4, Range=18-89) using T1-weighted MRI measurements of mean cortical thickness from 100 regions of interest (ROI). We first used R bestglm package to identify a subset of the top 20 ROIs in which mean cortical thickness was significantly associated with CA. These ROIs mapped to bilateral superior frontal, right medial temporal, and left superior temporal regions. Together predicted age (PA) by a linear model using mean cortical thickness in these regions was highly associated with CA in the training cohort (R=0.88; p<2.2e-16). In a cross-validation analysis using an independent cohort of 184 healthy controls we observed a similar association between PA and CA (R=0.92; p<2.2e-16), suggesting good reliability. To determine whether this "brain age" signature was related to age-associated neurodegeneration independent of tau pathology we applied our training model to a cohort of 76 clinically-diagnosed AD patients with cerebrospinal fluid evidence of amyloid pathology (<192pg/mL). Each patient also had phosphorylated tau (p-tau) and total tau (t-tau) CSF measurements available. P-tau levels in the CSF reflect the accumulation of misfolded tau as a result of neurofibrillary tangles (NFTs) in AD. T-tau levels in CSF are a marker of nonspecific axonal loss. Our AD analyses revealed significantly greater brain age discordance (PA > CA) in AD (M=14.00, SD=13.3) relative to our control cohort (M=-0.242, SD=9.88, t=9.05, p=4.00e-14) consistent with age-related neurodegeneration. CSF association analyses revealed a significant t-tau (R=0.24; p=0.036), but not p-tau (R=0.03; p=0.800) relationship with greater brain age discordance. These findings suggest that neurodegeneration in superior frontal and temporal cortex may occur due to aging independent of tau misfolding and provide proof-of-concept evidence for a reliable brain age algorithm that can be used to better understand the independent contributions of aging and tau misfolding in the context of AD and related disorders.

Disclosures: N.G. Kinney: None. **J.S. Phillips:** None. **D.G. Wakeman:** None. **L.M. Shaw:** None. **M. Grossman:** None. **D.A. Wolk:** None. **C.T. McMillan:** None.

Poster

125. Alzheimer's Disease and Other Dementias: Imaging Studies II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 125.16/C82

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Structural connectivity network of Alzheimer's disease and Lewy body disease including mixed disease

Authors: *K. BAIK[1], J.-J. YANG[2], B. YE[1];
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Abstract: Objectives: To identify the white matter tractography based structural connectivity network change in patients with Alzheimer disease (AD) and Lewy body disease (LBD) including mixed disease.
Methods: The study included 37 controls and 77 patients with AD-related cognitive impairment (ADCI) and/or LBD-related cognitive impairment (LBCI) who underwent clinical and neuropsychological assessment and diffusion tensor (DTI) MRI. There were 37 controls, 22 patients with pure ADCI, 19 patients with pure LBCI, and 36 patients with mixed ADCI and LBCI. Diagnoses of ADCI and LBCI were supported by ^{18}F-florbetaben PET and ^{18}F-N-(3-fluoropropyl)-2β-carboxymethoxy-3β-(4-iodophenyl) nortropane PET, respectively. We performed network analysis based on white matter tractography using DTI. The 92 nodes including 90 Automated anatomincal labeling (AAL) and bilateral substantia innominata were used. The edge was defined as multiplication of fractional anisotropy and number of fibers between 2 nodes. After we obtained connectivity matrix, graph theory based analysis was done. Both global and local measures were obtained. We used general linear model to find the independent and interactions of ADCI and LBCI on structural connectivity network.
Results: There was no interaction effect of ADCI and LBCI on global and local network measures. Independent LBCI effect was found in degree of right cingulate gyrus. In global network measures, scaled path length was significantly increased in mixed disease group compared to control group. With local network measures, LBCI group showed significantly increased betweenness centrality of right olfactory cortex and path length of right post cingulate gyrus compared with control group, which were inversely correlated with global cognitive function.
Conclusion: From white matter tractography based structural connectivity network analysis, we found that mixed disease group showed globally inefficient network. And LBD-related cognitive impairment might be related with dysfunction of white matter network.

Disclosures: K. Baik: None. **J. Yang:** None. **B. Ye:** None.

Poster

125. Alzheimer's Disease and Other Dementias: Imaging Studies II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 125.17/C83

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: RF1 AG041915
F31 AG059356
P50 AG005142
1R01AG058162

Title: Cardiovascular risk modifies the relationship of VEGF to cognition and regional glucose metabolism in Alzheimer's disease

Authors: *M. TUBI[1], M. HAPENNEY[1], K. KING[2], B. C. RIEDEL[3], W. MACK[4], P. M. THOMPSON[1], M. N. BRASKIE[1];
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Abstract: Introduction: Vascular endothelial growth factor (VEGF) exerts neuroprotective effects by regulating angiogenesis, neurogenesis, glucose transport, and inhibiting cell death. Atherosclerosis alters hemodynamic forces, such as shear stress, which modifies VEGF expression and endothelial cell proliferation. Variability in atherosclerosis severity may explain why studies in-vivo find discrepant relationships between VEGF levels and cognition in older adults. We hypothesize that a higher cardiovascular risk score - an established surrogate for atherosclerosis - will dampen the neuroprotective effect of VEGF on cognition and glucose metabolism in Alzheimer's disease (AD)-signature regions.
Methods: We evaluated 310 participants 55-90 years old (92 cognitively intact, 149 mild cognitive impairment, 69 AD) from the Alzheimer's Disease Neuroimaging Initiative (ADNI). We tested if the Framingham Risk Score (FRS) modified the association of CSF VEGF with 1) mean bilateral glucose metabolism (FDG-PET) in the posterior cingulate, entorhinal cortex, fusiform gyrus, lateral temporal cortex (superior, middle, and inferior temporal gyri), parietal cortex (superior and inferior parietal gyri, precuneus) and 2) neuropsychological composite measures (executive function and memory). We used multiple linear regression (co-varying for age, sex, education, APOE4 status, CSF T-tau, and diagnosis). All p-values presented are FDR corrected at a false discovery rate (FDR) of <5%. We used causal mediation (percentile bootstrap, B=1000) to evaluate whether the VEGF association with temporal lobe glucose metabolism mediated the relationship between VEGF and memory.
Results: Higher CSF VEGF was associated with better memory (β=0.416, p<0.001), and higher glucose metabolism in the lateral temporal lobes (β=0.935, p=0.014) and fusiform gyrus (β=0.854, p=0.043), but was not associated with executive function. The FRS score modified these associations (VEGF to memory: β=-0.186, p=0.003; VEGF to lateral temporal lobe SUVR β=-0.040, p=0.043; VEGF to fusiform SUVR: β=-0.040, p=0.043). FRS did not modify the effect of CSF VEGF on executive function. Mean bilateral temporal lobe FDG-PET signal mediated the relationship between VEGF and memory (estimated mediation effect=0.247; 95% CI [0.025, 0.54]; p=0.012).

Discussion: The negative interaction of FRS with CSF VEGF suggests that higher CSF VEGF levels were associated with better memory function and greater temporal lobe glucose metabolism in persons with lower FRS. Increased cardiovascular risk burden, an associated measure of atherosclerosis, may dampen the neuroprotective effects of VEGF.

Disclosures: M. Tubi: None. **M. Hapenney:** None. **K. King:** None. **B.C. Riedel:** None. **W. Mack:** None. **P.M. Thompson:** None. **M.N. Braskie:** None.

Poster

125. Alzheimer's Disease and Other Dementias: Imaging Studies II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 125.18/C84

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: JAES Foundation
Academy of Finland # 314497

Title: Altered cardiovascular brain pulsations in Alzheimer’s disease

Authors: T. TUOVINEN, J. KANANEN, Z. RAJNA, ***V. J. KIVINIEMI**;
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Abstract: Introduction. Cardiovascular pulsations drive both blood flow and perivascular metabolic transport in brain tissue[1]. A novel theory links Alzheimer’s disease (AD) to altered metabolic waste removal[2]. We investigated hypothesis that cardiovascular brain pulsations are altered in AD with functional MRI.
Methods. A total of 64+19+26 healthy controls (CON), 47+22+30 AD patients were scanned using 3T EPI (ADNI), 1.5 T EPI (local), and 0.1 sec MREG BOLD (local) respectively. Coefficient of physiological variations in BOLD signal (CV_{BOLD} = std/mean, c.f. Fig 1 a) were estimated at voxel level[3]. Cardiovascular brain pulse propagation was also analyzed using optical flow analytics of the fast MREG data[4].
Results. All three datasets presented an increase in CV_{BOLD} in AD subjects, Fig 1 b. The optic flow analysis showed more variability with overall increase in speed connected to cessation of cardiovascular pulse propagation in AD, while controls have more continuous pulse patterns, c.f Fig 1 c-f. **Conclusion.** The results indicate that cardiovascular pulsations driving the brain clearance are altered in AD.

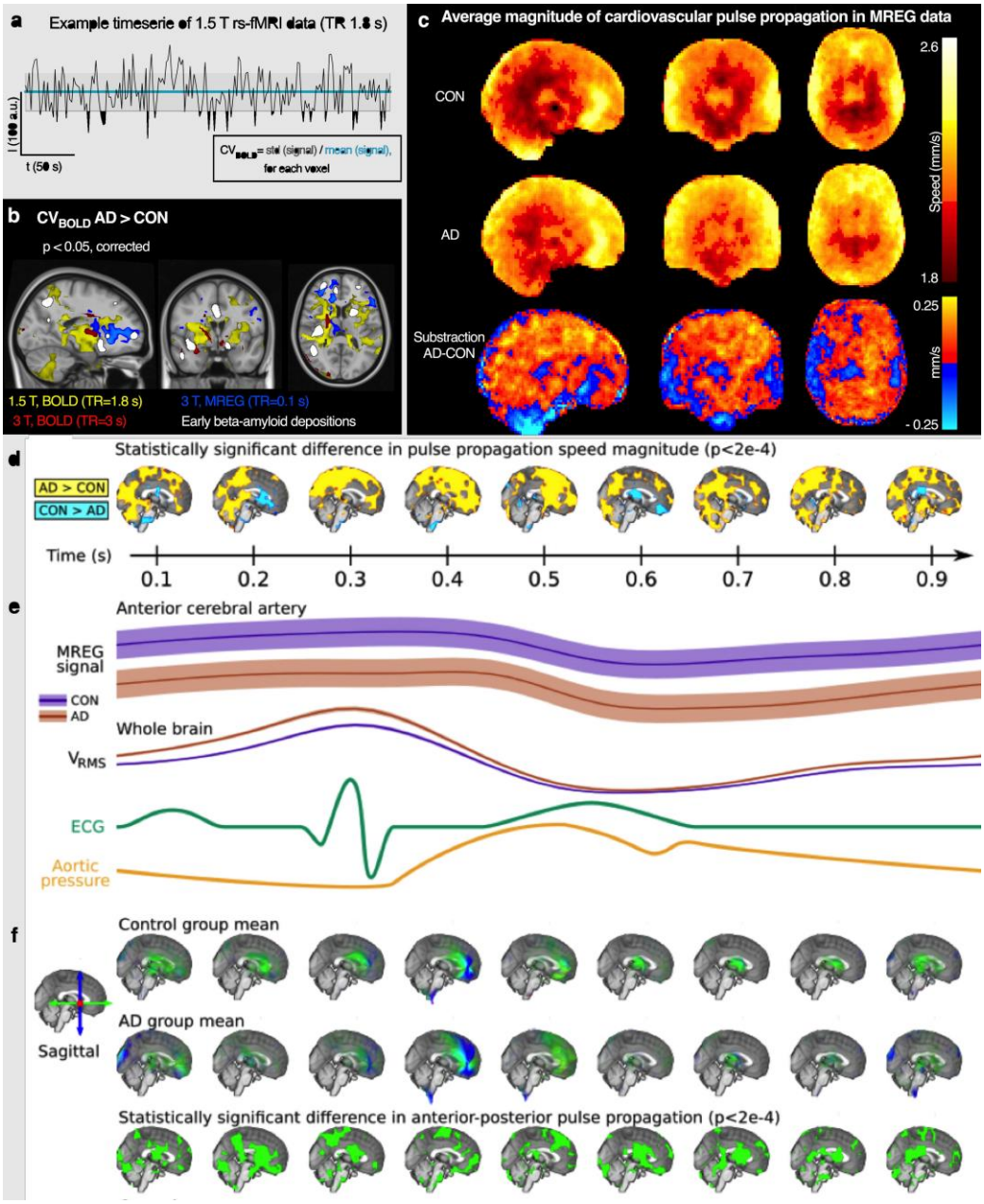

Figure 1. a) *CV_{BOLD} calculation from BOLD signal. b) Increased CV_{BOLD} ($p < 0.05$, FDR corr.) maps in all three AD datasets c) Average, and d) median cardiovascular pulse 0.9 sec (cycles N_{CON}=2580, N_{AD}=2519, p<0.0002) propagation speed magnitude in AD vs. Con, e) MREG signal from anterior cerebral artery, pulse propagation speed over whole brain (V_{RMS} scale 1.5-3.1 mm/s with 95% CI). f) Sagital cardiovascular pulse maps with directions for CON, AD and significant group speed. differences (p<0.0002).*

References. *[1]Mestre et al., 2018 Nat Com, [2]Rasmussen et al., 2018 Lancet Neurol, [3]Tuovinen et al., Front Hum NSci 2017, [4]Rajna et al., IEEE Trans Med 2019.*

Disclosures: **T. Tuovinen:** None. **J. Kananen:** None. **Z. Rajna:** None. **V.J. Kiviniemi:** None.

Poster

125. Alzheimer's Disease and Other Dementias: Imaging Studies II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 125.19/C85

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer Scotland
National Institute on Aging of the National Institutes of Health (R01-AG058853)

Title: Mechanical property alterations across the cerebral cortex due to Alzheimer's disease

Authors: *L. V. HISCOX[1,2], C. L. JOHNSON[1], M. D. J. MCGARRY[4], H. MARSHALL[3], E. J. R. VAN BEEK[3], N. ROBERTS[3], J. M. STARR[2];
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Abstract: Alzheimer's disease (AD) is a major public health concern and there is an urgent need for medical imaging techniques to better characterize the early stages of the disease and monitor its progression. Magnetic resonance elastography (MRE) is an emerging non-invasive imaging technique that can measure the mechanical properties of the brain *in vivo*. MRE combines mechanical wave propagation and MRI phase contrast imaging to record harmonic displacements through soft tissue, which are "inverted" to create maps of tissue viscoelasticity. A whole-brain, high-resolution MRE spiral sequence was used to acquire data at 1.6 mm isotropic resolution, which were analyzed using nonlinear inversion to produce maps of shear stiffness. For the first time, a voxel-wise MRE analysis and state-of-the-art post-hoc region of interest (ROI) approach was used to assess the volumetric and viscoelastic properties of the brain in AD (n=12;7M/5M; mean age 77 years) and healthy older adults (n=12;6F/6M; mean age 69 years). At the whole-brain level, reductions in volume (p=0.006) and stiffness (p=0.002) were observed in patients with AD relative to OA. Significant reductions in stiffness were also observed when white matter (p=0.021) and cortical gray matter (p<0.001) compartments were considered separately. Secondly, voxel-wise analyses of gray matter revealed significant local reductions in both volume and stiffness in the temporal lobe including fusiform, medial temporal, and superior temporal gyri. The affected regions, however, were not identical, suggesting that MRE detects changes in cerebral microstructural integrity that are independent of changes in cortical volume. Significant reductions in stiffness were also observed in the postcentral gyrus and precuneus in the parietal lobe. The results of the voxel-wise analysis were further investigated using an ROI approach in which relevant spatial information was incorporated within MRE inversion to increase sensitivity and reduce partial volume effects. The observations of stiffness reductions in medial temporal gyri (p=0.032), superior temporal gyri (p=0.002), postcentral gyri (p=0.026),

and precuneus (p=0.011) all remained statistically significant in AD even after correcting for regional volumes and age. The pattern of brain stiffness reduction observed in AD is supported by previous reports of MRE in AD and the known trajectory of disease. The new observation that stiffness reductions are localized to the temporal and parietal lobes and unrelated to volume reductions raises the possibility that MRE may provide unique insights regarding the neural mechanisms which underlie the development and progression of AD.

Disclosures: L.V. Hiscox: None. **C.L. Johnson:** None. **M.D.J. McGarry:** None. **H. Marshall:** None. **E.J.R. van Beek:** None. **N. Roberts:** None. **J.M. Starr:** None.

Poster

125. Alzheimer's Disease and Other Dementias: Imaging Studies II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 125.20/C86

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Changes in hippocampal-lateral prefrontal cortex resting-state anticorrelations following exercise interventions

Authors: *K. M. MCDONALD[1], S. ANTERAPER[2], M. VOSS[3], S. WHITFIELD-GABRIELI[1], K. I. ERICKSON[4], C. HILLMAN[1], E. MCAULEY[5], A. KRAMER[1];
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Abstract: Age-related cognitive decline and dementia risk are reduced if individuals participate in regular physical activity and have better cardiorespiratory fitness (Kramer, 2018). However, little is known about the mechanisms that underlie this relationship. We investigated participants who took part in an exercise intervention for 6 months and were evaluated for behavioral, structural, and functional brain changes before and after four different exercise regimens (Voss, et al., 2016). 162 older adults that had pre- and post-intervention MRI scans, fitness data, and behavioral data were included. All participants (Age range: 60 - 80 years, 70% female) were randomized to different exercise groups: walking (n=34), walking + nutritional supplement (n=39), dance (n=46), and stretching, strengthening, and stability (i.e., non-aerobic) active controls (n=43). Resting-state and structural MRI images were acquired on a 3T Siemens system during a single session before and after the interventions (Voss, et al., 2016). All resting-state preprocessing and analysis were conducted using CONN Toolbox (Whitfield-Gabrieli, et al., 2016). Anterior and posterior hippocampus served as seed-regions of interest (ROIs). Of the two ROIs, whole-brain seed-to-voxel analysis showed significant increases in Default Mode Network anticorrelations with lateral prefrontal cortex ($p<.001$) in the walking and walking + supplement groups post-intervention, only for the left anterior hippocampus seed. Previous studies have

shown that during normal aging, resting-state DMN anticorrelations are decreased (Keller, et al., 2015) in the lateral prefrontal cortex. For the walking (aerobic exercise) groups, the 6-month exercise intervention was both protective and restorative of hippocampal function. Further analysis will correlate hippocampal-dependent neuropsychological tasks and fitness measures with these findings and will determine whether functional connectivity acts as a mechanism by which exercise improves memory. Our findings provide an opportunity for cross-disciplinary communication at the SfN meeting, offering an example of the potential utility of neuroimaging studies for the development of therapeutic approaches for age-related cognitive decline.

Disclosures: K.M. McDonald: None. **S. Anteraper:** None. **M. Voss:** None. **S. Whitfield-Gabrieli:** None. **K.I. Erickson:** None. **C. Hillman:** None. **E. McAuley:** None. **A. Kramer:** None.

Poster

125. Alzheimer's Disease and Other Dementias: Imaging Studies II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 125.21/C87

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Locus coeruleus glucose metabolism in Alzheimer’s disease

Authors: *K. Y. LIU[1], J. ACOSTA-CABRONERO[2], D. HÄMMERER[3], R. HOWARD[1]; [1]Div. of Psychiatry, Univ. Col. London, London, United Kingdom; [2]Wellcome Ctr. for Human Neuroimaging, UCL Inst. of Neurol., London, United Kingdom; [3]Inst. of Cognitive Neurosci. UCL, London, United Kingdom

Abstract: Background: The earliest brain changes in Alzheimer’s disease (AD) occur in the locus coeruleus (LC), the major noradrenergic nucleus of the brain. The LC plays an integral role in the regulation of arousal, cognitive and autonomic function, thus degenerative changes within this structure and any associated central noradrenergic system dysfunction may contribute to cognitive and neuropsychiatric symptoms in AD. Optimal in vivo imaging of the LC is needed to evaluate its potential as a biomarker in dementia and to assess future treatments targeting the LC-noradrenergic system. Previous studies have employed T1-weighted and magnetization transfer (MT)-weighted magnetic resonance imaging (MRI) approaches to report lower LC signal intensity (considered to represent reduced structural integrity) in patients with AD compared to controls. However, it is unknown whether any AD-related alterations in LC neuronal activity can be measured in vivo as no previous FDG PET study has investigated LC cerebral metabolic rate in AD patients.
Methods:This study compared LC glucose metabolism in patients with AD and cognitively healthy controls (total N=571) using mean FDG PET and MPRAGE images obtained from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) database. MPRAGE image data were

corrected with a N4 bias field correction procedure and spatially coregistered to a common template space using the ANTS v2.1 software package. After each subject's FDG-PET image was coregistered to their bias corrected MPRAGE image using a non-linear coregistration routine in ANTS v2.1, mean cerebral metabolic rate of glucose (CMR-Glc) values were extracted from a volume of interest defined by previously published LC masks that were applied to the study-wise template. To minimize intersubject variability, these values were normalized to a cerebellar region of interest CMRglc. Statistical analysis involved unpaired t-tests (AD vs controls).
Results:Results will be presented.

Disclosures: K.Y. Liu: None. **J. Acosta-Cabronero:** None. **D. Hämmerer:** None. **R. Howard:** None.

Poster

125. Alzheimer's Disease and Other Dementias: Imaging Studies II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 125.22/C88

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: MH109104
Alzhiemer's Foundation of America

Title: Functional significance of cortical cholinergic circuits in cognitive decline

Authors: *M. R. ANANTH[1], C. DELORENZO[2], N. PALEKAR[2], R. PARSEY[2], D. A. TALMAGE[3], L. W. ROLE[4];
[1]Neurobio. & Behavior, Stony Brook Univ., Stony Brook, NY; [2]Psychiatry, Stony Brook Univ. Sch. of Med., Stony Brook, NY; [3]Natl. Inst. of Mental Hlth., Bethesda, MD; [4]Natl. Inst. of Neurolog. Disorder and Stroke, Bethesda, MD

Abstract: Basal forebrain cholinergic neurons (BFCNs) send extensive projections to the cortex and many subcortical regions of the brain. These highly branched axonal arbors are metabolically challenging to maintain, likely underlying their vulnerability to fragmentation and loss in aging. Studies of post-mortem brains from AD patients find significant loss of cholinergic fibers compared to age-matched, cognitively intact counterparts. One region known to be vulnerable early in aging is the entorhinal cortex (EC), a cortical region that receives extensive input from BFCNs. Using the EC as a model of early cortical dysfunction, we investigated the relationship between altered cholinergic integrity and cortical function in aging. First, in humans, we used Positron Emission Tomography with ligand [^{18}F]-VAT, a radiotracer that targets the vesicular acetylcholine transporter, a presynaptic cholinergic protein and marker of cholinergic synapse health. Recruiting healthy volunteers (HV) and patients with cognitive impairment

(MCI), we sought to visualize and quantify the pattern and density of cholinergic innervation in health vs disease. Our preliminary findings show lower [^{18}F]-VAT volume of distribution (V_T) in the EC between HV and MCI. Looking across a continuum of cognitive score, we find a significant correlation between cognitive performance and EC VAT V_T. To understand the mechanism underlying altered VAT uptake, we used a rodent aging model that exhibits accelerated aging pathology. Confirming that aging model mice have impaired performance on an EC-based memory task, we asked whether this was driven by alterations to cholinergic input to the EC. Our initial studies demonstrate dramatic fragmentation of EC cholinergic fibers and elevated firing rate in aging model mice, pointing to altered circuit dynamics. Ongoing studies are investigating the effects of altering endogenous acetylcholine levels, using opto- and chemogenetics, on firing rate and behavioral output. The strength of these studies lies in our ability to apply high-resolution techniques in both rodents and humans to better understand the cholinergic system. Using related markers of cholinergic terminal fields, albeit with dissimilar techniques, further strengthens the idea that the highly quantitative information gathered in rodents improves our interpretation of *in vivo* human imaging and allows us to make specific predictions about what altered VAT uptake can tell us about cognition and cognitive impairment.

Disclosures: **M.R. Ananth:** None. **C. DeLorenzo:** None. **N. Palekar:** None. **R. Parsey:** None. **D.A. Talmage:** None. **L.W. Role:** None.

Poster

125. Alzheimer's Disease and Other Dementias: Imaging Studies II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 125.23/C89

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Departments of Industry and Education from the Basque Government (KK-2017/14 Elkartek and IT975-16 Consolidated research groups grants)

Title: Sphingosine 1-phosphate receptor ($S1P_1$) activity in postmortem human brain

Authors: J. MARTÍNEZ-GARDEAZABAL[1], G. CUADRA-FERNÁNDEZ[1], I. BENGOETXEA DE TENA[1], I. FERNÁNDEZ-VEGA[2], M. MORENO-RODRÍGUEZ[1], A. LLORENTE-OVEJERO[1], L. LOMBARDERO[1], I. MANUEL[1], *R. RODRÍGUEZ-PUERTAS[1]; [1]Pharmacol., Univ. of the Basque Country (UPV/EHU), Bilbao, Spain; [2]Service of Pathology, Hosp. Universitario Central de Asturias, Oviedo, Spain

Abstract: Lipids in the brain have been identified mainly as structural molecules, but they contribute also to intracellular signaling and behave as neurotransmitters by binding to specific G protein-coupled receptors (GPCR), which can act as neurolipids modulating other systems. The $S1P_1$is the main sphingosine 1-phosphate receptor in brain, which is coupled to $G_{i/o}$proteins and

is involved in cell proliferation, growth or neuroprotection. Therefore, the main aim of our study is to quantify and identify the cerebral distribution of the activity mediated by $S1P_1$in *postmortem* human brain samples to know if the distribution of the activity mediated by these receptors is compatible with different neurodegenerative diseases such as the Alzheimer's (AD). [^{35}S]GTPγS binding is measured with microscopic resolution to get the $S1P_1$activity by functional autoradiography stimulated by the selective agonist CYM-5442 (10 μM), in multiple brain areas implicated in the control of learning and memory and/or cholinergic areas, including hippocampus, prefrontal cortex, amygdala and caudate-putamen of *postmortem* human brain samples.The results showed that $S1P_1$-mediated activity is one of the highest recorded for any GPCR subtype in most of grey matter areas of the human brain (range 50%-500% stimulation over the basal values) only comparable to that elicited by other neurolipid receptors such as the CB_1cannabinoid. Moreover, these results suggest that this receptor could be involved in areas that regulate learning and memory processes and could be modified in neurodegenerative diseases as AD. Therefore, the specific distribution of $S1P_1$activity supports that this receptor could be one of the most abundant and/or efficient GPCR expressed in the brain and related to higher brain functions.

Disclosures: J. Martínez-Gardeazabal: None. **G. Cuadra-Fernández:** None. **I. Bengoetxea de Tena:** None. **I. Fernández-Vega:** None. **M. Moreno-Rodríguez:** None. **A. Llorente-Ovejero:** None. **L. Lombardero:** None. **I. Manuel:** None. **R. Rodríguez-Puertas:** None.

Poster

126. APP Metabolites in Alzheimer's Disease

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 126.01/C90

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Canadian Institutes of Health Research
Alberta Prion Research Institute
Alberta Synergies in Alzheimer's and Related Disorders (SynAD)

Title: Characterization of exosomes-derived from cholesterol accumulated astrocytes and its significance in Alzheimer's disease pathology

Authors: *Q. WU[1], L. CORTEZ[1], R. KAMALI-JAMIL[2], V. SIM[1], H. WILLE[2], S. KAR[1]; [1]Dept. of Med., [2]Dept. of Biochem., Univ. of Alberta, Edmonton, AB, Canada

Abstract: Intracellular accumulation of cholesterol within neurons can increase the level/production of beta-amyloid (Aβ) peptide which plays an important role in the development of Alzheimer's disease (AD). A number of recent studies have shown that exosomes, which are small vesicles (40-100 nm diameter) of endocytic origin secreted by most cells including neurons

and glial cells, represent a novel form of intercellular communication in various physiological and pathological settings. Neuronal exosomes containing Aβ peptides have been shown to influence not only the function/vulnerability of neurons but also in “prion-like” propagation of AD pathology. Unlike neurons, the significance of glial exosomes, particularly those derived from astrocytes, remain unclear. Recently we reported that cholesterol accumulation within cultured astrocytes triggered by U18666A treatment can increase the level/secretion of Aβ-related peptides. Thus, as a follow up, we are now establishing the significance of exosomes derived from cholesterol accumulated astrocytes in the development of AD pathology. Exosomes were purified from control and U18666A-treated astrocytes culture supernatant by using differential centrifugation polyethylene glycol precipitation. While secreted exosomes were characterized by electron microscopy, dynamic light scattering and DiI labeling, the content of various proteins related to AD pathology was defined by dot blotting, Western blotting and ELISA. We observed that cholesterol accumulation following U18666A treatment significantly decreased the release of exosomes compared to control cultured astrocytes as revealed by electron microscopy, dynamic light scattering and DiI labeling. However, exosomes derived from U18886A-treated astrocytes, as detected by dot blotting and Western blotting, contain higher levels of amyloid precursor protein (APP) and APP-cleaved products (i.e., α-CTF and β-CTF) in contrast to control exosomes. The levels of Aβ1-40 peptide, as measured using ELISA, also found to be increased in exosomes of U18666A-treated astrocytes compared to control astrocytes. Cholesterol accumulation in cultured astrocytes can decrease the secretion of exosomes but enhance the levels of APP and Aβ-related peptides in secreted exosomes.

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Poster

126. APP Metabolites in Alzheimer's Disease

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 126.02/C91

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The analysis for APP-βCTF mediated traffic impairment in AD

Authors: *N. KANESHIRO[1], T. HASHIMOTO[2], T. SAKURAI[3], T. UEHARA[1], N. TAKASUGI[1,3];
[1]Okayama Univ., Okayama, Japan; [2]Univ. of Tokyo, Tokyo, Japan; [3]Juntendo Univ., Tokyo, Japan

Abstract: Background: Recently, Amyloid β (Aβ) independent pathology is focused attention in Alzheimer’s disease (AD) pathology. Among them, endocytic dysfunction is the early pathogenic event before Aβ aggregates. A body of evidence suggested that one of Aβ precursor

protein (APP) metabolites, β-carboxyl-terminus fragment (βCTF), accumulated in endosomes and impaired the endocytic trafficking in AD brain. However, the molecular mechanism is largely unknown. Previously, we identified TMEM30A (CDC50A) as the candidate partner for βCTF toxicity. TMEM30A is a subcomponent of lipid flippase which translocates phospholipids such as phosphatidylserine (PS) from outer to inner side of lipid bilayers. In this study, we aimed to analyze how TMEM30A and βCTF complex associates with vesicular trafficking in AD.
Methods: To investigate the molecular mechanism of βCTF mediated traffic impairment, we established BACE1 (β-secretase) stable expression in SH-SY5Y cells. We analyzed the event induced by complex formation of TMEM30A and βCTF using biochemical approach.
Results: We found that βCTF accumulation caused the interaction between TMEM30A and βCTF, which resulted in endosomal characteristic change in SH-SY5Y(BACE1). Moreover, this complex formation affected PS localization in endosomes, which indicated the alteration of lipid flippase activity.
Conclusion: Our study suggests that TMEM30A and βCTF complex can induce traffic impairment via lipid flippase dysfunction in AD. Although further analysis is required, our findings may contribute to the development of a novel AD therapy based on the improvement of vesicular transports.

Disclosures: **N. Kaneshiro:** None. **T. Hashimoto:** None. **T. Sakurai:** None. **T. Uehara:** None. **N. Takasugi:** None.

Poster

126. APP Metabolites in Alzheimer's Disease

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 126.03/C92

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Identification of a novel BACE1 dependent cleavage on amyloid precursor protein

Authors: *X. NIU, M. LUO, X. HOU, Y. GENG, Y. CHEN;
Shanghai Inst. of Organic Chem., Shanghai, China

Abstract: Point mutations of Amyloid Precursor Protein (APP) could increase Abeta production and cause early onset Alzheimer’s disease. Whereas APP Iceland mutation reduces Abeta production and decrease AD risk. These evidence strongly support Abeta hypothesis for AD. However, some mutations on gamma secretase reduce Abeta production but still cause familial AD. There are also studies that suggest these AD causing Presenilin 1 mutations are loss of function mutations. Given the recent difficulties on the development of Abeta targeting therapy, doubts have been casted on the original hypothesis. One possibility is that Abeta is not the sole disease-causing production of APP. We studied metabolism of APP and identified a novel BACE1 dependent cleavage that is more dominant in disease-causing APP mutants. This

suggests that product of this cleavage might contribute to the pathogenesis of familial APP mutations. Further studies have been done to identify the mechanism responsible for this cleavage with a novel proximal labeling biochemical approach. This could provide new mechanism and potential drug targets for AD.

Disclosures: **X. Niu:** None. **M. Luo:** None. **X. Hou:** None. **Y. Geng:** None. **Y. Chen:** None.

Poster

126. APP Metabolites in Alzheimer's Disease

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 126.04/D1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: JSPS Research Fellowships for Young Scientists 18J14653

Title: Structural dynamics of presenilin 1, a protein regulating Alzheimer’s disease associated aβ42 peptide production

Authors: *T. CAI, T. TOMITA;
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Abstract: Alzheimer disease (AD) is triggered by the long chronic accumulation of Amyloid-β peptide (Aβ), which is produced by γ-secretase. γ-Secretase is an intramembrane aspartic protease comprised of four components (i.e., Presenilin 1 (PS1), Nicastrin, Aph-1, and Pen-2} with a hydrophilic catalytic pore. γ-Secretase executes the final cleavage process in Aβ production and is responsible for the determination of C-terminal length of Aβ, which impacts on the neuronal toxicity and aggregability. Recent advances in the field of single-particle cryo-electron microscopy enables us to understand the detailed structure of γ-secretase complex at an atomic level. However, the molecular dynamics of the γ-secretase remained unclear. In this study, we investigated the structure-activity relationship of PS1, which is the catalytic subunit of γ-secretase, utilizing substituted cysteine accessibility method (SCAM). SCAM is a labeling experiment to analyze the structure of intramembrane proteases using MTS reagents that specifically bind to cysteine residues facing aqueous environment. The strongest point of this approach is that the target protein can be kept in physiological condition since intact cells or membrane fractions are the samples in this method. SCAM also enables us to detect the molecular movement of target residue by using small compounds that modify γ-secretase activity (i.e., inhibitors and modulators). In this study, we elucidated that hydrophilicity around transmembrane domain (TMD) 3 of PS1 changes corresponding to the production ratio of Aβ42, which is highly aggregable Aβ species, by SCAM. We revealed that TMD3 locates at proximity to TMD7 in which catalytic aspartate resides. Moreover, we found that conformationally stabilized PS1 by MTS crosslinker at TMD3 and TMD7 losses the sensitivity to the Aβ42

reducing γ-secretase modulator. These results indicate that the structural dynamics of TMD3 is involved in the regulation of Aβ42 production.

Disclosures: **T. Cai:** None. **T. Tomita:** None.

Poster

126. APP Metabolites in Alzheimer's Disease

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 126.05/D2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Canadian Institutes of Health Research Grant
Queen Elizabeth II Graduate Student Scholarship
Faculty of Medicine and Dentistry 75th Anniversary Award
Government of Alberta Graduate Student Scholarship

Title: Thermal response of amyloidogenic elements in cultured N2a cells: Potential relevance to Alzheimer's disease pathology

Authors: *A. SCHMAUS[1], S. KAR[2];
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Abstract: Alzheimer's Disease (AD), the most prevalent cause of dementia afflicting the elderly, is characterized by the accumulation of Tau-positive neurofibrillary tangles (NFTs) and amyloid-β (Aβ) containing extracellular plaques within the diseased brain. Production of these aggregated proteins correlate to the neurodegeneration and loss of cognitive function seen in AD patients. While a subset of cases may originate intrinsically via genetic mutations in genes encoding proteins such as the amyloid precursor protein (APP), the vast majority of disease arises sporadically and with potential extrinsic or environmental influences. Correspondingly, the greatest risk factor for developing AD is age, as normal functioning systems that regulate homeostasis weaken. Recent studies have indicated that perturbations in body temperature may have a role in protein aggregation and the development of pathological features associated with AD. Thus, in this current project, our aim is to evaluate the influence of ambient temperature on the cellular processes that drive neurodegeneration observed in AD.
We used wild-type murine neuroblastoma (N2a) and Swedish APP mutant N2a cells to investigate the effects of ambient temperature within a cellular context on the production, secretion, and degradation of APP-derived products, such as Aβ-peptides. Cells grown in a normothermic (37C) environment or subjected to hypothermic and hyperthermic conditions were assayed for amyloidogenic markers by western blot, ELISA, and fluorescent immunocytochemistry. Additionally, we evaluated how these conditions influence cell viability,

which may enhance neurodegeneration in AD.
Our results show an inverse relationship between temperature and APP processing in both wild-type and mutant cells, indicated by an increased production of Aβ-related peptides such as α/β-C-terminal fragments. The secretion of Aβ peptides as well as typical clearance routes via the endolysosomal system are found to be similarly altered in a temperature dependent manner, with an increased colocalization of APP and endolysosomal markers in a reduced temperature. Additionally, we showed that a hypothermic condition can significantly decrease cell viability. The results that we have obtained so far indicate that a cellular thermal response may directly influence AD-related pathology by altering the production and secretion of AD-associated molecules. These results may contribute to an understanding of environmental influences on AD pathogenesis and the development of alternate therapeutic avenues which are inclusive of both intrinsic and extrinsic factors.

Disclosures: **A. Schmaus:** None. **S. Kar:** None.

Poster

126. APP Metabolites in Alzheimer's Disease

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 126.06/D3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R21 NS109884
NSF 1557414

Title: New evidence that amyloid precursor protein can signal via the heterotrimeric G protein Go to regulate different aspects of neuronal motility

Authors: *P. F. COPENHAVER[1], J. M. RAMAKER[2], H.-J. LEE[2], G. WALKER-STEVENSON[2], C. BATES[2];
[1]Cell & Developmental Biol., Oregon Hlth. and Sci. Univ., Portland, OR; [2]Cell & Developmental Biol., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Amyloid Precursor Protein (APP) is the source of beta amyloid (Aβ) peptides that accumulate in Alzheimer’s Disease (AD), but APP may serve important functions in the brain, including synaptogenesis, remodeling, and regrowth responses following injury. In addition, neurotoxic forms of Aβ can directly bind APP, suggesting that Aβ might also provoke neurodegeneration by perturbing the normal functions of APP. Although APP may interact with a variety of signaling molecules, growing evidence supports the model that APP can function as an atypical G protein-coupled receptor, specifically interacting with the heterotrimeric G protein Goα to regulate neuronal responses. Early studies in cell culture showed that APP can bind and activate Goα via a conserved cytoplasmic motif, while chronic stimulation of this pathway can

provoke Ca^{2+} overload and apoptosis. APP mutations linked with AD can also hyperactivate Goα, while the severity of AD symptoms in patients corresponds with elevated G protein activity. However, authentic roles for APP-Goα signaling have remained controversial. Using insect discovery models, we showed that APP family proteins regulate Goα-dependent guidance responses, providing the first demonstration of this conserved pathway *in vivo*. Subsequently, we used cultured murine hippocampal neurons to show that APP-Goα signaling could similarly regulate their motile responses. Specifically, we found that APP and Goα colocalized in their leading processes, while endogenous APP co-immunoprecipitated with Goα (but not other G proteins) from mouse and human brain lysates. Co-expressing APP and Goα as fusion proteins with complementary portions of Venus fluorescent also showed that the two proteins directly interact in cultured neurons, while stimulating APP signaling restricted growth cone motility in a G protein-dependent manner. To investigate how this pathway might affect neuronal remodeling, we have now found that APP and Goα colocalize in dendrites and synaptic spines of cultured rat hippocampal neurons, while pharmacological assays suggest that APP-Goα signaling may promote Ca^{2+}-dependent aspects of synaptic maturation. Conversely, acute treatment with neurotoxic Aβ oligomers resulted in reduced synaptic spine densities in a Goα-dependent manner. Pilot studies using human brain samples also suggest that APP endogenously interacts with Goα in cortical neurons, an interaction that is diminished in AD. These results provide the framework for our ongoing investigations into the normal functions of APP-Goα signaling during neuronal development, and whether disrupting APP-Goα signaling contributes to the neuropathology of AD.

Disclosures: P.F. Copenhaver: None. **J.M. Ramaker:** None. **H. Lee:** None. **G. Walker-Stevenson:** None. **C. Bates:** None.

Poster

126. APP Metabolites in Alzheimer's Disease

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 126.07/D4

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Repeated hypoxia exposure decreases *O*-GlcNAcylation to indeed amyloid-beta and p-tau accumulation in the brain of adult zebrafish

Authors: *J. PARK, Y. LEE, S.-M. KIM, J.-W. LEE, I.-O. HAN;
Dept. of Physiol. and Biophysics, Inha Univ., Incheon, Korea, Republic of

Abstract: Exposure to repetitive hypoxia elicits a cognitive dysfunction but underlying mechanism by which hypoxia causes cognitive impairment is remains unknown. In this study, we show that chronic intermittent hypoxia impairs cognitive functions in the adult zebrafish. As the numbers of hypoxic episode increased, defects in learning and memory (L/M) ability in

zebrafish getting severed. Repetitive hypoxia triggered downregulation of *O*-GlcNAcylation of nucleocytoplasmic proteins and accumulation of β-amyloid (Aβ) and p-Tau in the brain of zebrafish. At the molecular level, repetitive hypoxia decreased mRNA and protein levels of *O*-GlcNAc transferase (OGT) along with increase in *O*-GlcNAcase (OGA) expression. Glucosamine (GlcN) recovered hypoxia-induced decrease in *O*-GlcNAyclation and protected from cognitive dysfunction and brain pathologies after hypoxia. Furthermore, an *O*-GlcNAcase (OGA) inhibitor, Thiamet G, significantly recovered *O*-GlcNAyclation as well as cognitive dysfunction and brain pathologies in response to repetitive hypoxia. Our results suggest that hexosamine biosynthetic pathway could be an important therapeutic target for hypoxic brain damage.

Disclosures: **J. Park:** None. **Y. Lee:** None. **S. Kim:** None. **J. Lee:** None. **I. Han:** None.

Poster

126. APP Metabolites in Alzheimer's Disease

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 126.08/D5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG042178
AG047812
NS105473

Title: Novel microRNA-455-3p and its protective effects against abnormal APP processing and amyloid beta toxicity in Alzheimer’s disease

Authors: *S. KUMAR[1], A. REDDY[2], X. YIN[3], P. REDDY[1];
[1]Intrnl. Med., [2]Pharmacol. & Neurosci. Dept., [3]Garrison Inst. on Aging, TTUHSC, Lubbock, TX

Abstract: The purpose of our study is to understand the protective role of miR-455-3p against abnormal amyloid precursor protein (APP) processing, amyloid beta (Aβ) formation, defective mitochondrial biogenesis/dynamics and synaptic damage in AD progression. In-silico analysis of miR-455-3p has identified the APP gene as a putative target. Using mutant APP cells, miR-455-3p construct, biochemical and molecular assays, immunofluorescence and transmission electron microscopy (TEM) analyses, we studied the protective effects of miR-455-3p on - 1) APP regulation, amyloid beta (Aβ)(1-40) & (1-42) levels, mitochondrial biogenesis & dynamics; 3) synaptic activities and 4) cell viability & apoptosis. Our luciferase assay confirmed the binding of miR-455-3p at the 3’UTR of APP gene. Immunoblot, sandwich ELISA and immunostaining analyses revealed that the reduced levels of the mutant APP, Aβ(1-40) & Aβ(1-42), and C99 by miR-455-3p. We also found the reduced levels of mRNA and proteins of mitochondrial biogenesis (PGC1α, NRF1, NRF2, and TFAM) and synaptic genes (synaptophysin and PSD95)

in mutant APP cells; on the other hand, mutant APP cells that express miR-455-3p showed increased mRNA and protein levels of biogenesis and synaptic genes. Additionally, expression of mitochondrial fission proteins (DRP1 and FIS1) were decreased while the fusion proteins (OPA1, Mfn1 and Mfn2) were increased in cells that express miR-455-3p and mutant APP. Our TEM analysis showed a decrease in mitochondria number and an increase in the size of mitochondrial length in mutant APP cells transfected with miR-455-3p. Based on these observations, we cautiously conclude that miR-455-3p regulate APP processing and protective against mutant APP-induced mitochondrial and synaptic abnormalities in AD.
Key words: microRNA-455-3p, Alzheimer's disease, Amyloid Precursor Protein, Amyloid Beta, Mitochondrial Biogenesis, Synaptic Proteins.

Disclosures: **S. Kumar:** None. **A. Reddy:** None. **X. Yin:** None. **P. Reddy:** None.

Poster

126. APP Metabolites in Alzheimer's Disease

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 126.09/D6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG060238
AG033570
AG033570-S2
AG062251
AG061628
CCTS UL1TR002003
T32AG057468

Title: Loss of caveolin-1 promotes Alzheimer's disease in type-2- diabetes

Authors: *A. SHETTI[1], J. BONDS[2], A. BHERI[1], Z. CHEN[1], A. DISOUKY[1], L. TAI[1], M. MAO[3], B. P. HEAD[5], M. BONINI[4], J. HAUS[6], R. MINSHALL[1], O. LAZAROV[1];
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Abstract: Type 2 Diabetes (T2D) is a risk factor for the development of Late Onset Alzheimer's disease (LOAD). AD is characterized by loss of memory and cognitive decline. The hallmarks of AD are amyloid deposition and neurofibrillary tangles. However, the cause of LOAD is not well understood. Caveolin-1 (Cav-1) is a 22 kDa coat protein of caveolae, a subset of lipid rafts, and a major player in transport and signaling across the Blood Brain Barrier (BBB). Here we show loss of Cav-1 in the brains of T2D patients (n=3) concomitantly with a significant increase in β-

Amyloid (Aβ) levels compared to healthy aging controls. These samples also show reduced levels of endothelial nitric oxide synthase (eNOS) which is tightly regulated by Cav-1 specifically in endothelial cells, suggesting loss of Cav-1 dependent signaling in endothelial cells. Loss of Cav-1 and increased Aβ was recapitulated in the brains of the diabetic mouse model db/db. Levels of amyloid precursor protein (APP), β-secretase-1 (BACE-1) and hyperphosphorylated tau species were increased as well. These mice exhibit cognitive deficits at 12 weeks of age. Restoration of Cav-1 via Cav-1-expressing adenovirus rescues learning and memory impairments and AD-linked pathology. In summary, this study provides evidence that depletion of Cav-1 underlies the development of AD in T2D and suggests that restoration of Cav-1 may be a therapeutic target for the prevention or attenuation of AD.

Disclosures: A. Shetti: None. **J. Bonds:** None. **A. Bheri:** None. **Z. Chen:** None. **A. Disouky:** None. **L. Tai:** None. **M. Mao:** None. **B.P. Head:** None. **M. Bonini:** None. **J. Haus:** None. **R. Minshall:** None. **O. Lazarov:** None.

Poster

126. APP Metabolites in Alzheimer's Disease

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 126.10/D7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR grant to SK
SynAD fellowship to MA

Title: Effects of temperature on APP processing in cultured astrocytes and its potential implication in Alzheimer's disease pathology

Authors: *M. M. ALAM[1], G. KARTHIVASHAN[2], Q. WU[2], D. VERGOTE[2], G. BAKER[1], S. KAR[1,2];
[1]Psychiatry, [2]Med., Univ. of Alberta, Edmonton, AB, Canada

Abstract: Alzheimer’s disease (AD), the most common type of dementia, is characterized neuropathologically by the presence of extracellular β-amyloid (Aβ)-containing neuritic plaques and intracellular *tau*-positive neurofibrillary tangles in selected regions of the brain. Aβ peptides are generated from the amyloid precursor protein (APP) by amyloidogenic processing mediated *via* the enzymes β- and γ-secretases. Some recent studies reported that ambient temperature may influence disease progression and/or pathology in animal models and AD patients. However, very little is known about the mechanisms by which temperature can influence APP metabolism in cells. In the present study, we used astrocytes, to evaluate the impact of temperature on APP metabolism-related proteins. We investigated the cell viability using the MTT assay and determined the alterations in the protein levels related to APP metabolism in hypo-/hyper-

thermic conditions using Western blotting (WB). Our MTT results indicated that the viability of astrocytes decreased substantially under hypothermic conditions but increased at hyperthermic conditions compared to cells grown at ambient temperature. From our WB data, the astrocyte marker-GFAP was observed to decrease in a time-dependent fashion under hyperthermic conditions. While the levels of APP holoprotein were significantly increased under hyperthermic conditions compared to hypothermic conditions, the levels of APP cleaved products, i.e. α- and β-CTFs, were found to be differentially altered as a function of time with temperature. The steady-state levels of the APP-processing enzymes ADAM10 and BACE1 were also altered variably following exposure of cultured astrocytes to different temperature conditions. Interestingly, markers (LC-3II and LAMP1) of the autophagic/lysosomal system which regulates APP metabolism were found to be time-dependently increased under both hypo- and hyperthermic conditions. This study indicates that alteration in temperature can influence AD pathology by regulating APP-metabolism in cultured astrocytes.

Disclosures: **M.M. Alam:** None. **G. Karthivashan:** None. **Q. Wu:** None. **D. Vergote:** None. **G. Baker:** None. **S. Kar:** None.

Poster

126. APP Metabolites in Alzheimer's Disease

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 126.11/D8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR Grant

Title: Investigation of cerebrovascular proteins involved in amyloid-beta disposition in a mouse model of sporadic Alzheimer's disease

Authors: *K. A. TRESIDDER[1], B. M. BENNETT[2];
[1]Ctr. for Neurosci. Studies, [2]Dept. of Biomed. and Mol. Sci., Queen's Univ., Kingston, ON, Canada

Abstract: The identification and validation of molecular mechanisms underlying Alzheimer’s disease (AD) is of critical importance for the development of effective therapeutics. Therapeutic development, however, has been hampered by the lack of animal models which mirror the age-related progression of sporadic AD pathologies. Our laboratory has developed an oxidative stress-based mouse model of age-related cognitive impairment with AD-like biochemical and structural pathologies (*Aldh2*$^{-/-}$ mice). Importantly, *Aldh2*$^{-/-}$ mice display increases in oxidative stress markers (i.e., 4-hydroxynonenal protein adducts) in both the brain and the cerebral vasculature, including age-related increases in amyloid-β (Aβ) deposition in cerebral microvessels (CMVs). The accumulation of Aβ in cerebral blood vessels, known as cerebral

amyloid angiopathy, is associated with cognitive decline and occurs in 80-95% of AD patients. Our objective was to assess whether vascular oxidative stress alters cerebrovascular proteins involved in Aβ disposition. Using a mechanical dispersion and filtration technique, we collected cerebral microvessels (CMVs) from 3, 6, 9, and 12-month old *Aldh2*$^{-/-}$ (KO) mice and age-matched, wild-type (WT) littermates. Immunoblot analysis of CMVs shows an absence of neuronal and oligodendrocyte markers such as NeuN and Olig2, the presence of the astrocyte marker GFAP, and a strong signal for smooth muscle alpha-actin. We assessed the basal levels of a number of proteins involved in the formation (nicastrin), catabolism (neprilysin), or transport (LDL-receptor-related protein 1, LRP1) of Aβ, hypothesizing that alterations in the levels of these proteins may contribute to the increased deposition of Aβ observed in CMVs. However, the immunoblot data did not reveal significant differences between WT and KO (n=2-4 per group) for any of these proteins at any of the ages assessed, indicating that CMV Aβ deposition is likely not due to differences in the levels of the three proteins examined. We are currently following this up with activity assays to determine whether there are differences in the function/activity of these proteins which could explain the increases in Aβ deposition, which in turn could contribute to the cognitive impairment observed in *Aldh2*$^{-/-}$ mice.

Disclosures: K.A. Tresidder: None. **B.M. Bennett:** None.

Poster

126. APP Metabolites in Alzheimer's Disease

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 126.12/D9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant NS055223
NIH Grant AG042762
NIH Grant AG019070
NIH Grant P01CA166009
Alzheimer's Association Grant IIRG-06-26148
Alzheimer's Association Grant NIRG-15-342442
BrightFocus Foundation Grant A2012386

Title: APP-mediated signaling rescues memory impairment in presenilin knock-in mice carrying familial Alzheimer’s disease variant

Authors: C. DEYTS[1], M. CLUTTER[1], N. PIERCE[1], G. BESANT[1], V. SPRUILL[1], P. CRUZ[2], G. THINAKARAN[1], T. GOLDE[2], ***A. PARENT**[1];
[1]Univ. of Chicago, Chicago, IL; [2]Univ. of Florida, Gainesville, FL

Abstract: We previously described that familial Alzheimer Disease knock-in mice that carry presenilin 1 M146V mutation (PS1-KI) exhibit an increase of axodendritic outgrowth in the hippocampal area (Deyts et al., eLife 2016). We determined that increased levels of APP intracellular C-tail fragment (APP-CTF) are a consequence to this outcome, which encompasses a direct coupling of APP-CTF with Gαs protein and subsequent activation of adenylate cyclase and CREB signaling. We were able to capture the intricate function of APP-CTF using an experimental construct containing APP intracellular domain, which is tethered to the membrane via a lipid anchor (named mAICD; Deyts et al., J Neuroscience 2012). We recently reported that overexpression of mAICD in the brain prevents memory decline in amyloidogenic AD mice (Deyts et al., Cell Reports 2019). By experimentally targeting APP-CTF at the membrane by expressing mAICD (devoid of the Aβ sequence), it allowed us to achieve constitutive activation of APP-mediated signaling. In order to establish if mAICD overexpression is sufficient to overcome memory impairment in a non-amyloidogenic mouse model, we tested if a recombinant adeno-associated virus (rAAV)-mediated expression of mAICD could also prevent memory decline in PS1-KI mice. We performed intracerebroventricular delivery of rAAV-mAICD at birth. Six months later, memory performances were analyzed using novel object recognition and contextual fear conditioning tasks. We observed that PS1-KI mice exhibit a profound cognitive deficit in spatial working memory that is rescued by mAICD brain expression. We also found that sustained APP-mediated signaling through overexpression of mAICD produced a significant increase in cognitive performance in PS1-KI mice that lack APP expression. Based on our findings, we conclude that targeting APP-CTF at the membrane early during brain development is sufficient to reverse cognitive deficit seen in mouse models that do not exhibit amyloidosis pathology during the course of their life. Although we found that sustained APP-mediated signaling promotes non-amyloidogenic processing pathway, APP expression is not required to improve cognitive performance in mouse models that do not accumulate Aβ. Therefore our results suggest that intrinsic property of APP-CTF is sufficient to heighten memory.

Disclosures: C. Deyts: None. **M. Clutter:** None. **N. Pierce:** None. **G. Besant:** None. **V. Spruill:** None. **P. Cruz:** None. **G. Thinakaran:** None. **T. Golde:** None. **A. Parent:** None.

Poster

126. APP Metabolites in Alzheimer's Disease

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 126.13/D10

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Insulin reduces APP endocytosis by decreasing its localization in lipid rafts

Authors: *O. KWON, Y. CHO, S. CHUNG;
Sungkyunkwan Univ. Sch. of Med., Suwon city, Korea, Republic of

Abstract: Alzheimer's disease (AD) is a chronic and progressive neurodegenerative disease caused by the accumulation of neurotoxic amyloid-β (Aβ) peptides. Aβ is derived from the sequential proteolytic cleavage of amyloid precursor protein (APP), which can undergo two different metabolic pathways: non-amyloidogeinic, and amyloidogenic. In non-amyloidogenic pathway, APP is cleaved by α-secretase and γ-secretase at the plasma membrane, excluding Aβ production. Alternatively, APP at the plasma membrane is internalized via endocytosis, and delivered to early endosomes and lysosomes, producing Aβ via β-secretase and γ-secretase. Recently, some studies have shown that insulin in the periphery crosses the blood-brain barrier and plays important roles in the brain. Furthermore, impaired insulin signaling has been linked to the progression of AD. Intranasal insulin administration improves memory impairments and cognition. Recently, we reported that insulin increases the level of cell surface APP, decreasing the endocytosis rate of APP. Insulin reduced Aβ generation through up-regulation of APP O-GlcNAcylation via Akt insulin signaling. In a separate report, we also showed that a significant amount of APP is localized in lipid rafts, and that increasing cholesterol level increases the localization of APP in lipid rafts. Since recent studies have suggested that membrane trafficking plays a key role in the regulation of APP processing, we hypothesize that the reduction of APP endocytosis by insulin is caused by the change in the APP localization from lipid raft to non-raft fractions via O-GlcNAcylation. To test this possibility, we used SH-SY5Y cells stably expressing wild-type APP and BACE1. When antibody for caveolin was used for the marker for lipid raft, insulin decreased the level of co-localization of APP with caveolin. Consistent with this result, insulin promoted the translocation of APP from raft fractions to non-raft fractions when we used discontinuous sucrose gradients to separate lipid raft fractions. The insulin effect on APP lipid raft localization was blocked by Akt inhibitor, indicating that insulin effects on APP localization in lipid raft is via Akt signaling. When we labeled the surface APP and measured the internalized APP, insulin decreased the internalized APP levels in the lipid raft fractions. These results suggest that insulin reduces the rate of lipid raft-dependent APP endocytosis by decreasing its localization in lipid rafts.

Disclosures: O. Kwon: None. **Y. Cho:** None. **S. Chung:** None.

Poster

126. APP Metabolites in Alzheimer's Disease

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 126.14/D11

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Cholesterol enriched lipid raft is a platform for APP endocytosis

Authors: *Y. CHO, O. KWON, S. CHUNG;
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Abstract: Alzheimer's disease (AD) is the most common and irreversible neurodegenerative disorder characterized by progressive loss of cognitive, memory, and behavioral impairments. The hallmark of pathogenesis of AD is cerebral elevation and deposition of Aβ peptides, which are generated via the sequential proteolytic cleavage of amyloid precursor protein (APP) by β-, γ-secretases. APP at the plasma membrane is internalized via endocytosis, and delivered to early endosomes and lysosomes, producing Aβ. As the specialized cholesterol-enriched microdomains, lipid rafts are considered as platforms for various cell signaling and protein-protein interactions. It is widely believed that β- and γ-secretases as well as their substrate APP are localized in lipid raft microdomains. In our previous study, we found that CHO PS1 ΔE9 cells showed increased cellular cholesterol levels, and the elevation of cellular cholesterol is associated with a significant shift in localization of APP toward cholesterol-enriched lipid raft fractions. Since membrane trafficking plays a key role in the regulation of APP processing, we investigate whether the cellular cholesterol level affects the endocytosis of APP in lipid raft microdomains. We found that PS1 ΔE9 mutant cell showed increased rate of APP endocytosis compared to PS1 WT cell using two different APP antibodies to separate internalized APP from APP in the plasma membrane. Reducing the cholesterol levels to the comparable level of WT cells by MβCD-cholesterol decreased the rate of APP endocytosis in CHO PS1 ΔE9 cells. Also, the amount of colocalization of APP with caveolin, a lipid raft marker, is increased in CHO PS1 ΔE9 cells. When we labeled the surface APP and measured the internalized APP, larger amount of labeled APP was located in lipid raft indicating that APP localized in lipid raft fractions preferentially undergoes endocytosis compared to APP in non-raft fractions. Our results suggest that cholesterol-enriched lipid raft microdomain is a platform for the endocytosis of APP, and that the regulation of APP localization in the lipid raft is important for Aβ production.

Disclosures: Y. Cho: None. **O. Kwon:** None. **S. Chung:** None.

Poster

126. APP Metabolites in Alzheimer's Disease

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 126.15/D12

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: NIH Grant R01AG51086

Title: Dysregulation of the aβ degrading enzyme neprilysin (MME) could contribute to Alzheimer's disease

Authors: *R. WANG, N. CHOPRA, B. MALONEY, D. K. LAHIRI;
Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Background: Alzheimer's disease (AD) is the foremost world-wide cause of age-related dementia. Its global prevalence is expected to due to an aging population. AD is characterized by accumulation of extracellular amyloid β (Aβ) plaques and intracellular aggregates of hyper-phosphorylated microtubule-associated protein tau (p-τ) in brains. Nevertheless, the underlying pathogenesis of AD is presently unknown, particularly the most common form of AD, late-onset. Even though genetic, epigenetic, and environmental factors play critical roles in AD etiology (Maloney and Lahiri, *Lancet Neurol*-2016), specific triggers are still unknown. We hypothesize that AD results from dysregulation of key biochemical *pathways*, particularly through *regulatory* molecules, such as non-coding RNAs, including microRNA (Long, Maloney, Rogers and Lahiri, *Mol. Psychiatry*-2018). We further posit that disruption of miRNA regulation of membrane metallo-endopeptidase (MME), or neprilysin could contribute to Aβ accumulation, hence AD pathogenesis, since MME is an important Aβ-clearing enzyme. **Method:** Using the bioinformatics tools we predicted miRNA binding sites on the MME mRNA 3'-untranslated region (3'-UTR). Weneuronallydifferentiated human neuroblastoma (SK-N-SH) cells with all-*trans* retinoic acid (ATRA) and transfected differentiated cells with either siRNA against MME or candidate miRNAs. We assayed levels of MME mRNA and protein by qRT-PCR and Western blotting, respectively. We also measured MME protein levels in several human fetal tissues. MME mRNA and miR-181d levels in AD and control brain samples were determined by qPCR. **Result:** While several miRNAs were predicted to target the MME 3'-UTR, many did not alter MME mRNA or protein levels. We found that miR-181d down-regulates MME mRNA and protein levels in neuronal cultures. Its specificity was further confirmed by anti-sense oligomer inhibitors (target protection). In addition, miR-9 and miR-218 also found to down-regulated MME protein in neuronal cultures. We found significant differences in MME mRNA levels by Braak stage in AD brain samples. MME is also expressed in a variety of human fetal organs, with highest expression in kidney, while relatively low expression in brains. **Conclusion:** miR-181d, miR-9, and miR-218 can down-regulate MME through specific target sited within the MME 3'-UTR. As MME is a key Aβ-degrading enzyme involved in AD, Dysregulation by miR-181d could contribute to AD pathogenesis.

Disclosures: R. Wang: None. **N. Chopra:** None. **B. Maloney:** None. **D.K. Lahiri:** None.

Poster

126. APP Metabolites in Alzheimer's Disease

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 126.16/D13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant P01 AG017617
NIH Grant R01 AG056732

Title: Extracellular vesicles: Where the amyloid precursor protein carboxyl-terminal fragments accumulate and amyloid-beta is not generated

Authors: *Y. KIM[1,2], R. PÉREZ-GONZÁLEZ[1,2,5], C. MILLER[1], M. PAWLIK[1], E. LEVY[1,2,3,4]; [1]Ctr. for Dementia Res., Nathan S. Kline Inst., Orangeburg, NY; [2]Dept. of Psychiatry, [3]Dept. of Biochem. & Mol. Pharmacol., [4]Neurosci. Inst., New York Univ. Langone Hlth., New York, NY; [5]Dept. of Neurol., Ctr. for Networked Biomed. Res. on Neurodegenerative Dis. (Ciberned), Hosp. de la Santa Creu i Sant Pau, Barcelona, Spain

Abstract: The amyloid β precursor protein (APP) is a single-pass transmembrane protein whose proteolysis by α- and β-secretases generates α- and β-carboxyl-terminal fragments (APP-CTFs), respectively. γ-secretase cleavage of APP-CTFs generates amyloid-β (Aβ), the major component of the amyloid deposits in the brain of Alzheimer's disease patients. The involvement of extracellular vesicles (EVs) in Aβ amyloidosis was proposed because full-length APP, APP cleaving enzymes, APP-CTFs, and a minute fraction of Aβ were identified in association with EVs. Here we undertook to determine whether in addition to Aβ binding to EVs in the extracellular space as was previously shown, EVs are a source of Aβ. We investigated the processing of APP in exosome-enriched EVs isolated from the brain of wild-type and APP overexpressing Tg2576 mice, aged 4 to 6 months. EVs were isolated from brain extracellular space using a method developed in our laboratory. The EVs were incubated in human CSF for different time periods at 37°C and analyzed by Western blotting. We found that APP was enzymatically processed in isolated brain EVs following 24h incubation, generating APP-CTFs. We not only confirmed the presence of the α- and β-secretases, ADAM10 and BACE1 in the EVs, but also found the presence of all the γ-secretase subunits. Interestingly, treatment of EVs with the γ-secretase inhibitor, L-685, did not affect the levels of APP-CTFs, suggesting no further processing of APP-CTFs in EVs. Lastly, we found a decrease in the level of the 4 kDa monomeric Aβ accompanied by an increase in the level of Aβ signals at 8-9kDa following 24h incubation. This indicates the recruitment of EV-associated Aβ into dimers, which have been suggested as a building block of toxic assemblies. As a result, our data show that while exosomes are not a major source of Aβ generation, it seeds oligomeric Aβ.

Disclosures: Y. Kim: None. **R. Pérez-gonzález:** None. **C. Miller:** None. **M. Pawlik:** None. **E. Levy:** None.

Poster

126. APP Metabolites in Alzheimer's Disease

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 126.17/D14

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: NIH Grant AG046200

NIH Grant AG062378
Alzheimer Association IIRG 10-173180
MUSC interprofessional

Title: Gut microbiota manipulation by dietary restriction to treat sensory and cognitive changes in an Alzheimer animal model

Authors: *K. SAMBAMURTI[1], V. PADMARAJU[1], E. AMELLA[2], M. A. PAPPOLLA[4], C. VASU[3], N. BHAT[1], D. K. LAHIRI[5], N. H. GREIG[6];
[1]Neurosciences, [2]Nursing, [3]Microbiology, Med. Univ. of South Carolina, Charleston, SC; [4]Neurol., Univ. of Texas, Galveston, TX; [5]Dept. of Psychiatry, Indiana Univ. Sch. of Med., Indianapolis, IN; [6]Drug Design & Develop. Section, LNS, Intramural Res. Program, Natl. Inst. On Aging, NIH, Baltimore, MD

Abstract: High serum homocysteine is a risk factor for Alzheimer Disease (AD) and has been reported to influence the accumulation of its characteristic lesions - senile plaques (SP) and neurofibrillary tangles (NFT) - containing amyloid β protein (Abeta) and microtubule-associated protein Tau (MAPT), respectively. It is also a known risk factor for retinal degeneration and typically increases in the aging population. Our hypothesis is that metabolic failure with aging might drive conditions that increase homocysteine and favor SP formation. Reduced food or calorie intake or branch chain amino acids is already known to benefit AD model mice. Restriction of Met, an essential aa, is known to contribute to increase in longevity, without calorie restriction. We used treated APP/PS1 transgenic mice with a 75% Met restricted (MR) diet and compared it with a isocaloric isonitrogenous complete diet (CD) for up to 12 months. MR diets significantly alter gut microbiota. As expected, the bacteria that metabolize Met and Cys are reduced significantly. Furthermore, several major species of bacteria were reduced significantly, and the overall diversity of microbiota appears to be increased. Our data show that the treatment significantly drops brain soluble Abeta at 3 and 6 months. Older animals continue to show trends towards reduction of Abeta, but the levels are not significantly different. This is not unexpected as the levels are determined by protein accumulation, with limited contribution from fresh synthesis, which is a target for MR. Nevertheless, older mice show significant improvement in behavior in a novel object recognition task after MR treatment. MR treatment is known to be senolytic and therefore, we are currently examining related processes, such as preservation of lysosomal capacity, changes in metabolome, mRNA and miRNA profiles. Senolysis and improvements in AD pathology may also be mediated by reduced inflammation due to the increased diversification of the microbiome. Thus, such diets may play an important role in strategies to prevent AD, retinal degeneration and other age-associated disorders.

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Poster

126. APP Metabolites in Alzheimer's Disease

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 126.18/D15

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: NIH Grant U01 AG15001

Title: Associations between amyloid and tau burden in Down syndrome using positron emission tomography

Authors: *M. ZAMMIT[1], C. LAYMON[2], D. TUDORASCU[2], K. CODY[1], S. JOHNSON[1], S. ZAMAN[3], M. SABBAGH[4], D. MINHAS[2], A. COHEN[2], W. KLUNK[2], B. HANDEN[2], B. CHRISTIAN[1];
[1]Univ. of Wisconsin-Madison, Madison, WI; [2]Univ. of Pittsburgh, Pittsburgh, PA; [3]Univ. of Cambridge, Cambridge, United Kingdom; [4]Barrow Neurolog. Inst., Phoenix, AZ

Abstract: Background: Adults with Down syndrome (DS) are at increased risk to develop Alzheimer's disease (AD). Using PET imaging, β-amyloid (Aβ) and neurofibrillary tau extent can be characterized *in vivo*. The aim of this study is to determine a threshold of Aβ burden in which significant tau spread is evident in DS. **Methods:** N=117 adults with DS (39±8yrs; 63M/54F) underwent T1w-MRI in addition to [11C]PiB PET scans to measure brain Aβ and [18F]AV-1451 scans to measure tau. Tau burden was assessed using FreeSurfer (v5.3.0) defined regions associated with Braak 3 tau staging (parahippocampal, fusiform, lingual gyri and amygdala). Tau levels were measured using standard uptake value ratios (SUVR) in which AV-1451 PET signal in the target Braak 3 regions were normalized to a cerebellum reference region. A threshold for tau-positivity (tau(+)) was established using k-means clustering with resampling on the AV-1451 SUVR data. From the [11C]PiB data, Amyloid Load ($A\beta_L$) was used to assess the percent global Aβ burden based on the total brain carrying capacity for Aβ. Participants were deemed positive for Aβ (Aβ(+)) if their $A\beta_L$ exceeded 26.1%. A linear regression model was used to assess associations between AV-1451 SUVR and $A\beta_L$, and an estimate of the $A\beta_L$ threshold for tau(+) was derived from the regression fit. The regression was repeated to correct for age, sex, and scanner site. **Results:** The cutoff for tau(+) of 1.4 SUVR was determined from k-means clustering (Fig 1a). A statistically significant association between $A\beta_L$ and AV-1451 SUVR was observed ($p<0.001$) with a slope estimate of 0.016[0.014, 0.018]. The inclusion of sex and scanner site did not influence model variability. From the regression fit, the $A\beta_L$ corresponding to tau(+) was estimated as 27.4% (Fig 1b). **Conclusion:** These exploratory results indicate that significant tau spread in DS occurs after participants are positive for Aβ, consistent with what is observed in sporadic AD. Future work will involve using longitudinal Aβ change as a predictor for when this tau threshold is reached during the course of AD.

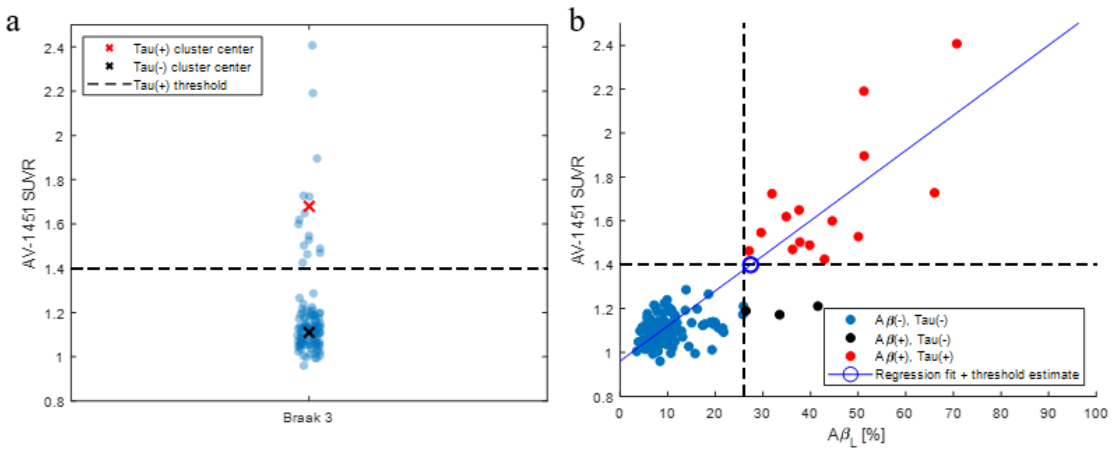

Figure 1. (a) Determination of the cutoff for tau(+) based on k-means clustering with resampling. (b) Linear regression of the PET data estimating the Aβ threshold for tau(+). Data points are colored based on their Aβ and tau status. Dashed lines represent the cutoff for Aβ(+) and tau(+).

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Poster

126. APP Metabolites in Alzheimer's Disease

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 126.19/D16

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG044714
NIH Grant P50-AG033514
NIH Grant UL1TR002373
DOD Grant W81XWH-16-1-0082
FRAXA Research Foundation

Title: J20 Alzheimer's disease mice exhibit an altered circadian actigraphy profile

Authors: P. R. WESTMARK, M. J. FILON, E. WALLACE, S. WRIGHT, R. K. MAGANTI, *C. J. WESTMARK;
Neurol., Univ. of Wisconsin, Madison, WI

Abstract: Study Objectives: Sleep disturbances are common in people with Alzheimer’s disease (AD), fragile X syndrome, autism and other neurological disorders. Accumulating evidence suggests a strong association between sleep, deposition of amyloid-beta, and AD. Actigraphy is a sensitive, noninvasive, reliable biomarker to measure rest-activity cycles. We sought to

determine if: (1) sleep deficit is a significant phenotype in J20 AD mice, and (2) metabotropic glutamate receptor 5 (mGluR5) inhibitors could rescue sleep deficit. Methods: Circadian activity levels were measured by actigraphy in J20 mice. The J20 mouse is an established rodent model for the study of AD that expresses the human amyloid protein precursor (*hAPP*) gene containing both the Swedish and Indiana familial mutations. J20 exhibit greatly exacerbated amyloid-beta production and cognitive deficits. Rest-activity rhythms were assessed under standard lighting conditions in Plexiglas chambers containing infrared sensors mounted on the underside of the lids. Mice were individually housed during actigraphy, and each gross movement of the animal was recorded as an activity count with VitalView Q4 Optical Beam software. Activity counts were binned in 60 sec epochs and scored on an activity scale (0-50). Data were analyzed with ACTIVIEW Biological Rhythm Analysis software. A Chi-square periodogram method was used to determine the diurnal rest-activity period. Results: Three independent experiments were performed on cohorts of wild type (WT) and J20 littermate mice at 8 months of age to assess the effect of genotype and season on activity levels. The J20 mice consistently exhibited increased hyperactivity with a pronounced shift in activity levels during the “lights off” phase. Specifically, the J20 mice had an approximately 4.5 hr delay in peak activity compared to WT littermates. The mGluR5 inhibitors fenobam and CTEP did not rescue hyperactivity or reverse the delay in peak activity. Conclusions: J20 mice are hyperactive during the mid to late part of the dark phase of the circadian cycle with a significant delay in peak hyperactivity that is prolonged over a 4 hr period compared to wild type littermates. This aberrant hyperactivity pattern correlates with “sundowning” in human patients with AD.

Disclosures: P.R. Westmark: None. **M.J. Filon:** None. **E. Wallace:** None. **S. Wright:** None. **R.K. Maganti:** None. **C.J. Westmark:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Angelini Pharma.

Poster

126. APP Metabolites in Alzheimer's Disease

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 126.20/D17

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: NIH Grant R01AG051086

Title: Specific microRNAs (miRNAs) play major roles in human diseases

Authors: *D. K. LAHIRI[1], R. WANG[1], B. MALONEY[1], J. BECK[3], N. CHOPRA[1], D. K. SOKOL[2], N. H. GREIG[4], K. SAMBAMURTI[5], S. E. COUNTS[3];
[1]Psychiatry, [2]Neurol., Indiana Univ. Sch. of Med., Indianapolis, IN; [3]Transl Sci. and Mol Med,

Family Med., Michigan State Univ., Grand Rapids, MI; [4]Drug Design & Develop. Section, LNS, Intramural Res. Program, Natl. Inst. On Aging, NIH, Baltimore, MD; [5]Neurosciences, MUSC, Charleston, SC

Abstract: Alzheimer's disease (AD) results from alterations of key biochemical pathways ultimately leading to classic AD pathology: brain extracellular neuritic plaques generated from aggregates of amyloid-β peptide (Aβ), cleaved from Aβ precursor protein (APP), and intracellular neuronal tangles of hyperphosphorylated microtubule-associated protein tau (MAPT). Dysregulation of proteins involved in Aβ production, such as APP and β-secretase (or BACE1), and/or Aβ degradation, such as membrane metallo-endopeptidase (MME or neprilysin), contribute to excess Aβ deposition. MicroRNAs (miRNAs) are non-coding small RNAs that typically downregulate mRNA translation via mRNA 3'-untranslated region (UTR) sequences, and, consequently, many cellular processes. Notably, miRNA expression is often dysregulated in human sporadic disorders, including AD. Recently we discovered specific miRNAs in brain tissues with altered levels in AD patients vs. controls. Our mechanistic studies revealed how specific miRNA species regulate genes involved in AD, using human primary mixed cell cultures and brain tissues from control and AD subjects. In particular, we observed regulation of APP by miR-101 and miR-153; BACE1 by miR-9 and miR-339-5p; MAPT by miR-298; and MME by miR-181d, via the respective target's mRNA-3'UTR. Our results also revealed fundamentally novel regulatory interactions. Surprisingly, we found that miR-346 uniquely *upregulates* APP levels via the APP mRNA *5'UTR* in human cultures (Long et al, *Mol. Psychiatry*-2019) and that this activity was part of iron (Fe) homeostasis. Besides AD, we have shown that APP metabolites differ in subjects with autism spectrum disorder vs typical developing (TD) controls (Ray et al *Sci Report-2016*; Sokol et al *Frontiers*-2019). In particular, the differences were a "mirror image" of those found in AD. Specifically, neuroprotective APP products (sAPPα) were elevated in ASD, and are reduced in AD, while neurodegenerative APP products (Aβ) were reduced in ASD and elevated in AD. Other groups have shown miR-101 to be *upregulated* in ASD (Vasu et al *Mol Autism*-2014), and in ASD mouse models supplementing miR-153 alleviated autism symptoms (You et al *Biosci Rep*-2019). Dysregulation of miRNAs, due to multiple factors, may cause pathological consequences. Together, these multiple unique regulatory interactions may serve as novel therapeutic targets and enable the development of treatment strategies beneficial against human diseases, including AD and ASD. We acknowledge grant supports from the National Institute on Aging (NIH).

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Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.01/D18

Topic: C.02. Alzheimer"s Disease and Other Dementias

Title: The effects of traumatic brain on behavior in double transgenic APP/tau mice

Authors: *J. M. FLINN[1], N. COSCHIGANO[2], F. BARRIENTOS[1], C. M. HERNANDEZ[1], T. DIMOPOULOS[1], T. GERVASE[1], R. BARKEY[1], A. BOOTH[1], D. CERRI[1], K. M. CRAVEN[1]; [1]Psychology, George Mason Univ., Fairfax, VA; [2]Psychology, George Mason, Fairfax, VA

Abstract: Alzheimer’s disease (AD) is a neurodegenerative disorder affecting nearly 6 million individuals in the United States alone. The hallmarks of AD include declines in spatial and episodic memory as well as the build-up of amyloid plaques and tau tangles. It is well documented that traumatic brain injury (TBI) is a risk factor for Alzheimer’s disease. However, the effect of repetitive mild TBI on adolescent AD mice has yet to be studied. This work determined the effect of repetitive mild TBI, 5 hits with 48-hour intervals, on 8-week old mice containing genes for amyloid precursor protein (APP) and tau. Behavior was assessed starting 24 hours post TBI and at 3.5 months of age. The effects on spatial memory, anxiety, and motor ability were determined. Overall Alzheimer’s mice demonstrated spatial memory deficits compared to Wildtype mice as evidenced by an increased latency to platform ($p < 0.001$), less time spent in target quadrant ($p < 0.001$), and fewer platform crosses on probe trials in Morris Water Maze ($p < 0.001$). Additionally, AD mice spend more time near the edges of the pool, indicating anxiety ($p < 0.001$). This effect is corroborated by open field results that demonstrate that AD mice spend significantly less time in the center of the open field compared to Wildtype mice ($p = 0.006$). Elevated zero results showed that AD mice subjected to rmTBI have significantly more head-dips than AD sham mice ($p = 0.01$) as well as Wildtype mice subjected to rmTBI ($p < 0.001$), demonstrating increased risk-taking behavior. Effects of rmTBI on MWM may have been masked by the very strong genotype effect. This study shows that repetitive mild TBI’s during adolescence can contribute to worse outcomes in the context of AD.

Disclosures: J.M. Flinn: None. **N. Coschigano:** None. **F. Barrientos:** None. **C.M. Hernandez:** None. **T. Dimopoulos:** None. **T. Gervase:** None. **R. Barkey:** None. **A. Booth:** None. **D. Cerri:** None. **K.M. Craven:** None.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.02/D19

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: The Kathryn Brooks Scholarship from the Oscher Lifelong Learning Institute
George Mason Department of Psychology
OSCAR Undergraduate Research Scholars Program Grant

Title: Instinctual behaviors in double transgenic young adult mice are impaired following repetitive mild TBI

Authors: *T. N. GERVASE, K. M. CRAVEN, N. T. COSCHIGANO, F. J. BARRIE, J. M. FLINN;
George Mason Univ., Fairfax, VA

Abstract: Alzheimer’s disease (AD) is the most common neurodegenerative disorder, with traumatic brain injury (TBI) as one of the known risk factors. This study observed young double transgenic (dual Tg) mice that contain genes for human amyloid and human tau. They were subjected to five days of repetitive mild TBI (rmTBI) to observe the combined effects of both AD and TBI on instinctual behaviors. There were four conditions for the mice: wildtype (WT) mice with or without rmTBI, and AD mice with or without rmTBI. The current study investigated the interactions between AD and rmTBI for nesting, burrowing and circadian rhythm (CR) patterns. All three behaviors were evaluated at two timepoints after TBI: T1 was two weeks after TBI, and T2 was 6.5 weeks after TBI. Results for nesting for both measurements show a significant effect for genotype in which AD mice scored significantly worse than wildtype mice for T1 and T2 ($p < .001$). Similar effects were observed for burrowing ($p < .001$). There was a main effect for TBI at light onset in which mice that had TBI kept running later than mice that did not ($p < .05$). There was a main effect with AD mice having significantly more activity regardless of TBI ($p < .01$). Additionally, there was an interaction between genotype and TBI for total activity between 3am and 8am (lights on at 8am) in which WT mice that had TBI showed decreased activity compared to WT mice without TBI whereas AD mice with TBI had increased activity compared to those that did not ($p = .003$). Interestingly, there was also an interaction between genotype and sex in which AD females had significantly more total activity than AD males ($p = .001$), whereas WT females had more activity, but it was not significant. An ELISA test will also be run to measure the levels of melatonin in the blood to help explain differences between genotypes. These results show the importance that TBI has on AD-type mice when evaluating instinctual behavior in three different ways: nesting, burrowing, and circadian rhythms. These results demonstrate that TBI can affect the progression of non-

cognitive behaviors of AD-type mice which can reflect similar difficulties in daily living for human populations.

Disclosures: **T.N. Gervase:** None. **K.M. Craven:** A. Employment/Salary (full or part-time):; Was a full time employee at GMU as a Graduate Teaching Assistant during this project. **N.T. Coschigano:** A. Employment/Salary (full or part-time):; Full time employee at GMU as a Graduate Teaching Assistant. **F.J. Barrie:** None. **J.M. Flinn:** A. Employment/Salary (full or part-time):; Full time employee at GMU as faculty member.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.03/D20

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: Kathryn Brooks Scholarship from the Osher Lifelong Learning Institute
George Mason University Psychology Department

Title: Traumatic brain injury increases tau hyperphosphorylation in the prefrontal cortex and hippocampus of double transgenic APP/tau mice

Authors: *R. E. BARKEY[1], K. M. CRAVEN[1], N. T. COSCHIGANO[1], C. M. HERNANDEZ[2], D. D. CERRI[1], F. J. BARRIENTOS[1], T. N. GERVASE[1], J. M. FLINN[3];
[2]Cognitive & Behavioral Neurosci., [3]Psychology, [1]George Mason Univ., Fairfax, VA

Abstract: Alzheimer’s Disease (AD) affects nearly 6 million people in the United States and is characterized by the formation of amyloid plaques and tau tangles in the brain. Traumatic brain injury (TBI) is a known risk factor for developing AD. Increases in tau protein, amyloid precursor protein (APP), and inflammatory markers are seen in TBI and may play a role in the development of cognitive deficits, similar to those seen in AD pathology. Adolescents are at an increased risk of sustaining TBI, with a higher likelihood of sustaining additional TBIs after the first one. This study subjected a dual transgenic (Tg) mouse model that displays both APP and tau pathology (J20 hAPP bred with rtg4510 from Jackson Laboratories) to a rotational repetitive mild TBI (rmTBI) paradigm to determine the effects of rmTBI on an AD mouse model. Adolescent dual Tg mice sustained 5 closed-head injuries with 48 hours between each hit, using a Controlled Cortical Impact (CCI) device on a dropping platform to produce rotational effects. A subset of mice from each group were removed for analysis of brain changes. The following significant changes were seen in AD mice with rmTBI compared to AD sham mice: 1. greater quantity of larger and more intense amyloid plaques within the hippocampus (Congo red staining/Image J), 2. an increase in phosphorylated tau and total tau (Western blot), 3. an increase in tau tangles in the prefrontal cortex (Thioflavin-S staining), and 4. relatively more

tangles in the prefrontal cortex than the hippocampus (Thioflavin-S staining). This study provides a comprehensive view of the effect of rmTBI on the progression of AD in a mouse model containing both tau and amyloid and allows for comparison of their possible interactions. IHC is currently being performed to localize the presence of amyloid-β 40, amyloid-β 42, total tau etc. to further understand of the impact of rmTBI on the progression of AD.

Disclosures: R.E. Barkey: None. **K.M. Craven:** None. **N.T. Coschigano:** None. **C.M. Hernandez:** None. **D.D. Cerri:** None. **F.J. Barrientos:** None. **T.N. Gervase:** None. **J.M. Flinn:** None.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.04/D21

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG025493 to RY
NIH Grant NS074256 to RY
NIH Grant RFAG058261 to RY
NIH Grant AG046929 to RY
AD Association Grant AARF-17–504724 to MGS

Title: Accumulation of lysosomal vesicles and ferritin in dystrophic neurites and their selective degradation in Alzheimer’s disease mice brain

Authors: *M. SHAROAR, X. HU, R. YAN;
Neurosciences, Univ. of Connecticut Hlth. Ctr., Farmington, CT

Abstract: Dystrophic neurites (DNs) is a component of neuritic plaque and its formation is a distinguishing feature of Alzheimer’s disease (AD) patients’ brain. Recently, we have shown that DNs in AD mice brain are formed in three sequential layers with accumulation of distinct proteins and organelles. A preautophagy protein, ATG9A and mature autophagy proteins, LC3 and RAB7 as well as tubular endoplasmic reticulum (ER) proteins are involved in forming DNs. However, it was not clear whether lysosomes are actively involved in forming DNs. To reveal how lysosomes are involved in forming DNs during plaque growth, we utilized different ages of APP-knock in (NL-G-F) and 5xFAD mice and investigated the functional and non-functional lysosomes accumulation in DNs. We found that LAMP1 and LAMP2 stained lysosomal vesicles, those devoid of cathepsin B and D, massively accumulates during the formation of DNs by ATG9A. An iron storage protein ferritin was also enormously accumulated at this stage and it was largely colocalized with ATG9A and LAMP1. We observed that a few microglia are accumulates near the plaque and located outside of first layer of DNs during initial plaque

development. At later stage, a circle structure with many microglia were formed at the inner side of the first layer of DNs and they encapsulate β-amyloid deposited core. Interestingly, both LAMP1 and ferritin staining were reduced at a later stage of plaque development in older AD mouse brains, but their reminiscences were largely colocalized with microglia marker IBA1 and cathepsin B and D. The intensity of ATG9A- and RTN3- marked DNs were increased during plaque growth but rarely colocalized with LAMP1, IBA 1 or astrocytes markerd in older mouse brains. Our results suggest that dysfunctional lysosomes and ferritin are massively accumulated in DNs at initial stage, but they were degraded at the later stage of neuritic plaque development. We are investigating whether microglia have a role in degrading those DNs in AD brains.

Disclosures: **M. Sharoar:** None. **X. Hu:** None. **R. Yan:** None.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.05/D22

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: VA Merit Review

Title: Metabolic syndrome exacerbates amyloid pathology in a novel comorbid mouse model by decreasing the expression of insulin degrading enzyme

Authors: *S. PUGAZHENTHI[1,2], A. TYAGI[1,2];
[1]Rocky Mountain Regional VA Med. Ctr., Aurora, CO; [2]Univ. of Colorado-Anschutz Med. Campus, Aurora, CO

Abstract: Alzheimer’s disease (AD) often coexists with other aging-associated diseases including obesity, diabetes, hypertension, and cardiovascular diseases. The early stage of these comorbidities is known as metabolic syndrome (MetS) which is highly prevalent in mid-life. An important cause of MetS is the deficiency of SIRT3, a mitochondrial deacetylase which enhances the functions of critical mitochondrial proteins by deacetylation. Global deletion of Sirt3 leads to acceleration of MetS. In a recently published study, we demonstrated in the brain of $Sirt3^{-/-}$ mice (i) downregulation of metabolic enzymes by hyperacetylation, (ii) decreased brain mitochondrial respiration and (iii) formation of inflammasome. These findings suggested a novel pathway that could link mitochondrial hyperacetylation to neuroinflammation, an important cause of Alzheimer’s pathogenesis. Therefore, we hypothesized that MetS and amyloid pathology interact through converging pathways of metabolic dysregulation and neuroinflammation in comorbid AD. To investigate these interactions, we crossed $Sirt3^{-/-}$ mice with APP/PS1 mice and successfully generated APP/PS1/$Sirt3^{-/-}$ mouse model with MetS and amyloid pathology. We observed exacerbation of insulin resistance, amyloid plaque deposition, markers of

neuroinflammation including elevated expression of IL-1β, TNF-α and cox-2 and microglial proliferation in both male and female mice of the comorbid AD model. To further investigate the converging pathways, induced by MetS and amyloid pathology, we performed RNA-seq analysis with the brain samples. The heat map of cluster analysis, Venn diagrams and Volcano plots showed distinct changes in gene expression patterns as a result of Sirt3 gene silencing in APP/PS1/Sirt3$^{-/-}$ mouse brain. GO and KEGG pathway analysis of modulated genes revealed novel interactions between metabolic dysregulation and inflammatory pathways. A key finding was that Sirt3-deletion decreased the expression of insulin degrading enzyme (IDE) which plays an important role in Abeta clearance by ~50%. Our observations suggest a novel mechanism by which MetS may exacerbate amyloid pathology during the cellular phase of AD. Therapeutic targeting of SIRT3 in AD with comorbidities may produce beneficial effects when combined with treatments that reduce amyloid pathology.

Disclosures: S. Pugazhenthi: None. **A. Tyagi:** None.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.06/D23

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: KAKEN Grant-in-Aid for scientific research (B) 17H04193

Title: Soluble high-molecular weight oligomers of amyloid-beta peptide derived from the brains of APP transgenic mice induce cerebral amyloid-beta deposits

Authors: *T. HASHIMOTO[1], Y. NAKA[2], M. HAKOZAKI-KASHIWAGI[2], T. TAJIRI[2], T. IWATSUBO[2];
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Abstract: Spatiotemporal spreading of deposition of amyloid beta peptides (Abeta) as senile plaques is a key pathogenic process in the brains of patients with Alzheimer’s disease; however, the molecular characteristics of Abeta strains that initiate the Abeta spreading *in vivo*are not fully understood. To identify the Abeta strains responsible for the deposition and spreading of Abeta *in vivo*, we extracted the Tris-buffered saline (TBS)-soluble fractions from the brains of 18-month-old APP transgenic (tg) mice (A7 line) by size-exclusion chromatography using a Superdex 75 column, and found that the Abeta -positive peaks detected by an Abeta42-specific ELISA in the TBS-soluble fractions were separated into three fractions, eluting at ~200-250 kDa (peak 1 Abeta), ~50-70 kDa (peak 2 Abeta) and ~10-20 kDa (peak 3 Abeta). To examine whether these Abeta strains are capable of propagating Abeta deposition *in vivo*, we inoculated

peak 1 or peak 2 Abeta into the hippocampus of 9-month-old APP tg mice, and found that peak 1 Abeta induced a characteristic pattern of Abeta propagation in the hippocampus after 4 months, whereas peak 2 Abeta did not. We also found that immunodepletion of peak 1 Abeta with anti-Abeta antibodies completely abolished the Abeta propagation induced by inoculation of peak 1 Abeta. These results suggested that the Abeta strains in peak 1 Abeta play a role as "aggregation seeds" *in vivo*. To further analyze the biological properties of peak 1 Abeta, we subjected the peak 1 Abeta to single antibody sandwich ELISAs using BAN50 or 82E1, and found that peak 1 Abeta contains Abeta oligomers. Furthermore, we found that the Abeta42levels in peak 1 Abeta quantified by ELISA positively correlated well with the percentage of Abeta-positive areas in the hippocampus, which led us to speculate that the peak 1 Abeta is associated with Abeta plaques. We showed that the soluble high-molecular weight Abeta oligomers of ~250-300 kDa in size (peak 1 Abeta) is the strain responsible for the induction of propagation of Abeta deposition, possibly through conformational changes, in the brains of APP tg mice. The findings may provide clues to the mechanism of spatiotemporal spreading of Abeta in the brain as well as therapeutic strategies to prevent Abeta deposition.

Disclosures: T. Hashimoto: None. **Y. Naka:** None. **M. Hakozaki-Kashiwagi:** None. **T. Tajiri:** None. **T. Iwatsubo:** None.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.07/D24

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Vanech Family Foundation

Title: Intravesicular retention of amyloid beta aggregates precedes plaque formation and changes in extracellular amyloid beta levels

Authors: E. A. ECKMAN, D. CLAUSEN, *J. PACHECO-QUINTO;
Biomed. Res. Inst. of New Jersey, Cedar Knolls, NJ

Abstract: Introduction: In Alzheimer's disease (AD), while insoluble beta-amyloid (Aβ)42 aggregates accumulate massively throughout the brain, levels of soluble Aβ42 progressively drop in CSF. Microdialysis studies have demonstrated that CSF levels directly reflect the dynamics of interstitial Aβ and, in AD mouse models, interstitial Aβ also decreases at the onset of plaque formation. By implementing a method to quantify intravesicular Aβ in brain, even under conditions of high Aβ deposition, we sought to establish whether reduced levels of soluble Aβ are associated with intraneuronal disturbances in Aβ metabolism.
Methods: To isolate intracellular vesicles, hemi-brains from TgCRND8 mice (1-6 months old,

both male and female) were homogenized in 0.32 M sucrose in HEPES buffer without detergents. Homogenates were cleared of cell debris and intracellular vesicles were then pelleted by centrifugation at 10,000 x g, washed to remove extracellular Aβ, and extracted in RIPA buffer for quantification of intravesicular Aβ. To validate that Aβ measurements were not artifactually influenced by Aβ binding to vesicles during the extraction process, intracellular vesicle preparations from wild type mice were incubated with Aβ extracts from a mouse with high Aβ burden, and analyzed in parallel. Size exclusion chromatography was used as an alternative to centrifugation for separation of intracellular vesicles from other Aβ-containing fractions. Levels of Aβ40, Aβ42 and Aβ oligomers were quantified by sandwich ELISA. Amyloid load was quantified in the other brain hemisphere by immunostaining followed by image analysis. **Results**: We found a progressive intraneuronal build-up of Aβ42 and Aβ oligomers before the onset of plaque formation, and before any significant changes in extracellular Aβ species were apparent. During the progression of Aβ deposition, intravesicular Aβ, especially aggregated species, increased exponentially. Our results support that intracellular retention of Aβ before secretion promotes the formation of intracellular aggregates and may contribute to decreased CSF Aβ levels.

Disclosures: E.A. Eckman: None. **D. Clausen:** None. **J. Pacheco-Quinto:** None.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.08/DP04/D25

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Blockparty the effects of connected cages on nesting behavior in APP Alzheimer's mice

Authors: *K. A. PEDEMONTE, R. BARKEY, J. M. FLINN;
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Abstract: Alzheimer’s disease (AD) is a major neurodegenerative disease characterized by the accumulation of two proteins in the brain: amyloid-β and tau. These protein accumulations lead to progressive cognitive and behavioral impairments including in activities of daily living (ADL). ADLs can be examined in mice by testing nest building behavior, an innate behavior in mice. Transgenic AD mice build worse nests than wildtype mice. Although, it’s not clear at what age nest building starts to deteriorate in AD mice. In current models of nest building rodents are housed individually in the testing cage, which has been seen to cause stress in animals. Animal Care Systems has recently released new cages called Blockparty, which are the same size as normal mouse cages, but allow for multiple cages to be connected by a tube. The present study

focused on the effects of Blockparty cages and group housing on nest building. Preliminary research on 5-8-month J20/hAPP transgenic mice with APP pathology (Jackson Laboratory) examined nest building behavior after 2, 12 and 24 hours when communally housed in Blockparty cages. It was found that the AD mice, which normally perform worse in individually housed nesting paradigms, are better able to build nests when communally housed in Blockparty cages. Impaired behavior in lab mice may be significantly ameliorated by the environment and could explain experimental differences.

Disclosures: K.A. Pedemonte: None. **R. Barkey:** None. **J.M. Flinn:** None.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.09/D26

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant DA044242
NIH grant U54AG054345

Title: Early emergence of impaired spatial learning and memory function in male TgF344-AD rats

Authors: *Z. DING[1,2], Y. ZHANG[1], A. M. SENTIR[1], C. M. INGRAHAM[3], B. T. LAMB[3,2], A. L. OBLAK[4,2];
[1]Psychiatry, [2]Paul & Carol Stark Neurosci. Res. Inst., [3]Med. and Mol. Genet., [4]Radiology and Imaging Sci., Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Alzheimer’s disease (AD) is associated with various neuropsychiatric symptoms, including motor disturbance and cognitive dysfunction. TgF344-AD rats express human APP with the Swedish mutation and human PSEN1 with the Δ exon 9 mutation. These rats develop amyloid pathology as early as 6 months and have been shown to exhibit heightened locomotor activity (LMA) and impaired cognitive function at late stage (~ 15 months). However, such dysfunctions have not been adequately studied at early stage with the emergence of AD pathology. The current study examined potential early motor and cognitive alterations in TgF344-AD rats. Male TgF344-AD rats and wild-type (WT) F344 rats (n=15-18/genotype; ~ 6.4 months old) were assessed for basal LMA in standard open field arenas for 9 daily 1-hr sessions with ambulatory distance recorded and analyzed. Rats were then tested for spatial learning and memory in a standard radial arm maze (RAM) for 21 daily sessions. Performance in RAM was reinforced by a piece of Froot Loops at the distal end of each arm. Three primary parameters were assayed: (1) entries-to-repeat (ETR) defined as the total number of arm entries before making an error by repeating an arm entry, (2) session time defined as either the maximum of

300 seconds or time during which rats finish entry to all arms, whichever comes first, and (3) Froot Loops consumed. TgF344-AD and WT rats did not differ in ambulatory distance travelled either during the 1st session or across all sessions (p = 0.28; ~ 1700 vs 1500 cm/hr). WT rats achieved significantly greater numbers of ETR during sessions 11 to 21 than AD rats (p = 0.000, ~ 6 vs 3/session). WT rats completed sessions significantly faster than AD rats during sessions 13-21 (p = 0.000, ~ 200 vs 280 s/session). In addition, WT rats consumed significantly more Froot Loops than AD rats during sessions 11-21 (p = 0.001, ~ 7 vs 3/session). These results indicate that TgF344-AD rats display impaired spatial learning and working memory function at early stage corresponding to the emergence of AD-specific brain pathology. However, there is no altered motor activity at this stage, suggesting that the development of motor disturbance in these rats may be age dependent.

Disclosures: Z. Ding: None. **Y. Zhang:** None. **A.M. Sentir:** None. **C.M. Ingraham:** None. **B.T. Lamb:** None. **A.L. Oblak:** None.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.10/D27

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Evidence of differential expression of AD related proteins in post natal stages of rat brain regions provide clue on SAD progression

Authors: *A. JAYACHANDRAN[1], M. PONNUSAMY[1], J. K. SWAMINATHAN[2]; [1]Biochem., [2]Bioinformatics, Bharathidasan Univ., Tiruchirappalli, India

Abstract: Research through decades investigate the molecular mechanism behind SAD pathology, however, the rationale for SAD progression is still under quest. AD related proteins are present even during early stages of life, though they have never led to the formation of SAD pathology. Therefore it is noteworthy to perform expression profile of AD related proteins in various regions of rat brain during post natal developmental stages that could pave way in understanding at what stage of life does these proteins enter into amyloidogenic phase leading to formation of pathological hallmarks of SAD condition. Therefore, analyzing the expression profile of key AD related proteins during postnatal developmental stages of rat brain could help us identify the role of vital AD proteins during the process of aging. Immunoblotting analysis of AD proteins viz., APP, BACE1, NCT, PS-1, PS-2, PEN2, Tau, GSK3α/β, AMPA receptors (GluR1-4, pGluR1 & pGluR2) in P30 day and P24 months rat brain regions (olfactory-bulb, frontal-cortex, parietal-cortex, temporal-cortex, occipital-cortex, hippocampus, cerebellum, hypothalamus-thalamus and pons-medulla-oblongata) were performed. Further, Immunofluorescence study was utilized to analyze the ADAM10 and BACE1 expression for

analyzing its sub-regional distribution. Immunoblotting results depicted that AD related proteins are highly expressed in P30 day samples and differ in their expression levels in brain regions. Particularly, pyramidal cell rich regions have abundant AD related proteins compared to other cell types. Interestingly, GluR4 in particular was only seen in cerebellum. On other hand, ADAM10 was uniformly observed in all the regions. However, immunofluorescence results revealed that significant variation in regional distribution of ADAM10 in different brain regions was observed. In specific, higher expression of ADAM10 in neocortex region was seen, whereas lesser expression was observed in the hippocampus and other regions. These results clearly demonstrate that AD related proteins were effectively found in entire brain regions, while ADAM10 was effectively found in grey matter and is perhaps due to its effective role in axonal growth and myelination of neuron. Further insights into the understanding of all vital proteins in various stages of life could help understand SAD progression.

Disclosures: **A. Jayachandran:** None. **M. Ponnusamy:** None. **J.K. Swaminathan:** None.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.11/D28

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA grant AG053524
NIA grant AG051753

Title: Episodic memory in a transgenic rat model of Alzheimer's disease

Authors: *D. PANOZ-BROWN[1], L. M. CAREY, IV[2], A. G. HOHMANN[2], J. D. CRYSTAL[3]; [1]Psychological & Brain Sci., [3]Dept Psychological and Brain Sci., [2]Indiana Univ., Bloomington, IN

Abstract: Alzheimer’s Disease (AD) is a neurodegenerative disease of aging characterized by profound impairments in episodic memory. Episodic memory has been characterized as autobiographical memory consisting of unique personal past events and experiences. Currently, we lack an animal model of AD that captures these critical features of episodic memory impairment exhibited by AD patients. Documenting episodic memory impairment in preclinical models of AD would promote therapeutic strategies that specifically target episodic memory function. The TgF344-AD (Tg-AD) rat is a transgenic Alzheimer model developed to express mutant human amyloid precursor protein (APPsw) and presenilin 1(PS1ΔE9) genes, each independent causes of familial AD. Tg-AD rats exhibit progressive increases in neuropathological changes as well as age-dependent deficits in spatial learning and memory. However, it is currently unknown if Tg-AD rats (1) have intact episodic memory function at

timepoints that precede advanced neuropathology development and (2) exhibit age-dependent episodic memory loss relative to wildtype littermate controls (WT). The current study was conducted to evaluate the presence of age-dependent episodic memory deficits in Tg-AD and WT rats using a novel behavioral assessment of episodic memory developed in our laboratory. Our approach documents that rats use episodic memory to remember multiple unique events and the context in which these events were encountered (items-in-context approach) while controlling for non-episodic memory, odor perception, and other non-specific factors. The goal of this research is to develop a preclinical model of AD episodic-memory impairment and characterize neuropathological correlates of cognitive decline. Here we show that young Tg-AD and WT rats remember multiple unique events and the contexts in which they occurred using episodic memory. When the memory of item in context was put in conflict with non-episodic familiarity cues, young Tg-AD and WT rats relied on item in context using episodic memory. Our findings suggest that episodic memory is intact in young Tg-AD and WT rats and support the hypothesis that intact episodic memory function precedes pathology-induced cognitive decline. Longitudinal studies elucidating episodic and non-episodic memory performance together with pathological AD markers in behaviorally trained Tg-AD and WT rats at middle and old age timepoints may provide new insights into AD-specific impairments.

Disclosures: D. Panoz-Brown: None. **J.D. Crystal:** None. **A.G. Hohmann:** None. **L.M. Carey:** None.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.12/D29

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 2R44AG043203-03
R44 NS086343-04
R43HL142315-01

Title: Tricyclic pyrone CP2 improves long term memory in the TgF344 AD rat of an Alzheimer's disease model

Authors: *B. ZOU[1], W. CAO[1], C. PASCUAL[1], K. ERICKSON[2], H. FAN[3], Z. TONG[3], A. V. D. VLIES[3], I. MAEZAWA[2], L.-W. JIN[2], D. H. HUA[3], X. XIE[1];
[1]AfaSci Res. Labs., Redwood City, CA; [2]Dept. of Pathology and Lab. Med., Univ. of California Davis, Sacramento, CA; [3]Dept. of Chem., Kansas State Univ., Manhattan, KS

Abstract: Alzheimer’s disease is a devastating neurological disease still lacking effective treatments. We recently reported that our novel tricyclic pyrone compound CP2 reduced both

intraneuronal and extracellular Aβ aggregates as well as hyperphosphorylated tau, restored axonal trafficking, and modulated hippocampal NMDA-mediated synaptic activity and plasticity. These multiple synergistic cellular actions, rather than anti-Aβ toxicity alone, can produce greater efficacy *in vivo*. To test this hypothesis, we started with characterization of a transgenic rat model, TgF344-AD which expresses mutant human amyloid precursor protein and presenilin 1 genes. TgF344-AD rats exhibited decreases in homecage activity, locomotion and rearing activity compared to non-transgenic (non-Tg) rats monitored by the SmartCage under normal light/dark condition. More relevant to the AD phenotype, Tg344-AD rats displayed learning and memory deficits using conventional water maze test and step-through passive avoidance test. During water maze test training, latency to find the underwater hidden platform was slightly longer in TgF344-AD rats compared to non-Tg ones. After 5 days training both genotypes had a similar latency and the probe test started. TgF344-AD rats spent significantly less time in the probe test to search for the hidden platform in the target quadrant. This long-term memory deficit in TgF344-AD rats improved after treatment with CP2 through drinking water (6-8 mg/kg/day) for 40 days compared to non-Tg rats. However, memory flexibility and short-term memory deficits were not improved by CP2 treatment. During the 4 repetitive probing tests, non-Tg rats progressively decreased probe time to search the target quadrant. By the 3rd test non-Tg rats spent the same probe time as TgF344-AD rats. The platform was re-introduced and rats were placed on the platform for 20s and then put into another quadrant to look for the missing platform. Non-Tg rats exhibited significantly more time to reach the platform in comparison with TgF344-AD rats. Impaired memory flexibility and short-term memory deficits are more pronounced and refractory to all current treatments in AD patients. Thus far we have explored a new test paradigm in TgF344-AD rats and can be employed to identify more effective therapeutics. After the water maze test, rats were subjected to mild foot shock experiments (70V, 6s) using the SmartCage as training. After 2 weeks, TgF344-AD male rats showed a lack of passive avoidance significantly ($p<0.05$) indicated by a shorter first-entry latency into the dark box. Effects of drug treatment on the cognitive function and brain pathology are ongoing.

Disclosures: B. Zou: None. **W. Cao:** None. **C. Pascual:** None. **K. Erickson:** None. **H. Fan:** None. **Z. Tong:** None. **A.V.D. Vlies:** None. **I. Maezawa:** None. **L. Jin:** None. **D.H. Hua:** None. **X. Xie:** None.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.13/D30

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Application of amyloid-beta oligomer to *Aplysia* buccal ganglia suppressed the feeding behavior

Authors: *T. NAGAHAMA[1], T. MINAGAWA[2], H. NAKAJIMA[2], M. TOKORO[2], M. WAKUTA[2], S. NAGAHAMA[3];
[1]Teikyo Heisei Univ/ Fac Hlth. Med. Sci., Tokyo, Japan; [2]Fac Phar Sci, Toho Univ., Fubnabashi, Japan; [3]Human Care, Teikyo Heisei Univ., Tokyo, Japan

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder affecting cognitive functions. It is widely accepted that the accumulation of amyloid-β peptide (Aβ) is a major cause of the pathogenesis of disease. Moreover, eating disorder is often reported in the AD patients. In the preliminary experiments, we injected Aβ 1-42 monomer (m-Aβ) into the *Aplysia* body cavity as a final concentration was 300 nM in the body fluid and the effect on the food intake was explored. The amount of food consumed was obtained by measuring the seaweed that remained in the cage at 2 or 4 hours after placing the seaweed in an individual animal cage every morning. Food intake was quantified as the percentage of body mass. The beginning of stable seaweed consumption determined the start of experiments. M-Aβ was applied on the fourth day (single application), or the fourth and sixth days (double application) after the start of experiments. In a single application, the amount of food intake decreased only on the next day after application. On the other hand, double application significantly diminished the amount of the food intake for five days at least after the first application. These results suggest that sufficient amount of m-Aβ may aggregate on the cell surface of *Aplysia* brain and reduced amount of food intake. In order to ascertain it, we next applied Aβ 1-42 oligomer (o-Aβ) directly on the surface of the buccal ganglia by surgical treatment on the fourth day after the start of experiments. Then small pieces of filter paper soaked in 1 or 3 μM o-Aβ solution (as monomer concentrations) were placed on the bilateral buccal ganglia for 1 hour, with exchanging the pieces for the fresh ones every 10 min. For the behavioral experiments the similar methods as before were performed. Application of 3 μM o-Aβ significantly diminished the amount of food intake for five days at least after the treatment while application of 1 μM o-Aβ scarcely affected it. These results suggest that application of sufficient amount of o-Aβ to the buccal ganglia caused decline of the food intake by affecting the feeding neural circuit and the synaptic dysfunction caused by o-Aβ may be useful for understanding of the behavioral disorder in AD.
Acknowledgment: We wish to thank Misses M. Hayashi and K. Imai for their helpful early experiments searching skillful techniques of Aβ application to the buccal ganglia by surgical treatment.

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Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.14/D31

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG057355

Title: APP-induced patterned neurodegeneration is exacerbated by APOE4 in *C. elegans*

Authors: *L. BROSE[1], W. SAE-LEE[1], L. L. SCOTT[2], T. SHI[1], J. T. PIERCE[1];
[1]Neurosci., [2]Waggoner Ctr. for Alcohol and Addiction Res., Univ. of Texas at Austin, Austin, TX

Abstract: Variations in the age of onset and progression of Alzheimer's disease (AD) has been linked to genetic variants in the amyloid precursor protein (*APP*) and apolipoprotein E (*APOE*). Both an extra copy or any number of gain-of-function mutations in *APP* considerably lower the age of AD onset. Additionally, the ε4 allele of *APOE* (*APOE4*) hastens and exacerbates the progression of early and late onset forms of AD compared to the other isoforms (*APOE3* and *APOE2*). Current *in vivo* models to study the interactions between APP and APOE4 to influence neurodegeneration are lacking. Previous studies in our lab have shown that expression of human *APP* induces specific, age-related neurodegeneration in the nematode *C. elegans*. Here we have genetically engineered worms to express both *APP* and *APOE4*. Live fluorescent microscopy confirms that APOE4 (but not APOE3) acts synergistically with APP to hasten and expand the pattern of cholinergic neurodegeneration caused by APP. Further, we show that APOE4 (but not APOE3) specifically affects a salient behavior, resulting in the phenotype bag-of-worms. However, motor coordination remains normal. The age-related decline and severity of egg laying correlates with degeneration of identified neurons critical for this behavior. We next seek to determine the specific pathways involved in neuronal cell death in the presence of APP and APOE4. This convenient worm model of neurodegeneration can further be used to determine the molecular mechanisms and cell-specific interactions underlying how APP and APOE4 function in both degenerating and intact neurons.

Disclosures: L. Brose: None. **W. Sae-Lee:** None. **L.L. Scott:** None. **T. Shi:** None. **J.T. Pierce:** None.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.15/D32

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 1 R15 NS101608-01A1

Title: Investigating a potential interaction between the chromatin remodeling protein kismet and APP in a *Drosophila* model of Alzheimer's disease

Authors: *N. L. LINSKEY, E. L. HENDRICKS, F. L. W. LIEBL;
Biol., Southern Illinois Univ. Edwardsville, Edwardsville, IL

Abstract: Alzheimer's disease is characterized by an abundance of plaques formed from amyloid β (Aβ) peptides. These plaques result in a loss of neurons and synaptic connections, which leads to impaired memory and places Alzheimer's disease as a leading cause of dementia in geriatric patients. The cell adhesion molecule amyloid precursor protein (APP) can be processed by β-site APP-cleaving enzyme (BACE). BACE cleaves APP and, through a series of other enzymatic functions, ultimately causes Aβ to be released from the plasma membrane to aggregate and form plaques. Overexpression of human APP and BACE1 in *Drosophila* larval motor neurons is used as a synaptic model for late onset Alzheimer's disease. The *Drosophila* protein Kismet (Kis), which is homologous to the chromatin remodeling enzymes CHD7 and CHD8, positively regulates the localization of synaptic cell adhesion molecules and BMP signaling. *APP; BACE1* overexpression in motor neurons leads to significant reductions in miniature endplate junctional current (mEJC) amplitudes similar to *kis* mutants. To investigate a potential connection between Kis and APP, we will examine the role of Kis in the regulation of synaptic APP-like expression and analyze neuroligin transcript levels in *kis* mutants and *APP; BACE1* overexpressing larvae. We will also compare BMP signaling after overexpressing *APP; BACE1* to the increased BMP signaling observed in *kis* mutants, and analyze endocytic function in both genotypes by testing the levels of calcium channel subunit transcripts.

Disclosures: N.L. Linskey: None. **E.L. Hendricks:** None. **F.L.W. Liebl:** None.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.16/D33

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: University of Auckland Doctoral Scholarship
Brain Research New Zealand Grant

Title: Progress towards creating a sheep (*Ovis aries*) model of Alzheimer's disease

Authors: *N. E. MCKEAN[1], R. G. SNELL[2], R. HANDLEY[2], H. WALDVOGEL[3], R. L. M. FAULL[3], S. BAWDEN[4], C. MCLAUGHLAN[4], H. ZETTERBERG[5], J. HARDY[6], J. GUSELLA[7], J. LIU[8];
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Abstract: Animal models of Alzheimer's disease (AD) have so far failed to provide the much sought after cure or prevention method that has been hoped for. One of the biggest problems has been translatability between the rodent models in which therapeutic compounds are tested and clinical trials. A number of different mutations have to be over-expressed in rodent models in order to generate a complete AD-like phenotype, some of which are not found in human AD. This leads to questions about the validity of rodent models and more valid models have recently been called for. This project aims to create a sheep (*Ovis aries*) model of Alzheimer's disease using CRISPR-Cas9 editing to introduce a single point mutation into the PSEN1 gene that will cause the E280A substitution found in the largest cohort of Familial AD patients. Sheep have much larger brains than rodents and our previous research has shown that plaques and tangles, which are the hallmark of AD, develop naturally in these animals with age. Sheep have a longer lifespan and as the gene will be expressed under its natural promoter it will most likely reflect the normal biological function of this allele in human patients. This will provide a more valid and robust model for which therapeutic agents can be tested prior to human clinical trials. After trialing several CRISPR-Cas9 methods we now have edited embryos heterozygous for the E280A substitution that have been implanted. We are in the process of generating more will start characterizing the animals after birth later this year. We are trialing sex-determination methods that can be used prior to implantation to preferentially generate ewes as our founder animals, as our reproductive technology enables the taking of oocytes from ewe lambs six weeks after birth to create a large number of animals for the next generation. The methods we have used so far and current results will be presented, including our studies in wild type sheep that confirm the suitability of sheep as a model of neurodegenerative disorders.

Disclosures: **N.E. McKean:** None. **R.G. Snell:** None. **R. Handley:** None. **H. Waldvogel:** None. **R.L.M. Faull:** None. **S. Bawden:** None. **C. McLaughlan:** None. **H. Zetterberg:** None. **J. Hardy:** None. **J. Gusella:** None. **J. Liu:** None.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.17/D34

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R43-OD023025

Title: Progress in Alzheimer's disease modeling: Intrathecal administration of amyloid-beta oligomers in the African green monkey

Authors: ***J. D. ELSWORTH**[1], M. R. WEED[1], A. M. KURIAN[1], M. S. LAWRENCE[1], S. E. PEREZ[2], E. J. MUFSON[2], E. N. CLINE[3], K. L. VIOLA[3], W. L. KLEIN[3], D. R. WAKEMAN[1];

[1]RxGen Inc, New Haven, CT; [2]Neurobio., Barrow Neurolog. Inst., Phoenix, AZ; [3]Neurobio., Northwestern Univ., Evanston, IL

Abstract: A principal obstacle to developing new treatment strategies for Alzheimer's disease (AD) is the inadequacy of available preclinical models to predict clinical outcomes. While transgenic mice are the mainstay of AD animal research, their shortcomings have likely contributed to the lack of clinical efficacy of promising candidate interventions identified in such models. As nonhuman primate (NHP) neurobiology shares unparalleled homology to humans in virtually all respects relevant to AD modeling, we have been refining an inducible NHP model of AD based on administration of soluble amyloid-beta oligomers (ABOs) in the African green monkey, a species that is free of major primate pathogens and naturally develop pathological features of AD. ABOs play a major role in provoking an AD-like pathological cascade that includes induction of phosphorylated tau (p-tau), leading to neurofibrillary tangles, amyloid plaques, loss of synapses and neurons, inflammation and cognitive decline. To learn more about the parameters determining ABO-induced pathology in the model, we have evaluated the impact of several critical experimental factors and expanded the range of endpoint measures. Rather than delivering ABOs by repeated intraparenchymal or ICV injections, we employ intrathecal (IT) administration of ABO using a subcutaneous access port to permit delivery to CSF. The latter procedure has the benefit of involving a less invasive and more reproducible surgery. We determined that IT administration of ABOs to awake, chair-trained monkeys induced a more reliable induction of AD-like pathology than dosing in ketamine-sedated monkeys, and attribute this to improved movement of oligomers in CSF. IT administration of 200 micrograms of standardized ABO, but not vehicle, 3-times a week for 4 weeks to young adult NHPs induces significantly elevated expression of p-tau in the medial temporal cortical memory circuit (e.g., hippocampus and entorhinal cortex), which persists for at least a month after the last injection. Immunostaining revealed the emergence of neuropil amyloid precursor protein aggregations with an increase in microgliosis in hippocampus and entorhinal cortex following ABO administration. MRI revealed a significant loss of hippocampus volume at 4 weeks after ABO injections compared with baseline measurements. In this NHP model, ABOs induce pathological features similar to those seen in sporadic AD and should be a valuable resource to advance our understanding of AD pathogenesis and enable testing of novel diagnostic and therapeutic strategies in a manner that will significantly de-risk clinical trials.

Disclosures: J.D. Elsworth: None. **M.R. Weed:** None. **A.M. Kurian:** None. **M.S. Lawrence:** None. **S.E. Perez:** None. **E.J. Mufson:** None. **E.N. Cline:** None. **K.L. Viola:** None. **W.L. Klein:** None. **D.R. Wakeman:** None.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.18/D35

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CONACyT Support

Title: Effect of a fiber-enriched diet in the gut microbiota of app/ps1 transgenic and wild type mice: Gender differences

Authors: *D. CUERVO;
Cinvestav, Mexico, Mexico

Abstract: Introduction: Alzheimer´s disease (AD) is an age-related dementia characterized by memory loss and cognitive impairment[1], with more prevalence in female subjects[2]. Recent attention has been paid to the relationship between soluble fiber intake and lower incidence of AD[3]. Moreover, fiber fed rodents present different gut microbiota (GM) composition[4] and improved cognitive performance[3] compared to control fed animals. However, up to day there is no data regarding gender differences nor on the impact of a fiber-enriched diet on GM of AD transgenic (Tg) mice.
Methods: Male and female wild type (Wt) and Tg 4-months old mice were fed for 2 months with control diet (AIN-93), AIN-93 + 5% fructans (5%-F) and 5%-F + antibiotic cocktail (ampicillin [1 g/l], neomycin [1 g/l], metronidazole [1 g/l], and vancomycin [0.5 g/l], diluted in drinking water [5%-F+Abx]). DNA was extracted from 200 mg of feces and 16S rDNA library was processed by Ion Torrent Semiconductor DNA massive sequencing. Microbial diversity was assessed through relative abundance, alpha diversity, beta diversity and linear discriminant analysis.
Results: Fiber-enriched diet increased Clostridiales order in male animals and Acetobacteraceae family in female animals [ANOVA, $p<0.01$] compared to AIN-93 fed animals, while *Oscillospira* and *Ruminicoccus* genders differed between male and female 5%-F fed animals [t-test, $p<0.01$]. There was an increased alpha-diversity in male and female Tg 5%-F fed animals compared to Tg AIN-93 [ANOVA, $p<0.05$] and Tg 5%-F+Abx fed animals [ANOVA, $p<0.01$]. In addition, 5%-F diet also increased Bacteroidetes phylum [ANOVA, alpha=0.01] compared to AIN-93 group. These changes were due to higher relative abundance of S24-7 family [ANOVA, $p<0.01$] and lower of *Lactobacillus* gender [ANOVA, $p<0.01$].
Conclusion: There were gender specific GM differences in Tg mice. Fiber-enriched diet restored Tg´s GM alterations to control values. Fiber-enriched diet favored a more diverse GM, perhaps related to lower incidence of AD.
References: [1]Jahn, (2013) DCN, 15:445; [2]Herbert et al. (2013) Neurology, 80:1778; [3]Syeda et al., (2013) JAD, 66: 1657, [4]Sánchez et al., (2017) SR, 7:4716.

Disclosures: D. Cuervo: None.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.19/D36

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AMED Grant JP19dm0107056

Title: Reversible effects of metabolic improvement on high-fat diet-induced amyloid accumulation in a mouse model of Alzheimer's disease

Authors: T. OCHIAI[1,3], T. WAKABAYASHI[1,2], K. YAMAGUCHI[1], K. MATSUI[1], T. SANO[1], *T. IWATSUBO[1];
[1]Dept. of Neuropathology, [2]Dept. of Innovative Dementia Prevention, Grad. Sch. of Medicine, The Univ. of Tokyo, Tokyo, Japan; [3]Pharmacol. Dept., Drug Res. Center, Kaken Pharmaceut. Co., LTD., Kyoto, Japan

Abstract: Deposition of amyloid-β peptides (Aβ) as senile plaques in the brain is the hallmark pathology of Alzheimer's disease (AD), and accumulation of Aβ is believed to be the primary cause of neurodegeneration in AD brains. Many epidemiological and experimental studies have indicated that type 2 diabetes mellitus (T2DM) is a risk factor of AD. In studies using mouse models of AD, high-fat diet (HFD) feeding causes insulin resistance, systemic diabetic phenotypes, and exacerbates amyloid pathology. Previous reports suggested that postmortem brain tissues from patients with AD show signs of insulin resistance. Thus, insulin resistance has been considered as a common pathophysiological feature shared by AD and T2DM. In this study, we examined whether metabolic improvement or upregulation of chaperone effects has reversible effects on HFD-induced exacerbation of amyloid pathology in AD model (A7-Tg) mice, either by dietary or pharmacological interventions that have been shown to improve insulin sensitivity. We fed A7-Tg mice with a HFD starting from 3 months of age. At 9 months, HFD-fed A7-Tg mice showed increases in body weight, fasting plasma insulin and glucose levels, and reduced insulin sensitivity. At this age, HFD feeding has already led to an increase in cerebral Aβ levels. For dietary intervention studies, HFD-fed A7-Tg mice were switched back to the standard chow diet (HFD-Chow) or 30% caloric restriction (HFD-CR) at 9 months of age. After switching the diet, body weight was rapidly decreased, and HFD-induced insulin resistance was improved in both HFD-Chow and HFD-CR mice. Immunohistochemical analysis of aged mouse brains showed a protective effect of dietary intervention on Aβ deposition corresponding to the degree of diet restriction. We also performed a pharmacological intervention study treating HFD-fed mice with tauroursodeoxycholic acid (TUDCA), a chemical chaperone with endoplasmic reticulum stress-relieving activity, and observed a decrease in body weight and improvement in insulin resistance, which was associated with a trend of lower levels of brain Aβ compared with

the control HFD-fed group. Altogether, our results suggested that metabolic improvement by controlling diet has reversible effects on amyloid pathology. Moreover, reducing metabolic stress by chemical chaperone may mitigate the progression of amyloid pathology, which could be a therapeutic target for prevention of AD.

Disclosures: **T. Ochiai:** A. Employment/Salary (full or part-time):; Kaken Pharmaceutical Co., LTD.. **T. Wakabayashi:** None. **K. Yamaguchi:** None. **K. Matsui:** None. **T. Sano:** None. **T. Iwatsubo:** None.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.20/D37

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Effect of a ketogenic diet on spatial learning performance in PS1+APP, APP, and wild type rats

Authors: *P. N. MICHENER[1], R. A. RICHARDSON[1], C. L. GANN[1], O. M. FAHR[1], I. M. NORTH[1], B. TUMMALA[1], C. AGCA[2], Y. AGCA[2], T. R. SCHACHTMAN[1];
[1]Psychological Sci., [2]Vet. Pathobiology, Univ. of Missouri, Columbia, MO

Abstract: The present study used transgenic rat models of Alzheimer’s Disease (AD) that overexpress amyloid precursor protein (APP) and APP and presenilin 1 (PS1) transgenes. These transgenic models produce mutations that result in the formation of amyloid-beta plaques, a marker of AD, which are associated with impaired mitochondrial functioning. A low carbohydrate, high fat ketogenic diet (KD) is thought to reduce amyloid beta and improve mitochondrial functioning. Therefore, the present study was an exploratory study comparing KD and standard lab chow on spatial learning performance in transgenic PS1+APP, APP, and wild type (WT) Fischer male rats. These PS1+APP and APP rats have previously been shown to have spatial learning deficits. Half of the rats of each type (PS1+APP, APP, and WT) were placed on a KD, while the other half remained on standard lab chow. The rats were 5 to 6 months old at the start of their respective diets. The rats were on the diets for 16 days before beginning testing in the Barnes maze, which is a spatial learning task that requires rats to locate a goal box located under one of the holes in the maze. The rats experienced 4 acquisition days. During acquisition, the rats experienced two 5-min trials per day in which the number of errors (nose poke into an incorrect hole) and latency were measured: 1) time until rats nose poked into the goal box; 2) time until they stepped down in the goal box; and 3) time to enter the goal box. Following this acquisition period, the rats experienced an 8-day retention interval. Following the retention interval, the rats experienced 2 retention test days, which were identical to the acquisition trials. Following retention testing, the rats experienced 4 days of reversal training. During reversal, the

goal box was moved to a location 180 from the original location, and errors and latencies were recorded. The results showed that overall, the KD significantly improved WT rat performance in the maze compared to WT rats on regular lab chow. However, the KD significantly decreased performance in both PS1+APP and the APP transgenic models. It is possible that while the KD was improving mitochondrial function in WT rats, the KD may not help to improve learning in this specific transgenic strain of rats. It is possible that the abundance of necrotic neurons (as well as amyloid plaques and their deposition) may result in AD animals not being able to effectively utilize ketone bodies as much as non-AD control animals. Also, KD is physiologically taxing for organisms and may severely affect the cognitive functioning of AD animals. It is also possible that a KD may not help to improve the spatial learning deficits of AD. More data collection is anticipated.

Disclosures: P.N. Michener: None. **R.A. Richardson:** None. **C.L. Gann:** None. **O.M. Fahr:** None. **I.M. North:** None. **B. Tummala:** None. **Y. Agca:** None. **C. Agca:** None. **T.R. Schachtman:** None.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.21/D38

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Program for Neurology Research & Discovery,
A. Alfred Taubman Medical Research Institute
Handelman Emerging Scholar Fund
Robert E. Nederlander Sr. Program for Alzheimer’s Research
National Institutes of Health (T32 DK101357)

Title: The effects of insulin and insulin-like growth factor-I on amyloid precursor protein phosphorylation in *in vitro* and *in vivo* models of Alzheimer's disease

Authors: *B. KIM[1], S. E. ELZINGA[2], R. E. HENN[2], L. M. MCGINLEY[2], E. L. FELDMAN[3]; [2]Neurol., [3]Dept. of Neurol., [1]Univ. of Michigan, Ann Arbor, MI

Abstract: Alzheimer’s disease (AD) is a growing problem worldwide, and there are currently no effective treatments for this devastating disease. The neurotrophic growth factors insulin and insulin-like growth factor-I (IGF-I) are currently being investigated as potential therapeutic approaches for AD in preclinical and clinical studies. However, given that the metabolic syndrome (MetS) and diabetes are risk factors for AD, it is unknown how associated insulin resistance (IR) in the brain may impact the effectiveness of these therapies for AD. In this report, we therefore investigated the mechanisms underlying the effects of insulin and IGF-I on AD-

associated pathology in the context of IR, with particular emphasis on phosphorylation of amyloid precursor protein (APP), a key step in promoting amyloid plaque formation in AD. Both insulin and IGF-I decreased APP phosphorylation in cultured primary cortical neurons, supporting their therapeutic use in AD. Induction of IR blocked the beneficial effect of insulin and reduced the effect of IGF-I on APP dephosphorylation. These effects were mediated by the phosphatidylinositol 3-kinase (PI3-K)/protein kinase B (Akt) pathway, as inhibition of this pathway during IR restored the effect of IGF-I on APP dephosphorylation. Finally, we established the translational relevance of these results *in vivo* by demonstrating that high fat diet fed mice, a robust model of IR and MetS, exhibited the expected increased brain APP phosphorylation. Overall, these data indicate that the beneficial therapeutic effect of insulin and IGF-I on APP phosphorylation is negatively impacted by IR, and suggest that insulin and IGF-I alone may not be appropriate therapies for AD patients with IR, MetS, or diabetes.

Disclosures: B. Kim: None. **S.E. Elzinga:** None. **R.E. Henn:** None. **L.M. McGinley:** None. **E.L. Feldman:** None.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.22/D39

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: GNT1143978
GNT1143848
GNT1136241
GNT1081916
GNT1132524

Title: Cognitive deficits in late stage Alzheimer's mice ameliorated by specific tau phosphorylation by p38gamma

Authors: *A. ITTNER, K. STEFANOSKA, J. BERTZ, P. ASIH, Y. LIN, A. VOLKERLING, F. DELERUE, L. ITTNER;
Dementia Res. Centre, Dept. of Biomed. Sci., Macquarie Univ., Sydney, Australia

Abstract: Current treatments for Alzheimer’s disease (AD) show only symptomatic relief and cannot revert cognitive/memory impairment. Toxic gain-of-function of the neuronal tau protein through non-specific/indiscriminate hyperphosphorylation has been suggested as central pathomechanism in AD. We have recently shown that site-specific phosphorylation of tau at Threonine-205 (T205) by MAP kinase p38gamma reduces toxic signals in AD and prevents memory deficits. Here, we show that this concept can be exploited to improve cognitive/memory

performance in a mouse model of Alzheimer's disease even at advanced stage. Using adeno-associated virus (AAV)-based gene therapeutic approach, cognitive/memory deficits in Alzheimer's mice at advanced stage are reverted by increasing activity of T205 tau kinase p38gamma in neurons. Using genome editing, we show that endogenous tau T205 can modulate memory deficits in Alzheimer's mice. Ablation of this kinase renders human tau in mice more toxic and incurs memory deficits. Thus, site-specific phosphorylation of tau may serve as a novel concept to improve cognition under conditions of advanced tau-mediated dementia.

Disclosures: **A. Ittner:** None. **K. Stefanoska:** None. **J. Bertz:** None. **P. Asih:** None. **Y. Lin:** None. **A. Volkerling:** None. **F. Delerue:** None. **L. Ittner:** None.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.23/D40

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 AG030142
BrightFocus Foundation

Title: Tau hyper-phosphorylation in two Alzheimer's disease mouse models

Authors: *S. KEMAL[1], C. HALDAR[2], R. J. VASSAR[1];
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Abstract: Alzheimer's disease (AD) is famously characterized by neuronal tau inclusions and extracellular beta amyloid (Aβ) plaques in the brains of AD patients. Surrounding the plaques are damaged, or dystrophic, neurites, and severe neuroinflammation as evidenced by the presence of microglia and reactive astrocytes. AD mouse models recapitulate some, but not all, aspects of the disease that are observed in human AD patients. These models serve as a valuable tool to understand the cellular and molecular mechanisms underlying disease progression and in doing so, potentially identify novel therapeutic targets for intervention. Given the failure of most AD drug trials, exploring new avenues of treatment is imperative.
The 5XFAD mouse line overexpresses mutant amyloid precursor protein (APP) and presenilin-1 (PS1), resulting in rapid (within ~2 months) amyloid deposition, neuroinflammation, and dystrophic neurite formation. More recently, another model, the APP^{NL-G-F} mouse, employs a knock-in approach to express mutant APP at endogenous levels, thereby circumventing any potential artifacts of overexpression. The APP^{NL-G-F} line also exhibits early plaque development accompanied by neuroinflammation. Less is known about dystrophic neurite formation in these mice. One aspect of human AD pathology that has not been consistently observed in the AD mouse models is the formation of neurofibrillary tau tangles. Tau is a microtubule-associated

protein that has a role in microtubule stability. Hyper-phosphorylation of tau causes it to disengage from microtubules and form aggregates. We separately crossed the 5XFAD and the APP^{NL-G-F} models with a tau-knockout line to assess the effect of tau removal on brain pathology in these two AD models. In this study, we report on the tau pathology observed in both the 5XFAD and the APP^{NL-G-F} models and also expand on the tau-independent role of Aβ in disease progression.

Disclosures: S. Kemal: None. **C. Haldar:** None. **R.J. Vassar:** None.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.24/D41

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Project no. LQ1605 from the National Program of Sustainability II (MEYS CR)
Technology Agency of the CR (TA CR), project no. TG02010048

Title: Axonal transport during injury

Authors: *V. LACOVICH, V. M. POZO DEVOTO, K. TEXLOVÁ, M. NOVAKOVÁ, M. FEOLE, G. B. STOKIN;
Translational Neurosci. and Aging Program (TAP), FNUSA-ICRC, Brno, Czech Republic

Abstract: Amyloid precursor protein (APP) and microtubule-associated protein tau have been shown to play a major role in the pathogenesis neurodegenerative disease such as Alzheimer's disease (AD), traumatic brain injury (TBI) and more recently in chronic traumatic encephalopathy (CTE). Injuries have been linked to the development of neurodegenerative diseases further in life as mechanical stress has been shown to favor the amyloidogenic cascade thus suggesting the direct link between TBI and AD. Moreover, APP has been a golden standard as a surrogate marker for diffuse axonal injury (DAI) and axonal swellings, however the molecular mechanisms leading up to APP accumulation and its role in the pathogenesis of the onset of neurodegeneration remain unclear. We aim to investigate the role of perturbed axonal transport in neurodegeneration with particular emphasis on dynamic changes in APP and tau following injury, since knowledge about these changes will also contribute to a better understanding of frequently equally dynamic behavioral and cognitive changes in AD and CTE. We have developed a novel cell culture paradigm to assess *in vivo* dynamic changes in axonal APP and tau following injury. The setup consists of a microfluidic chamber, populated with human stem cell derived neurons, coupled to an electronically controlled syringe pump, which induces sheared stress in the axons. The system allows for real time axonal transport imaging in response to injury. Results obtained with this cell culture paradigm are coupled to a well-

established mouse traumatic brain injury model to allow for confirmation of cell culture findings in an *in-vivo* setting. We found that sheared stress induced axonal injury results in selective and immediate perturbation of the axonal transport of APP, mitochondria and cytoskeletal proteins. To further define the role of APP a KD cellular model has been used to investigate the molecular mechanisms underlying the changes following injury. Considering our recent results identified subtle perturbations in tau isoforms as sufficient to trigger perturbations in the axonal transport of APP we next addressed whether axonal perturbations of APP also result in changes in tau. Our finding disclose an intimate bidirectional link between APP and tau in AD and CTE, which sheds new light to the mechanisms involved in the development of neurodegeneration.

Disclosures: V. Lacovich: None. **V.M. Pozo Devoto:** None. **K. Texlová:** None. **M. Novaková:** None. **M. Feole:** None. **G.B. Stokin:** None.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.25/D42

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: P01 AG012411-17A1
1R01AG062254-01
VA Merit-BX001655
Inglewood Foundation Scholars Program

Title: A hierarchy of preferred protein-protein adhesions, inferred by cross-linking of Alzheimer-like aggregates, predicts novel drug targets

Authors: *M. BALASUBRAMANIAM[1], S. AYYADEVARA[1], A. GANNE[1], S. KAKRABA[1], N. R. PENTHALA[2], X. DU[4], P. A. CROOKS[2], S. T. GRIFFIN[5], R. J. SHMOOKLER REIS[3]; [1]Geriatrics, [2]Pharmaceut. Sci., [3]Dept. of Geriatrics, Reynolds Inst. on Aging 4120, Univ. of Arkansas For Med. Sci., Little Rock, AR; [4]Dept. of Bioinformatics & Genomics, Univ. of North Carolina at Charlotte, Charlotte, NC; [5]Geriatrics, Univ. of Arkansas for Med. Sci., Little Rock, AR

Abstract: Diagnosis of neurodegenerative diseases hinges on detection of “seed” proteins in disease-specific aggregates. In addition to known seed proteins, such as $A\beta_{1-42}$ or tau, these inclusions contain diverse constituents, adhering through aberrant interactions that our prior data indicate are nonrandom. To define preferential protein-protein contacts mediating aggregate coalescence, we created click-chemistry reagents that link neighboring proteins within human neuroblastoma-cell (SH-SY5Y-APP_{Sw}) aggregates. These reagents incorporate a biotinyl group to efficiently recover linked tryptic-peptide pairs. Mass-spectroscopy outputs were screened for

all possible peptide pairs catalogued in the aggregate proteome from the same cells. These empirical linkages, ranked by abundance, implicate a protein-adherence network termed the "aggregate-contactome." Critical hubs and hub-hub interactions were assessed by RNAi-mediated rescue of chemotaxis in aging nematodes that express $A\beta_{1-42}$ in all neurons. Proteins influential in driving aggregation were inferred by multivariate-regression and neural-network approaches. Aspirin, while disrupting aggregation roughly 2-fold, simplified the aggregate contactome to a much greater degree. This approach implicates a dynamic model of aggregate accrual, and reveals the architecture of insoluble-aggregate networks while highlighting influential proteins as novel targets to ameliorate protein-aggregation diseases.

Disclosures: M. Balasubramaniam: None. **S. Ayyadevara:** None. **A. Ganne:** None. **S. Kakraba:** None. **N.R. Penthala:** None. **X. Du:** None. **P.A. Crooks:** None. **S.T. Griffin:** None. **R.J. Shmookler Reis:** None.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.26/D43

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Allosteric regulation of a7 nAChRs by high and low AB(1-42) concentrations

Authors: *J. B. ANDERSON[1,2], K. DEBOEUF[1,2], J. PANCHAL[2,3], M. ISLAM[2], I. MCFATRIDGE[2], J. FARLEY[1,2];
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Abstract: Several studies (including our own) have found that high (nM - uM) concentrations of amyloid beta (Aβ) peptides inhibit alpha 7 (α7) nicotinic acetylcholine (ACh) receptor (nAChR) activity (e.g., Wu et al., 2004; Pym et al., 2005). In contrast, recent studies suggest that picomolar concentrations interact with α7 Rs in a facilitatory way, demonstrating potentially positive roles for Aβ in certain learning- and memory-related processes (Puzzo et al., 2008). Previous work in our lab has shown that 100pM Aβ(1-42) increased peak amplitudes and slowed desensitization of ACh-evoked α7 R currents, expressed in Xenopus oocytes, similar to but less strongly than the type II positive allosteric modulator (PAM) PNU 120596. 100pM Aβ(1-42) was never observed to directly activate α7 Rs. PNU 120596 (but not the type I PAM, genistein) also occluded the effects of 100pM Aβ. Collectively, these results suggest that 100pM Aβ acts as a type II PAM at α7 Rs. Consistent with this view, 2 to 4-hour 100pM Aβ exposure did not potentiate currents of the α7 M276L mutant receptor originally demonstrated by Young et al. (2008) to suppress potentiation by type I and II PAMs. Surprisingly, higher concentrations of Aβ(1-42) [100 and 500 nM] that partially inhibited ACh-evoked currents through wt α7 Rs failed to inhibit M276L α7 Rs. These results suggest that modulation of wt α7 R activity by both

inhibitory and facilitatory Aβ(1-42) concentrations relies on the M276 residue and, therefore, occur via the same/overlapping (rather than distinct) binding site. In addition to signaling as prototypical LGICs, α7 Rs are also capable of signaling through a G-protein binding cluster (GPBC: RMKR) located on the M3-M4 loop (King et al., 2015). To determine if GPCR-signaling was involved in the PAM-like facilitation effects of 100pM Aβ(1-42), we examined Aβ's effects on the RMKR-lacking α7344-347 mutant, and found facilitation to be unaffected. As others have suggested (e.g. Gulisano et al., 2018; Ono et al., 2009), we believe the opposing actions of high (nM) and low (pM) Aβ concentrations on α7 are the result of differential action of distinct tertiary species, though the nature of the species and how they differentially interact with the M276 residue remain to be determined.

Disclosures: **J.B. Anderson:** None. **K. Deboeuf:** None. **J. Panchal:** None. **M. Islam:** None. **I. McFatridge:** None. **J. Farley:** None.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.27/D44

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Astellas Foundation for Research on Metabolic Disorders
KAKENHI Fostering Joint International Research (B)

Title: Ammonia-mediated induction of Alzheimer's disease pathology in astrocytes

Authors: A. KOMATSU, S. KISHIKAWA, G. ITO, I. IIDA, ***M. TERUNUMA**;
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Abstract: Alzheimer's disease (AD) is the most common neurodegenerative disease characterized by cognitive decline and irreversible memory loss. Excessive amount of ammonia has been detected in the brain and serum of AD patients, and the toxicity of ammonia has been thought as a factor which contributes to the progression of AD. In the central nervous system, astrocytes play a key role in ammonia metabolism by expressing the enzyme called glutamine synthetase, which catalyzes the synthesis of glutamine from glutamate and ammonia, and supply glutamine to neurons for the production of neurotransmitters. Here we report that prolonged NH_4Cl treatment accumulates amyloid precursor protein (APP) in astrocytes. Enhanced APP levels was detected in both the plasma membranes and intracellular compartments. Immunocyto-chemistry revealed that NH_4Cl induces the accumulation of APP in the endoplasmic reticulum, Golgi apparatus and recycling endosome. APP gene epression was not induced by NH_4Cl treat-ment however, the elongated half-life of APP was determined. To determine if amyloid beta (Aβ) production is induced by NH_4Cl, we examined the expression of two enzymes BACE1 and

presenilins (PS1 and PS2) which cleave APP and produce Aβ40 and/or Aβ42. The NH_4Cl treatment did not alter the levels of BACE1 and presenilins (PS1 and PS2) and Aβ40, however, the amount of intracellular Aβ42 was significantly increased. Together, our data suggests that ammonia induces the production and accumulation of APP and Aβ in astrocytes, and possibly exacerbate AD pathology.

Disclosures: A. Komatsu: None. **S. Kishikawa:** None. **G. Ito:** None. **I. Iida:** None. **M. Terunuma:** None.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.28/D45

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Health and Medical Research Fund
Research Grants Council Hong Kong
CUHK direct grant scheme
United College endowment fund

Title: Phosphorylation of cellular adaptor GULP1 regulates Alzheimer’s disease amyloid precursor protein processing

Authors: *K.-F. LAU, J. C.-K. NGO, D. D.-L. CHAU;
The Chinese Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: Amyloid-β (Aβ) is derived from the proteolytic processing of amyloid precursor protein (APP), and the deposition of extracellular Aβ to form amyloid plaques is a pathological hallmark of Alzheimer’s disease (AD). Engulfment adaptor PTB domain containing 1 (GULP1) is a molecular adaptor that has been shown to interact with APP to alter Aβ production. Therefore, the modulation of GULP1-APP interaction may be an alternative approach to reducing Aβ. However, the mechanisms that regulate GULP1-APP binding remain elusive. As GULP1 is a phosphoprotein, and because phosphorylation is a common mechanism that regulates protein interaction, we anticipated that GULP1 phosphorylation would influence GULP1-APP interaction and thereby Aβ production. In fact, we have found that phosphorylation of a residue within the GULP1 PTB domain reduces GULP1-APP interaction and suppresses the stimulatory effect of GULP1 on APP processing. Moreover, the kinase for the residue has been identified. Overexpression of the kinase reduces both GULP1-APP interaction and GULP1-mediated Aβ generation. Additionally, our X-ray crystal structure of GULP1 PTB-APP intracellular domain (AICD) peptide has revealed that the GULP1 phosphorylatable residue is

not located at the GULP1-AICD binding interface. Together, we have identified a mechanism for regulating GULP1-mediated APP processing.

Disclosures: K. Lau: None. **J.C. Ngo:** None. **D.D. Chau:** None.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.29/D46

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AGO55497

Title: Direct and indirectly-transformed human neurons from AD patients display pathogenic calcium signaling patterns and protein aggregations

Authors: *S. SCHRANK[1], J. MCDAID[2], C. A. BRIGGS[1], V. BOTTERO[1], A. HOUCEK[3], K. MAIGLER[4], A. D. EBERT[5], R. A. MARR[6], G. E. STUTZMANN[7];
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Abstract: Alzheimer's disease (AD) is the leading cause of dementia in persons over 65, and is the 6th leading cause of death in the USA. Despite the investment of significant resources in AD research, few effective therapeutic interventions exist, in part due to a lack of adequate model systems. Human-induced neurons (HiNs) may fill this need and advance the field, and can be used to identify mechanisms of AD pathology, including the production of Aβ, tau hyperphosphorylation, synaptic dysfunction, and Ca^{2+} dyshomeostasis. To this end, we have generated HiNs from several AD patients and age-matched healthy persons using two different technical approaches termed Direct and 2-Step reprogramming using lentiviral vectors. This strategy also allows us to test the contribution of epigenetic factors which are largely maintained in the Direct approach, yet lost in the 2-step reprogramming during the reversion to an iPSC stage. Using the 2-Step method, we have generated 11 iPSC clones (through transfection of Yamanaka Factors; 5 healthy, 6 Familial AD), and demonstrated their successful conversion to HiNs (by lentiviral transduction with NGN2(Zhang 2013)) through both expression of mature neuronal proteins, as well as electrically-excitable membranes, and spontaneous and evoked synaptic activity. Furthermore, we have observed increased Aβ production and tau-hyperphosphorylation in the AD HiNs relative to the healthy neurons. As has been shown in numerous mouse and cellular models of AD, the AD HiNs also display largely exaggerated RyR-

evoked calcium responses that are mitigated by treatment with dantrolene, a RyR negative allosteric modulator. Furthermore, we have been successful in generating direct reprogramming neurons (Mertens 2015). Conversion from fibroblast to mature neuron occurs over approximately 1 month of time, with dramatic changes in morphology within two weeks. We have also observed calcium waves, and voltage-gated calcium responses using the genetically encoded calcium indicator, GCamp6f, delivered by lentiviral vector. This model system offers the possibility of modeling sporadic AD by bypassing the iPSC intermediate and retaining key epigenetic markers from the fibroblast state. This work demonstrates the power of the induced neuron platform to study human-specific and aging-related diseases. Furthermore, our results confirm that several key upstream pathogenic mechanisms exist in human AD neurons such as dysregulated intracellular calcium handling and generating of aberrant protein products, and provide an avenue for more impactful therapeutic testing in a relevant species.

Disclosures: **S. Schrank:** None. **J. McDaid:** None. **C.A. Briggs:** None. **V. Bottero:** None. **A. Houcek:** None. **K. Maigler:** None. **A.D. Ebert:** None. **R.A. Marr:** None. **G.E. Stutzmann:** None.

Poster

127. Alzheimer's Disease: APP/Abeta Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 127.30/E1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Research Funding for Longevity Sciences (19-17) from the National Center for Geriatrics and Gerontology (NCGG), Japan.
the Japan Agency for Medical Research and Development (AMED) under Grant Number JP19ak0101047s0104
Grant-in-Aid for Scientific Research (B) Grant Number JP17H03574 from Japan Society for the Promotion of Science

Title: Upregulation of autophagy interrupts exosome secretion to augment endocytic disturbance and enhance intracellular accumulation of abeta

Authors: *N. KIMURA[1], S. KOINUMA[2], N. SHIMOZAWA[3], Y. YASUTOMI[3];
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Abstract: Accumulating evidence suggest that abnormal accumulation of β-amyloid protein (Aβ) is the key factor for Alzheimer's disease (AD) pathogenesis. Especially, intraneuronal

accumulation of Aβ precedes extracellular deposition, and several studies showed that intracellular Aβ has much toxicity than extracellular one. Recent findings showed that autophagy is downregulated in AD brains, and experimental induction of autophagy ameliorates Aβ pathology in AD model mouse brains. These findings suggest that the induction of autophagy would be beneficial to prevent Aβ pathology. On the other hand, we have previously shown that aging attenuates dynein-mediated axonal transport, which is indispensable for the fusion of lysosomes and autophagosomes, and that dynein dysfunction reproduces age-related endocytic disturbance, leading to intracellular accumulation of Aβ. Hence, in the present study, we aim to clarify whether the induction of autophagy can ameliorate intracellular accumulation of Aβ even when endocytic disturbance occurs. In Neuro2a cells, rapamycin treatment efficiently induced autophagy influx, and it strongly augmented chloroquine-induced endocytic pathology to enhance intracellular accumulation of Aβ. Noteworthy, rapamycin treatment alone induced intracellular accumulation of Aβ. In contrast, the levels of extracellular Aβ were significantly decreased with rapamycin treatment. Previous studies showed that Aβ is released extracellularly via exosome. Evidently, the induction of autophagy strongly decreased exosome secretion, and siRNA-induced knockdown of ATG5 ameliorated intracellular accumulation of Aβ by enhancement of exosome secretion. Taken together, these findings suggest that the induction of autophagy would be not beneficial for aged brain, and the downregulation of autophagy in AD brains may be a compensatory response to reduce intracellular Aβ via enhancement of exosome secretion.

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Poster

128. Neurodegenerative Disorders and Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 128.01/E2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: GW4 MRC BioMed DTP Studentship

Title: DNA methylation signatures in early Alzheimer’s disease blood

Authors: ***J. A. Y. ROUBROEKS**[1], R. SMITH[1], E. PISHVA[1,2], Z. IBRAHIM[3,4], P. PROITSI[3], S. LOVESTONE[3], I. KŁOSZEWSKA[5], P. MECOCCI[6], H. SOININEN[7], B. VELLAS[8], S. NEWHOUSE[3], R. DOBSON[3,4], J. MILL[1], D. L. A. VAN DEN HOVE[2,9], K. LUNNON[1]; [1]Univ. of Exeter, Exeter, United Kingdom; [2]Maastricht Univ., Maastricht, Netherlands; [3]King's Col. London, London, United Kingdom; [4]Univ. Col. London, London, United Kingdom; [5]Univ. of Łódź, Łódź, Poland; [6]Univ. of Perugia, Perugia, Italy; [7]Univ. of Eastern Finland, Kuopio, Finland; [8]Univ. of Toulouse, Toulouse, France; [9]Univ. of Würzburg, Würzburg, Germany

Abstract: Epigenetic mechanisms, and DNA methylation in particular, have been indicated to play a role in the etiology and progression of Alzheimer's disease (AD) by an increasing number of studies. To elucidate the role that DNA methylation may play in early stages of the disease, and examine its potential for the development of biomarkers, it is important to study both individuals with AD and individuals with mild cognitive impairment (MCI), who frequently progress to AD.
The current study examined genome-wide DNA methylation signatures in the blood of 301 individuals from the AddNeuroMed cohort, comprised of subjects with AD (n=94), MCI (m=111), and elderly controls (CTL; n=96), who have detailed clinical, genetic, and expression data available. A subset of MCI subjects (n=44) progressed to AD within one year after baseline measurement. DNA methylation was assessed using Illumina HumanMethylation 450K arrays, and differentially methylated regions (DMRs) were subsequently identified using linear models, controlling for age, sex, cell type proportions and batch. By applying weighted gene correlation network analysis (WGCNA) we further defined clusters ('modules') of highly co-methylated sites that are altered in disease and identified underlying biological pathways.
We identified five significant DMRs related to AD and MCI (in *HOXB6, DHX16, CNKSR1, COL11A2, MOV10L1),* and four DMRs associated with future conversion from MCI to AD (in *CPT1B, EML3, TMEM184A, PRDM1)*. Of the WGCNA-identified modules four were associated with MCI, two were associated with number of *APOE*-ε4 alleles, and another two were related to number of education years. Pathways altered in MCI included neurogenesis and wnt-signalling. We further investigated the relationship between methylation levels within the identified DMRs and expression levels of the corresponding genes, and discovered an interaction effect of disease on this relationship in the genes *DHX16* and *CPT1B.* This study will further identify methylation quantitative trait loci (mQTL) using available genetic data.
Taken together, this study identified DNA methylation alterations that can be detected in AD and MCI blood, as well as changes associated with future progression to AD. These results suggest that changes related to cognitive decline may occur at an early stage, and the epigenetic signatures nominated in this study may be useful for identifying individuals at an early stage of AD.

Disclosures: J.A.Y. Roubroeks: None. **R. Smith:** None. **E. Pishva:** None. **Z. Ibrahim:** None. **P. Proitsi:** None. **S. Lovestone:** None. **I. Kłoszewska:** None. **P. Mecocci:** None. **H. Soininen:** None. **B. Vellas:** None. **S. Newhouse:** None. **R. Dobson:** None. **J. Mill:** None. **D.L.A. van den Hove:** None. **K. Lunnon:** None.

Poster

128. Neurodegenerative Disorders and Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 128.02/E3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: J.V.S.-M is supported by a SYNAPSIS Foundation Advanced PostDocs fellowship and Heidi Seiler-Stiftung foundation.
The laboratory of J.G. laboratory is supported by the SYNAPSIS Foundation, the Béatrice Ederer-Weber Stiftung, the Floshield Foundation, and the Alzheimer's Association (NIRG-15-363964). J.G. is an MQ fellow and a NARSAD Independent Investigator.

Title: Comprehensive analysis of PM20D1 QTL locus in Alzheimer's disease

Authors: *J. SANCHEZ-MUT, J. GRAFF;
UPGRAEFF, Brain and Mind Institute, EPFL, Lausanne, Switzerland

Abstract: *Alzheimer's disease* (AD) is a multifactorial disorder caused by a combination of genetic and non-genetic factors, investigated by genome- (GWAS) and epigenome- (EWAS) wide association studies, respectively. Over the past years, multiple loci have been found to show genetic-epigenetic interactions, so-called quantitative trait loci (QTLs). Recently, we have identified the first QTL association with AD, centered on *Peptidase M20 Domain Containing 1 (PM20D1).* We have observed that *PM20D1* DNA methylation, RNA expression, and genetic background are correlated. We provided mechanistic insights for such correlations (i.e., the promoter of *PM20D1* is coupled to a chromatin loop that puts in contact a non-risk haplotype, which displays enhancer-like characteristics), and have shown that AD-associated stressors increase *PM20D1* expression, and that *PM20D1* is upregulated in symptomatic AD-mice and in non-risk haplotype human AD patients. Finally, we demonstrated that by genetically increasing and decreasing PM20D1 levels, AD-related pathologies were decreased and accelerated, respectively (Sanchez-Mut et al., 2018, Nature Medicine).
However, since the *PM20D1* QTL region also includes other genes, namely *Nuclear Casein Kinase And Cyclin Dependent Kinase Substrate 1 (NUCKS1), RAB7, member RAS oncogene family-like 1 (RAB7L1),* and *Solute Carrier Family 41 Member 1 (SLC41A1),* we cannot completely rule out the possibility that they also have an effect on AD progression.
To test this possibility, we have performed a comprehensive analysis of *PM20D1* QTL region at the DNA methylation, RNA expression and genetic-background level. In addition, we have assayed whether AD-related stressors modify the expression of those genes, and whether they are dysregulated in AD-mouse models and human AD postmortem samples. Finally, we also tested the effects of their overexpression and provided deeper insights on our previous AD association.

Disclosures: **J. Sanchez-Mut:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); J.V.S.-M holds a patent for the use of PM20D1 methylation and haplotype as biomarkers for Alzheimer's disease (European Patent No. 16180434.9). **J. Graff:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); J.G. holds a patent for the use of PM20D1 methylation and haplotype as biomarkers for Alzheimer's disease (European Patent No. 16180434.9)..

Poster

128. Neurodegenerative Disorders and Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 128.03/E4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Zhejiang Provincial Natural Science Foundation of China (LZ19C090001 to B.S.),
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the Major State Basic Research Program of China (2014CB964602 to B.S.,2013CB530902 to Y.Z
the Science and Technology Planning Project of Zhejiang Province (2017C03011 to B.S.)
Chinese Ministry of Education Project 111 (B13026)

Title: Deleting adult neural stem cells ameliorates synaptic and cognitive deficits in animal models of Alzheimer's disease

Authors: *Y. MEI[1], X. ZHANG[1], Y. HE[1], D. WANG[1], E. YANG[1], X. WEI[1], D. ZHOU[1], J. WANG[1], H. SHEN[2], Q. SHU[3], Y. ZHOU[1], B. SUN[1];
[1]Dept. of Neurobio., Inst. of Neurosci., Hangzhou, China; [2]Dept. of Physiol. and Pharmacol., Med. Sch., Ningbo, China; [3]Children's Hosp., Hangzhou, China

Abstract: The development of adult neural stem cells (aNSCs) was impaired in the hippocampus of animal models of Alzheimer's disease (AD). However, whether and how aNSCs and new neurons derived from aNSCs affect AD neuropathology is unclear. Here, we found that genetic or drug-induced ablation of aNSCs significantly ameliorated deficits of synaptic plasticity and cognitive functions in two mouse models of AD. On the other hand, whole cell recording revealed that deleting aNSCs prevented the increased inhibition in the dentate granule cells of AD mice. Furthermore, deleting aNSCs did not affect Aβ levels but prevented the calbindin depletion in the hippocampus of AD mice. Knocking down calbindin in the hippocampus abolished the effects of deleting aNSCs on synaptic and cognitive functions in AD mice. Taken together, our data suggest that deleting aNSCs improved synaptic plasticity and cognitive functions in AD mice by regulating the activity of dentate granule cells via calbindin.

Disclosures: Y. Mei: None. **X. Zhang:** None. **Y. He:** None. **D. Wang:** None. **E. Yang:** None. **X. Wei:** None. **D. Zhou:** None. **J. Wang:** None. **H. Shen:** None. **Q. Shu:** None. **Y. Zhou:** None. **B. Sun:** None.

Poster

128. Neurodegenerative Disorders and Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 128.04/E5

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Calcification of the basal ganglia in centenarians

Authors: *T. IWASE[1], M. YOSHIDA[2], Y. HASHIZUME[3], I. YAZAWA[4];
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Abstract: Pathological calcification of the basal ganglia is known as Fahr's syndrome. Fahr's syndrome is sometimes used to encompass both Fahr's disease (familial idiopathic calcification of the basal ganglia) and basal ganglia calcification secondary to other disorders. In the elderly, mild calcification of the basal ganglia is common and an incidental finding. It is not clear that Fahr's syndrome is an extension of incidental senile basal ganglia calcification. In our study of Fahr's syndrome, we clarified that vascular calcification of the basal ganglia was continuously spread over the surrounding white matter into the cortex. Axonal loss, myelin sheath loss and gliosis were observed in the white matter with severe vascular calcification. In this study, we investigated calcification of the basal ganglia in centenarians. The autopsied brains from 44 centenarians were studied. We found calcification of the basal ganglia in 20 cases, yielding an overall prevalence of 45.6%. Calcification appears histologically as basophilic (blue to blue-black) in routine hematoxylin-eosin staining. The localization of calcium deposits in vessels and capillaries was similar to Fahr's syndrome. Tiny globular deposits of relatively homogenous size aligned in rows along the capillaries, and deposits in the arterioles at the initial stage were confined to the border zone between the tunica media and adventitia. The calcification centered in the anterior part of the globus pallidus. The calcification was not spread into the surrounding white matter; rather, it was confined to the globus pallidus. Our findings indicate that there is a high prevalence of calcification of the basal ganglia with limited extension in centenarians. Involvement of surrounding white matter will be useful for the distinction between "pathological" and "physiological" basal ganglia calcification.

Disclosures: **T. Iwase:** None. **M. Yoshida:** None. **Y. Hashizume:** None. **I. Yazawa:** None.

Poster

128. Neurodegenerative Disorders and Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 128.05/E6

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Semantic and episodic memory in neurodegenerative disease

Authors: *S. I. DEV[1,2], B. C. DICKERSON[1,2], R. ECKBO[1], D. PUTCHA[1,2], B. WONG[1,2], J. A. COLLINS[1,2];
[1]Massachusetts Gen. Hosp., Charlestown, MA; [2]Harvard Med. Sch., Boston, MA

Abstract: Imaging studies suggest that semantic and episodic memory are supported by distinct neuroanatomical substrates that are differentially impacted across neurodegenerative syndromes. However, few studies have directly compared these two abilities within a single task with the same stimuli and response types. The purpose of this study was to examine semantic and episodic memory for faces and explore their relationships with cortical thickness in patients with typical Alzheimer's disease (AD), semantic variant primary progressive aphasia (svPPA), logopenic variant PPA (lvPPA), and posterior cortical atrophy (PCA). Nine cognitively normal (CN) adults and 40 patients (17 AD, 12 svPPA, 5 lvPPA, and 6 PCA) were administered a novel face memory task assessing semantic (discriminating between famous vs. non-famous faces) and episodic (recognizing previously studied faces regardless of fame) memory. Structural MRI scans were acquired for a subset of 21 participants (7 AD, 4 svPPA, 4 lvPPA, and 6 PCA). Relative to CN, svPPA and AD patients demonstrated worse semantic and episodic discriminability (d'). Semantic d' was worse in svPPA relative to AD; there was no significant difference in episodic d' between these two groups. Exploratory analyses suggest better episodic d' in lvPPA relative to all other patient groups. Vertex-wise analyses revealed that lower semantic d' was associated with cortical thinning in several nodes of the language network (temporal pole, rostral superior, middle and inferior temporal gyri, inferior parietal lobule). Worse episodic d' predicted cortical thinning in nodes of both language and default mode networks (entorhinal cortex, precuneus, anterior middle frontal gyrus). This study is consistent with others suggesting that semantic and episodic memory are differentially impacted across distinct clinical phenotypes. Results suggest that semantic and episodic face memory are supported by primary nodes in neural networks disrupted in neurodegenerative disease. Future studies will include larger sample sizes and a-priori regions of interest to study the relationship between semantic and episodic memory and cortical thickness.

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Poster

128. Neurodegenerative Disorders and Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 128.06/E7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIMH R21 R21MH109953
Brain & Behavior Research Foundation NARSAD
NNIMHD 2G12MD007592

Title: Genetic and non-genetic factors for sleep and inhibitory control

Authors: *E. B. SALDES, P. R. SABANDAL, A. ARZOLA, K. A. HAN;
Univ. of Texas at El Paso, El Paso, TX

Abstract: Dysfunctional executive functions are associated with brain disorders such as attention deficit hyperactivity disorder (ADHD), autism, dementia, substance abuse and addiction. However, the underlying neural and cellular mechanisms responsible remain unclear. To address this, we screened for novel genetic factors important for inhibitory control using a Go/No-Go test in *Drosophila melanogaster*. We found that *Shaker* and *Hyperkinetic*, which encode potassium channels, and *kekkon5*, which codes for a cell adhesion molecule with tyrosine kinase activity and important for synaptic plasticity. To identify the neural site where these genes play roles in inhibitory control, we knocked them down in either all neurons or a subset of neurons including mushroom body (MB) and central complex neurons known to be important for high order brain functions. We also explored non-genetic factors influencing inhibitory control. Previous studies indicate that loss of sleep increases mistakes in the human subjects performing the Go/No-Go test, which is used to assess impulsivity. In addition, approximately 60-80% of adults with ADHD show sleep anomaly. Consistently, *Shaker* and *Hyperkinetic* mutants have abnormal sleep, suggesting a role of sleep in inhibitory control. We disrupted sleep in wild-type flies, which resulted in impaired inhibitory control. This supports the notion that abnormal sleep causes impulsivity. This study will begin to clarify the mechanism by which genetic and non-genetic factors affect inhibitory control.

Disclosures: E.B. Saldes: None. **P.R. Sabandal:** None. **A. Arzola:** None. **K.A. Han:** None.

Poster

128. Neurodegenerative Disorders and Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 128.07/E8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG045571

Title: Detection of mitogen-activated protein kinase kinase 3 (MAP2K3) immunoreactivity in human cerebral cortex

Authors: *Y. PAN[1], A. BAHRAMI[1], I. AYALA[1], R. SHAHIDEHPOUR[1], *C.-K. WU[3], E. BIGIO[2], E. ROGALSKI[1], M. MESULAM[1], C. GEULA[1];
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Abstract: SuperAgers are defined as individuals aged 80 and above whose episodic memory performance is at least at the level of cognitively average individuals in their 50s and 60s. Our previous exome wide investigation revealed that the SuperAging phenotype is associated with variants in the *mitogen-activated protein kinase kinase 3 (MAP2K3)* gene. MAP2K3 is a dual specificity protein kinase that belongs to the mitogen-activated protein kinase signaling pathway. It is activated by different forms of stressful stimuli and inflammatory cytokines residing in a biological pathway linked to memory. To determine if presence of *MAP2K3* gene variants influence levels of its protein, it will be necessary to measure the levels of MAP2K3 protein in tissue. Detection of MAP2K3 protein levels have been reported in animal tissue and in human cells in culture. There is no information on the levels of MAP2K3 in the human brain. We sought to explore methods for detection of this protein in human cortical tissue using two commercially available antibodies in western blot and immunohistochemical experiments. We used brain tissue from the middle frontal gyrus of three cognitively normal aged individuals. To determine whether we can detect effects of neurodegeneration, we also used tissue from four patients with frontotemporal lobar degeneration (FTLD) with TDp-43 pathology. We were able to identify MAP2K3 in cortical tissue with both methods. Immunhohistochemistry in aged controls showed robust staining in neurites. Western blot analyses confirmed presence of MAP2K3 in cortical tissue homogenates and revealed a 47% decrease in protein levels in FTLD. Our findings indicate that MAP2K3 protein can be detected in human cortical tissue via both western blot and immunohistochemistry, and can be used to detect alterations in its levels.

Disclosures: Y. Pan: None. **A. Bahrami:** None. **I. Ayala:** None. **R. Shahidehpour:** None. **E. Bigio:** None. **E. Rogalski:** None. **M. Mesulam:** None. **C. Geula:** None. **C. Wu:** None.

Poster

128. Neurodegenerative Disorders and Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 128.08/E9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: MH109382
MH106967
NS041202
GM008076

Title: Transgenerational and immediate effects of E2F1 loss on development of neurocognitive impairment

Authors: *C. MEURICE[1], D. P. JACKSON[1], S. K. RYAN[1], M. A. ERICKSON[2,3], E. D. HARNESS[1], K. L. JORDAN-SCIUTTO[1];
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Abstract: The transcription factor E2F1 modulates the G1-S phase transition of the cell cycle, DNA damage response, and apoptosis. Neuronal E2F1 is upregulated in neurodegenerative diseases; however, E2F1's roles in other CNS-resident cell types during neurodegeneration have not been fully explored. Interestingly, our lab found that human iPSC-derived microglia infected with HIV have reduced E2F1 gene expression. Since adult E2F1 mutant mice lacking the DNA binding domain ($E2F1^{tm/tm}$) exhibit age-dependent memory impairment, we hypothesized that E2F1 loss in microglia results in a disease-associated phenotype that promotes the development of HIV-associated neurocognitive disorders (HAND) and other neurodegenerative diseases. To test this hypothesis, constitutive E2F1 knockout mice were generated by breeding floxed E2F1 mice ($E2F1^{fx/fx}$) with a tamoxifen-inducible Cre recombinase mouse under control of the gene trap ROSA 26 promotor ($GtROSA26Cre^{ERT2}$). Due to the ubiquitous expression of the Cre driver, tamoxifen injection in pregnant dams at 12 days post-coitus (dpc) resulted in embryonic recombination of E2F1. After confirming heritability of the Cre-recombined product, the resulting mice were bred with wildtype B6;129S F2 hybrid mice (+/+) to eliminate the $GtROSA26Cre^{ERT2}$ alleles. Middle-aged (12-13mth old) $E2F1^{+/+}$, $E2F1^{+/-}$ and $E2F1^{-/-}$ littermates were tested in novel object recognition, light/dark box, and open field assays to confirm object recognition impairment and anxiety-like behavior previously reported in $E2F1^{tm/tm}$ mice. Results indicate that mice born from $E2F1^{+/-}$ crosses, regardless of genotype, display reduced body weight and impaired object recognition memory compared to +/+ and $E2F1^{fx/fx}$, while only $E2F1^{-/-}$ mice exhibit anxiety-like behavior. Preliminary data indicate that maternal E2F1 heterozygosity

may play a role in the phenotypic differences observed in $E2F1^{+/+}$, as modifying the breeding scheme by mating $E2F1^{+/-}$ sires with $E2F1^{+/+}$ dams produces $E2F1^{+/+}$ offspring with normal body weights. Future studies will determine if and how phenotypic differences in $E2F1^{+/+}$ can result from dam E2F1 loss and if microglial phenotype is altered in $E2F1^{+/-}$ and $E2F1^{-/-}$ mice by single-cell RNA-sequencing (scRNA-seq) when compared to $E2F1^{+/+}$ as well as $E2F1^{fx/fx}$. This research will elucidate whether E2F1 loss impacts memory impairment by a direct effect as observed in our anxiety assay and/or due to latent changes in expression of specific genes initially primed at the developmental stage of life.

Disclosures: C. Meurice: None. **D.P. Jackson:** None. **S.K. Ryan:** None. **M.A. Erickson:** None. **E.D. Harness:** None. **K.L. Jordan-Sciutto:** None.

Poster

128. Neurodegenerative Disorders and Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 128.09/E10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSFC 81870852
NSFC 81471101

Title: GABA-B receptor induced BDNF hypermethylation in dorsal hippocampus impairs postoperative cognition of middle-aged mice

Authors: *C. GAO, T. WU, X.-Y. SUN, J.-R. HAO;
Xuzhou Med. Univ., Xuzhou, China

Abstract: Clinical researches have showed that the level of BDNF was reduced in cognition impaired patients after anesthesia and surgery. However, the molecular mechanisms remain largely unknown. The abnormal epigenetic regulation of BDNF may lead to variety of psychiatric disorders. The GABA receptors play an important role in anesthesia and cognition. Here, we hypothesized that $GABA_B$ receptor mediated *Bdnf* hypermethylation and the decreased expression of BDNF in perioperative neurocognitive disorders. Laparotomy under isoflurane inhalation anesthesia model, various behavioral tests, western blotting, coimmunoprecipitation, bisulfite sequencing, fluorescence-activated cell sorting, quantitative real-time reverse transcription polymerase chain reaction, patch-clamp recording, chemical genetics and immunohistochemistry were used in this study. We found that anesthesia and surgery induced cognition impairment in middle-aged mice, which was associated with the decreased expression of BDNF in hippocampal neurons. Overexpression of BDNF reversed cognition impairment. Long-term BDNF repression over 24 h attenuated synaptic plasticity induced by *Bdnf* exon IV and VI hypermethylation. MeCP2 Ser80 played an essential role in *Bdnf* hypermethylation which

was activated by HIPK2 through AMPK pathway. The interaction between AMPKα and $GABA_B$ receptor mediated AMPK pathway activation. Inhibition of $GABA_B$ receptor activation and hypermethylation of *Bdnf* might be a new strategy for avoiding perioperative neurocognitive disorders in clinic.

Disclosures: **C. Gao:** None. **T. Wu:** None. **X. Sun:** None. **J. Hao:** None.

Poster

128. Neurodegenerative Disorders and Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 128.10/E11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant P01NS092525
NIH Grant R01AG055523
Alzheimer's Association: DSAD-15-363207

Title: Dysregulation of the endosomal/lysosomal pathway in Dp16 mice, a mouse model of Down syndrome

Authors: *X.-Q. CHEN, W. C. MOBLEY;
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Abstract: Changes in endosomal function are an emerging theme in the pathogenesis of neurodegenerative disorders. The increased size of early endosomes in both Alzheimer's disease (AD) and Down syndrome (DS) brains and the compelling role that this compartment plays in trophic signaling to support neuronal function and survival supports a pathogenetic role for dysregulation of endosomes in this disorder. An important question addresses the mechanism and consequences of endosomal enlargement. Our published studies pointed out a role for increased levels of full length APP and its 99 amino acid C-terminal fragment (C-99) in inducing activation of Rab5, the small GTPase that regulates early endosome size. This change induced deficits in NGF signaling, in the retrograde endosomal transport of NGF, and the atrophy of NGF-responsive basal forebrain cholinergic neurons. To explore the underlying mechanism we asked whether or not the changes due to Rab5 hyperactivation were confined to early endosomes. We measured the activity of Rab5 and other small GTPases that regulate elements of the endosomal/lysosomal pathwayin a well-established model of DS, the Dp16 mouse; this model is segmentally trisomic for genes on human chromosome 21 and harbors three copies of APP. As a result, the Dp16 is characterized by Rab5 hyperactivity and enlargement of early endosomes, both of which can be normalized by treatment of Posiphen through downregulating APP translation, further confirming the role of APP in inducing Rab5 hyperactivation. Remarkably, we found upregulation of activity of not just Rab5 but of other Rabs, including

Rab7, Rab4, Rab11 and Rab10, without any changes in the total protein level of these Rabs. The changes were present in both young and old animals, pointing to persistent dysregulation ofthe entire endosomal/lysosomal pathway. Importantly, all the changes detected were downstream of increased activation of Rab5 as all other increases in the Rabs tested were prevented by either reduction of APP gene dose to normal in Dp16 mice or lentivirus-mediated introduction of a dominant negative form of Rab5, Rab5:S34N in primary Dp16 neurons. Furthermore, γ-secretase inhibitor-induced hyperactivation of Rab7, Rab4 and Rab11 was also prevented by Rab5:S34N. We conclude that increased APP gene dose acts through Rab5 hyperactivation to induce widespread dysregulation of the endosomal pathway and argue that this contributes to neuronal dysfunction in AD in DS.

Disclosures: X. Chen: None. **W.C. Mobley:** None.

Poster

128. Neurodegenerative Disorders and Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 128.11/E12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH AG055707

Title: Dysregulation of the retromer complex during the progression of Alzheimer's disease in Down syndrome

Authors: *M. E. CURTIS, D. PRATICÒ;
Temple Univ. Lewis Katz Sch. of Med., Philadelphia, PA

Abstract: Down syndrome (DS) is a congenital disorder caused by partial or complete trisomy of chromosome 21. DS is characterized by intellectual disability, developmental delays, and several common comorbidities including an increased risk of developing Alzheimer’s disease (AD). By age 40, nearly all individuals with DS develop amyloid beta (Aβ) plaques and tau neurofibrillary tangles, the pathological hallmarks of AD. This increased susceptibility to Alzheimer’s disease in Down syndrome (AD-DS) has primarily been attributed to an over-dosage of APP, which generates neurotoxic Aβ fragments when cleaved by β-secretase (BACE-1). However, the complete molecular mechanisms of AD-DS are not well understood. In recent years evidence has emerged to support the role of dysfunction of the retromer complex, a multiprotein system responsible for the sorting and trafficking of proteins from endosomes to the trans-golgi or cell surface, as an underlying pathology in several neurodegenerative diseases. It has been hypothesized that in AD, deficient retromer function results in endosomal retention of APP, and, consequently, prolonged interaction with BACE-1 and increased generation of neurotoxic Aβ fragments. The objective of the current study is to investigate the role of the

retromer complex in the development of AD-DS. Human post-mortem brain tissue and fibroblasts from subjects with DS and euploid controls were examined for retromer component using RT-qPCR and western blot analysis. We found that retromer core proteins were decreased in DS compared to unaffected controls in an age-dependent manner. We hypothesize that this dysregulation of the retromer complex in DS contributes to the development of AD-like pathology and cognitive decline, and that the retromer may represent a potential therapeutic target for AD-DS.

Disclosures: **M.E. Curtis:** None. **D. Praticò:** None.

Poster

128. Neurodegenerative Disorders and Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 128.12/E13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NHMRC #1037746

Title: ATP-binding cassette subfamily a gene expression is altered in frontotemporal lobar degeneration with TDP-43 inclusions

Authors: *J. S. KATZEFF[1], S. BHATIA[1], G. M. HALLIDAY[2], W. S. KIM[1];
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Abstract: The expression of a number of ATP-binding cassette subfamily A (ABCA) transporters, *ABCA1, ABCA2,* and *ABCA7,* is dysregulated in Alzheimer's disease (AD), altering the movement of various lipids, including phospholipids and cholesterol, across membranes. Many cases with AD also have TDP-43 inclusions. Whether the expression of ABCA transporters is also altered in dementia patients with only TDP-43 inclusions, as seen frontotemporal lobar degeneration with TDP-43 inclusions (FTLD-TDP), is unknown. The aim of this study was to determine if the expression of ABCA transporters is altered in FTLD-TDP brain (n=10) compared to controls (n=11). Frozen brain tissue from 5 differentially affected regions per case (amygdala, inferior temporal cortex, superior frontal cortex, cerebellum and parietal cortex) was obtained from the Sydney Brain Bank following regulatory approvals. RNA was extracted from 20 mg samples and converted to cDNA, and quantitative PCR performed using the fluorescent dye SYBR Green. The level of expression for each of the 12 *ABCA* genes was calculated using the comparative threshold cycle value method to determine relative expression compared to three housekeeper genes, and disease relevant changes tested using multivariate linear regression statistics covarying for age, gender and post-mortem delay. The expression of *ABCA3-4, ABCA7-10* and *ABCA13* were all significantly upregulated across the

FTD-TDP brain. *ABCA2-4, ABCA7, ABCA9-10* and *ABCA13* all demonstrated region specific alterations. In earlier affected regions, expression of *ABCA4* was most elevated whilst later affected regions showed greater elevation in *ABCA13*. Overall, *ABCA13* was most drastically dysregulated, elevated 1.3-fold across the FTLD-TDP brain, 7-fold in inferior temporal cortex, 2.6-fold in cerebellum and 4.5-fold in parietal cortex. *ABCA2* and *ABCA7* dysregulation may relate to general neurodegenerative pathways rather than be specific to AD pathology, while *ABCA4 and ABCA13* appear more selective for TDP-43 deposition compared to *ABCA1* which is dysregulated in AD and not FTLD-TDP.

Disclosures: J.S. Katzeff: None. **S. Bhatia:** None. **G.M. Halliday:** None. **W.S. Kim:** None.

Poster

128. Neurodegenerative Disorders and Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 128.13/E14

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG052943

Title: Mean diffusivity in semantic primary progressive aphasia (svPPA): An alternative biomarker

Authors: M. MARTIN[1], D. G. WAKEMAN[1], J. BOVE[1], J. S. PHILLIPS[2], P. COOK[3], C. MCMILLAN[4], D. IRWIN[1], *M. GROSSMAN[5], J. GEE[3], M. D. TISDALL[3];
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Abstract: Semantic variant primary progressive aphasia (svPPA), a form of frontotemporal dementia (FTD), is characterized clinically by language impairment encompassing a loss of concept knowledge. It is most commonly associated with TAR DNA-binding protein of ~43kDa (TDP-43) pathologic inclusions. Neuroimaging consistently reveals left anterior temporal lobe atrophy in svPPA, an important biomarker of disease. While cortical thickness is commonly used to assess atrophy implicated in disease, this study utilizes mean diffusivity (MD) of diffusion-weighted imaging (DWI) to assess disease by projecting MD volumes across the inflated cortical surface using FreeSurfer's mri_vol2surf command. Cortical vacuolization is observed in TDP-43 pathology in svPPA. DWI studies of patients with Creutzfeldt-Jakob disease have previously found increased MD associated vacuolization upon pathology, we therefore hypothesized that MD calculated from DWI from the Human Connectome Project (HCP) imaging protocol may also be a sensitive biomarker in svPPA. We examined 10 patients with svPPA (4 males, 6 females, mean age=63.2 years, mean disease duration= 5.5 years) diagnosed with published criteria by an experienced neurologist. Subjects were scanned on a 3T Prisma scanner. Resolution for T1-weighted scans was 0.8mm isotropic; resolution for DWI was 1.5mm

isotropic. We found increased MD signal in svPPA compared to 5 healthy controls (3 males, 2 females, mean age= 55.2 years), indicating disease specifically in the region of the left superior temporal lobe. This overlapped with their typical left anterior temporal cortical thinning. Furthermore, increased MD signal in svPPA correlated with decreased scores on the MINT, a measure of object naming (average MINT for svPPA=5.3/32, controls=31.6/32). We propose that MD is a sensitive biomarker of disease in svPPA that is consistent with cortical vacuolation seen in their TDP-43 pathology.

Disclosures: **M. Martin:** None. **D.G. Wakeman:** None. **J. Bove:** None. **J.S. Phillips:** None. **P. Cook:** None. **C. McMillan:** None. **D. Irwin:** None. **M. Grossman:** None. **J. Gee:** None. **M.D. Tisdall:** None.

Poster

128. Neurodegenerative Disorders and Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 128.14/DP05/E15

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer Society Grant 290 (AS-PG-15b-018)
Alzheimer Society Grant 228 (AS-DTC-2014-017)
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Alzheimer Research UK (ARUK-PG2016B-6)
RS Macdonald Trust

Title: Tracking endothelial dysfunction and microglial dynamics *in vivo* to uncover pathological mechanisms in a model of vascular cognitive impairment

Authors: *J. R. BEVERLEY[1], J. KOUDELKA[1], K. ASKEW[1], L. HAMILTON[1], D. SIEGER[1], U. K. WIEGAND[2], J. J. R. RODRIGUEZ[5], M. GARCIA-ALLOZA[6], B. MCCOLL[3], A. TAVARES[4], R. KALARIA[7], K. HORSBURGH[1];
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Abstract: Vascular cognitive impairment (VCI) encompasses a diverse group of pathologies that are fundamental to the onset and progression of many dementias. Chronic cerebral hypoperfusion, resulting from large vessel occlusion and stenosis, is a prominent early feature of

VCI. Cerebrovascular dysfunction and endothelial activation have been identified as potential mechanisms by which cerebral hypoperfusion leads to neurodegeneration, white matter pathology and the onset of cognitive decline. Our previous work demonstrated, in a mouse model, that bilateral common carotid artery stenosis (BCAS), leads to hypoperfusion, glial-vascular disruption and diffuse white matter damage leading to impaired spatial memory. We hypothesised that carotid stenosis leads to vascular dysfunction and endothelial activation resulting in white matter damage and ultimately cognitive impairment through the activation of microglial cells. Carotid stenosis was modelled in mice through BCAS surgery which involves the application of micro-coils around both the common carotid arteries. Changes in vascular reactivity and endothelial activation were assessed via multiphoton microscopy in C57Bl/6J wild-type (WT) mice, following BCAS/sham surgery. The effect of carotid stenosis on microglial density/proliferation, vascular pathology and cognition was assessed using WT and $Cx3Cr1^{+/eGFP}$ mice, following BCAS/sham surgery. Changes in microglial structure and process motility were assessed longitudinally over 3 months by multiphoton microscopy in a subset of $Cx3Cr1^{+/eGFP}$ mice. Cortical cerebral blood flow was evaluated throughout the studies with the use of laser speckle imaging. We demonstrate that BCAS results in a significant and persistent reduction in blood flow, by 30-50% of control levels, both globally and at a single vessel resolution. Carotid stenosis is associated with reduced arterial pulsation as well as increased leukocyte trafficking, indicative of increased endothelial activation. BCAS is also associated with increased microglia/macrophages, particularly within white matter tracts, as well as impairments in spatial learning and memory. These data demonstrate the utility of imaging approaches to identify endothelial and microglial related changes in a mouse model of VCI. BCAS leads to sustained cerebral hypoperfusion, vascular dysfunction and endothelial activation which is associated with the onset of cognitive impairment. Furthermore, there appears to be a functional role of microglial cells in the development of white matter damage as well as the ensuing cognitive impairment.

Disclosures: **J.R. Beverley:** None. **J. Koudelka:** None. **K. Askew:** None. **L. Hamilton:** None. **D. Sieger:** None. **U.K. Wiegand:** None. **J.J.R. Rodriguez:** None. **M. Garcia-Alloza:** None. **B. McColl:** None. **A. Tavares:** None. **R. Kalaria:** None. **K. Horsburgh:** None.

Poster

128. Neurodegenerative Disorders and Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 128.15/E16

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: LSII

Title: Pilot study of distinguish between dementia with Lewy bodies and healthy subjects using voice

Authors: *Y. OMIYA[1], T. URAGUCHI[1], T. TAKANO[1], M. NAKAMURA[2], S. SHINOHARA[2], M. HIGUCHI[2], K. SUZUKI[2], N. MANOME[2], M. ISHIDA[3], Y. KUMAMOTO[3], S. MITSUYOSHI[2], S. TOKUNO[2];
[1]PST Inc., Yokohama, Japan; [2]The Univ. of Tokyo, Tokyo, Japan; [3]LSII, Tokyo, Japan

Abstract: The aging society is spreading globally, and prevalence of dementia or mood disorder tends to be increasing. To prevent this problem, early detection and care or cure before the disease gets worse is very important.
For diagnosing or screening of dementia, intelligence test, biomarkers and image diagnosis are used as a conventional method. However, intelligence test takes much time to conduct, and the method of biomarker has a problem of high inspection cost, requirement of special equipment and/or reagent, and invasiveness. The method of imaging diagnosis has a problem of high inspection cost, requirement of special equipment.
On the other hand, analysis using voice is advantageous in terms of providing diagnostic support for physicians, it is non-invasive, it can be performed remotely, and it doesn't require special equipment. In this study, we examined to distinguish between dementia with Lewy bodies (DLB) and healthy subjects using voice.
In the study, we collected voice (96kHz, 24bit, wav file) using portable recorder and a pin microphone from patients with DLB (n=20) and healthy subjects (n=20) in the three hospital's consulting room. Further, patients were excluded if they had been diagnosed with serious physical disorders or mood disorders diagnosed by a medical worker using The Mini-International Neuropsychiatric Interview. The voices were collected when the subjects read 13 fixed phrases.
In the evaluation, 6,552 acoustic features were calculated from the speech of each phrase using the openSMILE software. As a result of excluding features which were observed significant differences in a different recording place and the same category, 168 features were selected. In case the t-test was conducted between mean values of DLB and healthy subjects, significant differences ($t<=0.01$, $r>=0.1$) were observed in 16 features among them. Then, to develop an algorithm using the LightGBM algorithm with 10% hold-out and 5-fold cross validation method for classifying healthy subjects and patients with DLB. As a result, the classification performance of the algorithm relative to healthy subjects, patients with a dementia is 75.0% with recall of 0.745 and 0.755, respectively, and precision of 0.745 and 0.755, respectively. Consequently, the extracted features performed well in classifying patients with DLB and healthy subjects, which suggest the utility of the classification algorithm in estimating disease conditions based on speech. Future studies will be conducted for verifying another dementia with Alzheimer's type. Furthermore, we are planning to perform analyzation with data collected at other hospitals or to improve its accuracy.

Disclosures: Y. Omiya: None. **T. Uraguchi:** None. **T. Takano:** None. **M. Nakamura:** None. **S. Shinohara:** None. **M. Higuchi:** None. **K. Suzuki:** None. **N. Manome:** None. **M. Ishida:** None. **Y. Kumamoto:** None. **S. Mitsuyoshi:** None. **S. Tokuno:** None.

Poster

128. Neurodegenerative Disorders and Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 128.16/E17

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grants P01 NS015655, RO1 NS070856 & P50 NS091856.

Title: Differential vulnerability of cholinergic involvement of primary sensory thalamic nuclei in dementia with Lewy bodies

Authors: *P. KANEL[1], M. L. T. M. MÜLLER[1], R. A. KOEPPE[2], K. A. FREY[1], N. I. BOHNEN[1];
[1]Radiology, [2]Div. of Nuclear Med., Univ. of Michigan, Ann Arbor, MI

Abstract: Cholinergic system degeneration is a major driver of cognitive impairment in Dementia with Lewy bodies (DLB). Cognitive processes are integrated across large-scale neural networks that may disintegrate with development of dementia. Attentional deficits are prominent in DLB and appear to predominantly affect the visual domain. There is evidence that the lateral geniculate nucleus (LGN) plays a role in the visual saliency network. A recent probabilistic thalamic atlas now allows for definition of thalamic sub-regions (Iglesias et al. Neuroimage 2018;183: 314-326). The purpose of this study was to compare cholinergic integrity of the thalamic sensory subdivisions (visual: LGN; auditory: medial geniculate nucleus - MGN; and somatosensory: ventral posterolateral-ventral posteromedial nuclei - VP_L/VP_M) in 5 subjects with DLB (3F; 77.8±4.2 yrs, disease duration 4.5±2.1 yrs, MMSE 18.6±4.8) compared to age- and gender-matched controls. Vesicular acetylcholine transporter brain ^{18}F-FEOBV PET imaging was performed in all subjects using a delayed acquisition PET imaging protocol (6 frames total) following i.v. injection of 8 mCi ^{18}F-FEOBV. Distribution volume ratios (DVR) were calculated using supratentorial white matter as a reference region. Volumes of interest were determined using the probabilistic atlas of thalamic nuclei. Compared to control subjects, we found significantly reduced ^{18}F-FEOBV DVR in the left-LGN (35.02%, t= -6.208, P < 0.0001) and right-LGN (30.1%, t= -4.32, P < 0.0001) but not in left-MGN (p=0.079) and right-MGN (p=0.054), or left-VP_L/VP_M (p=0.055) and right-VP_L/VP_M (p=0.075). MR morphometry showed significant atrophy of the LGN but not of the MGN or VP_L/VP_M in DLB patients. Covariate analysis showed that despite significant LGN atrophy, ^{18}F-FEOBV DVR in the LGN remained significantly different between groups (t=-7.73, p=0.0001). We conclude that there is selective vulnerability of the primary visual sensory thalamus. Impaired cholinergic function of the LGN may not only affect relay of visual information from the retina to the visual cortex but may also be involved with modulation of visual attention focus. Further studies are needed to investigate

the role of the LGN in visual attention, visuoperception, cognitive functions, and visual hallucinations in DLB.

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Poster

128. Neurodegenerative Disorders and Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 128.17/E18

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ALS CANADA/BRAIN CANADA

Title: The C9orf72 repeat expansion associated with fronto-temporal dementia leads to early synaptic dysfunction in hippocampal CA1 pyramidal neurons

Authors: *A. ZAMORA-MORATALLA[1], R. FRANCAVILLA[1], L. TOPOLNIK[2];
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Abstract: Frontotemporal dementia (FTD), a type of early onset dementia caused by degeneration of the frontal and anterior temporal lobes, leads to cognitive deficits and behavioral and language abnormalities. Recently, the *C9orf72* GGGGCC expansion mutation has been reported as the most frequent genetic cause of FTD. In the present study, using the *C9orf72* BAC expansion mouse model with 500 repeats (C9-500) that presents the age-dependent neurodegeneration in the hippocampus, we examined whether the *C9orf72* GGGGCC genetic repeat can be associated with an early synaptic dysfunction in hippocampal CA1 pyramidal neurons (PYRs). We found a slight decrease in the amplitude and a significant increase in the frequency of miniature excitatory postsynaptic currents in CA1 PYRs of C9-500 mice in parallel with no change in the spine density. In addition, there was a trend towards a lower frequency and amplitude of miniature inhibitory currents in C9-500 mice. Furthermore, excitatory synapses formed by the *Schaffer collaterals* onto CA1 PYRs showed no change in the basal synaptic transmission but a significant frequency-dependent increase in the short-term facilitation and a higher NMDA/AMPA receptor ratio. In summary, our data indicate that, in hippocampal CA1 pyramidal neurons, *C9orf72* expansion mutation may lead to significant pre- and post-synaptic changes at both excitatory and inhibitory synapses. In particular, at the excitatory synapses, it was associated with an increased transmitter release and postsynaptic glutamate receptor redistribution. Ongoing detailed analysis of the synaptic dysfunction in *C9orf72* BAC mouse model will reveal underlying mechanisms and consequences of synaptic pathology for hippocampal mnemonic processing and FTD pathogenesis.

Disclosures: **A. Zamora-Moratalla:** None. **R. Francavilla:** None. **L. Topolnik:** None.

Poster

128. Neurodegenerative Disorders and Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 128.18/E19

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01NS093097
F31NS108579

Title: Altered intrinsic and synaptic properties in C9ORF72-associated FTD mouse models

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Abstract: Frontotemporal dementia (FTD) is associated with atrophy of frontal and temporal lobes and debilitating behavioral deficits including changes in personality, decreased sociability, loss of empathy, and compulsive behaviors. The pathogenic mechanisms at all levels, from molecular, cellular, synaptic and circuits are largely unknown. Neuroimaging studies have shown specific atrophy in the medial prefrontal cortex (mPFC) in patients with FTD. Layer V (LV) pyramidal neurons in the mPFC are the major output neurons and exert top-down control of neural circuits preferentially disrupted by FTD. Emerging evidence suggests neuronal excitability deficits and changes in synaptic AMPA receptors (AMPARs) in prefrontal circuits in FTD pathogenesis and behavioral symptoms. A hexanucleotide G4C2 repeat expansion (HRE) in the *C9ORF72* gene is the most common familial type of FTD.
A unique AAV mouse model expressing 66-(G4C2) HREs throughout the CNS recapitulate key clinical and neuropathological disease phenotypes, offering an excellent model for investigating the neurophysiological mechanisms underlying behavioral deficits of C9FTD. We found that aged (11 months, equivalent to late disease stage in patients) AAV-(G4C2)66 mice develop severe behavioral abnormalities that include hyper-locomotor activity, decreased sociability and reduced anxiety. Preliminary studies of mutant mice at an early, asymptomatic age (2 months) reveal an increase in depolarization elicited suprathreshold action potential firing in mPFC LV pyramidal neurons, indicating prefrontal hyperexcitability at earlier stage that precedes the onset of behavioral and pathological deficits at late stages. Consistent with these results, E18 rat primary hippocampal neuron cultures overexpressing 80 repeat-associated non-ATG (RAN) translated poly-(GR) and poly-(GA) dipeptide repeat proteins (DPRs) display a significant increase in spontaneous and elicited action potential firing. Additionally, using a new transgenic mouse strain expressing 80 poly-(GR) DPRs in the forebrain, we found significant impairments

in synaptic AMPAR efficacy in mutant mPFC LV neurons in slices. *Our results indicate (i) aged AAV-(G4C2)66 mice display behavioral deficits similar to late-stage clinical symptoms of C9FTD patients (ii) a hyperexcitability in mPFC neurons in asymptomatic AAV-(G4C2)66 mice and (iii) poly-(GR) and poly-(GA) DPRs significantly increase neuronal excitability and impair prefrontal synaptic efficacy.* Thus, the mPFC becomes hyper-functional in early C9FTD conditions, which may contribute to neuron vulnerability and behavioral decline over the progression of the disease.

Disclosures: H.L. Phillips: None. **Q. Ma:** None. **S. Choi:** None. **L. Petrucelli:** None. **F. Gao:** None. **W. Yao:** None.

Poster

128. Neurodegenerative Disorders and Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 128.19/E20

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NS085770
The Lewis Foundation

Title: Strain-specific accumulation of human transgenic TDP-43, inclusion formation and microglia activation in a mouse model for frontotemporal lobar degeneration

Authors: *R. SHAHIDEHPOUR[1], L. KUKREJA[1], G. KIM[1], K. SADLEIR[2], C. SPENCER[1], H. DONG[3], M. MESULAM[1], R. VASSAR[2], C. GEULA[1];
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Abstract: Accumulation of TDP-43 inclusions is one of the pathological hallmarks of frontotemporal lobar degeneration (FTLD). Mouse models have shown that overexpression of wild-type or mutant human TDP-43 (hTDP-43) results in the formation of inclusions and neuronal loss. To investigate the temporal sequence of inclusion formation and degeneration, we employed a conditional transgenic mouse model expressing hTDP-43 under the control of tetracycline operator sequences (tTA). TTA and hTDP-43 transgenic mice were initially bred on 129SVE and FVB backgrounds respectively. Transgenic pups were weaned, and brains examined after 14 and 28 days, as well as 8, 15, and 24 weeks of TDP expression and immunohistochemically stained for phosphorylated TDP-43 (pTDP-43), hTDP-43 and the microglia marker, Iba-1. Bigenic mice on the 129SVE/ FVB background showed inclusions and microglial activation as early as 5 days after TDP-43 expression, followed by a rapid increase in number and size of inclusions, peaking at 14 days of post-weaning expression. After 8 and 24 weeks of transgene expression, microglial activation was significantly decreased and inclusions

were rarely encountered, but the brains showed the most severe degeneration. Past studies have shown that in the 129SVE and FVB lines, tTA possesses toxicity independent of hTDP-43, which was rescuable by moving the transgene onto a congenic C57BL/6 background (B6). To avoid tTA-specific degeneration, we backcrossed hTDP-43 overexpressing mice with B6 mice for 6 generations and bred pups with B6 mice expressing the tTA transgene. Unlike 129SVE/ FVB transgenic mice, bigenic mice on the B6 background displayed pTDP-43 inclusions at a much later age (starting at 24 weeks of expression), and increased progressively over time. Protein analysis and staining for wild-type human TDP-43 confirmed significant slowing of hTDP-43 accumulation in B6 mice compared to the original 129SVE/ FVB mice. These observations indicate that backcrossing hTDP-43 overexpressing mice onto a full B6 background delays accumulation of hTDP and inclusion formation. Iba-1 staining suggests microglial activation in B6 mice mirroring the progression of pathology. Our TDP-43 mouse model serves as a valuable tool in examining the temporal sequence of TDP-43 inclusion formation and its association with neuronal degeneration.

Disclosures: **R. Shahidehpour:** None. **L. Kukreja:** None. **G. Kim:** None. **K. Sadleir:** None. **C. Spencer:** None. **H. Dong:** None. **M. Mesulam:** None. **R. Vassar:** None. **C. Geula:** None.

Poster

128. Neurodegenerative Disorders and Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 128.20/E21

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The National Natural Science Foundation of China (81673405, 81722045)
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Title: Tetradecyl 2,3-dihydroxybenzoate alleviates demyelination following chronic cerebral hypoperfusion through IGF-1 receptor

Authors: ***M. I. YOUSSEF**[1], Y. ZHOU[1], J. ZHANG[2], L. JIANG[1], W. HU[1], J. QI[1], Z. CHEN[1]; [1]Inst. of Pharmacol. & Toxicology, NHC and CAMS Key Lab. of Med. Neurobiology, Col. of Pharmaceut. Sciences, Sch. of Basic Med. Sciences, Zhejiang Univ., Hangzhou, Zhejiang 310058, China; [2]Dept. of Pharmacy, Sir Run Run Shaw Hospital, Sch. of Medicine, Zhejiang University, 3 East Qingchun Road, Hangzhou, Zhejiang 310016, China

Abstract: Currently, there is no effective therapy for subcortical ischemic vascular dementia (SIVD) induced by chronic cerebral hypoperfusion, which exhibits progressive white matter damage and cognitive deficits. Tetradecyl 2,3-dihydroxybenzoate (ABG-001) is a lead compound derived from gentisides with neuritogenic activity. In this study, we aimed to study the effect of ABG001 in an experimental SIVD model through right unilateral common carotid arteries occlusion (rUCCAO) in mice. We found that ABG001 remarkably alleviated white matter damage and cognitive deficits in novel object recognition and morris water maze tests after rUCCAO. The protection of ABG001 on white matter was due to an amelioration of the oligodendrocyte apoptosis and demyelination rather than the remyelination. Moreover, IGF-1 receptor antagonist also blocked the protection of ABG001 against oligodendrocyte damage after rUCCAO. The present study indicates that ABG001 alleviates demyelination following chronic cerebral hypoperfusion through IGF-1 receptor, which could be represented as an encouraging treatment for SIVD.

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Poster

128. Neurodegenerative Disorders and Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 128.21/E22

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NINDS/NIA R01 NS110749
AHA 16SDG27190001

Title: Sex differences in the effects of high fat diet on VCID

Authors: *A. SALINERO[1], L. S. ROBISON[1], O. J. GANNON[1], D. RICCIO[1], A. GALLOWAY[1], K. L. ZULOAGA[2];
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Abstract: Diabetes causes vascular dysfunction and is a major risk factor for vascular cognitive impairment including dementia (VCID). There is a sex difference in this risk: diabetic women are 19% more likely to develop VCID than diabetic men. Data on the effects of prediabetes, which is 3 times more prevalent than diabetes, are lacking. Therefore, we examined the effect of HF diet on cognitive function in middle-aged male and female mice. We modeled prediabetes in mice via long-term administration of a high fat (HF) diet, which causes glucose intolerance. In addition, half of the mice were also subjected to unilateral carotid artery occlusion surgery, which causes chronic cerebral hypoperfusion and models VCID. We hypothesized that high fat diet/prediabetes would lead to more adverse metabolic and cognitive effects in middle-aged

females compared to males in a mouse model of VCID. To assess this, middle-aged (8.5 months old) male and female C57BL/6J mice were placed on a HF or control diet for 6 months. At month 3, they received either VCID or sham surgery. Body weight and glucose tolerance were measured. After 6 months on the diet, cognitive function was assessed through novel object recognition and Morris water maze. Activities of daily living were assessed through a nest building test. Finally, blood flow was measured via laser speckle contrast imaging and brains were collected for histology. HF diet caused a greater percent change in body weight and greater impairment in glucose tolerance in females than males. HF diet, surgery, or a combination of the two impaired object recognition regardless of sex. Spatial memory was impaired in VCID males, regardless of diet; however, in females, both HF diet and VCID (or a combination of the two) impairs spatial memory. The impairment in spatial memory was more severe in VCID females compared to males (regardless of diet). Similar to the effects of diabetes on VCID risk observed clinically, prediabetes also appears to increase cognitive deficits to a greater extent in aged females compared to males.

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Poster

128. Neurodegenerative Disorders and Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 128.22/E23

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NINDS/NIA R01 NS110749
AHA 16SDG27190001

Title: Sex differences in the effects of high fat diet on hippocampal neurogenesis in mouse models of healthy aging and dementia

Authors: *L. S. ROBISON, N. M. ALBERT, L. CAMARGO, O. J. GANNON, A. E. SALINERO, M. A. THOMAS, K. L. ZULOAGA;
Dept. of Neurosci. and Exptl. Therapeut., Albany Med. Col., Albany, NY

Abstract: High fat diet and metabolic disease are associated with impaired cognitive function and increased risk for dementia, as well as exacerbated pathology and memory impairments in mouse models of dementia. Impairments in neurogenesis have been observed in both Alzheimer's (AD) and metabolic disease and may contribute to cognitive dysfunction in both conditions; however, studies exploring their synergistic effects on neurogenesis are lacking despite high rates of comorbidity. There is a sex difference in rates of AD, with women being at an increased risk relative to men. Additionally, while obesity and diabetes are more common

among men, these conditions put women at a disproportionate risk for dementia. This is in agreement with our previous findings that both wild-type (WT) and AD female mice fed high fat diet (HFD; prediabetes model) exhibit greater cognitive deficits compared to male counterparts. Sex differences in the effects of HFD on hippocampal neurogenesis, however, have yet to be thoroughly investigated as a possible mechanism. In the first study, male and female C57BL/6J mice were fed high fat (HF) or low fat (LF) diet from 3-7 months of age, resulting in a similar prediabetic phenotype (weight gain and glucose intolerance) in males and females. LF females had a greater number of proliferating cells (Ki67+) and immature neurons (DCX+) compared to LF males; however, HFD reduced these numbers in females to the levels of males, while diet had no effect on any aspect of neurogenesis in males. These effects were robust in the septal portion of the hippocampus. Further, the number of proliferating cells and immature neurons were inversely correlated with both weight gain and glucose intolerance in females only. In the second study, 3xTg-AD (AD model), 3xTg-AD with right unilateral carotid artery occlusion (rUCCAO) surgery to induce chronic cerebral hypoperfusion/vascular pathology [mixed-etiology dementia (MED) model], and B6129SF1/J mice (controls) were maintained on LF or HF diet from 3-7 months of age. HFD induced a prediabetic phenotype across groups, with metabolic impairments most severe in 3xTg-AD females (AD and MED groups). Preliminary data from this study suggests trends of a greater number of immature neurons in females compared to males in WT and AD groups, and severely diminished numbers of immature neurons in AD and MED groups compared to WT mice. These findings demonstrate the vast effects of sex, metabolic disease, and dementia on hippocampal neurogenesis, and highlight the need for further investigation of potential mechanisms and targeting neurogenic deficits to treat cognitive decline associated with these conditions.

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Poster

128. Neurodegenerative Disorders and Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 128.23/E24

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH
MDA

Title: Studies on the role of DNA dynamics in neurodegeneration

Authors: *J. RAO[1], M. L. HEGDE[2], J. KOSAGISHARAF[3];
[1]Biol., Univ. of Missouri Kansas City-Umkc, Kansas City, MO; [2]Houston Methodist, Houston, TX; [3]Ctr. for Neurosci., INDICASAT AIP, Panama City, Panama

Abstract: DNA is a dynamic and crucial molecule whose conformation kinetics plays a major role in biological function. Reports from our lab and elsewhere indicated the presence of non-B-DNA forms of conformations in neurodegenerative diseases like Fragile X-syndrome, Huntington's chorea, Alzheimer's and others. Recently, our laboratory discovered the presence of Z-DNA in the hippocampal region of severely affected Alzheimer's disease (AD) brain samples and modified B-conformation in Parkinson disease. The alternate purine-pyrimidine bases are the potential sequences adopting Z-DNA, and these are present in the promoter regions of AD-specific genes like amyloid precursor protein (APP), Presenilin and ApoE. We hypothesized that Z-DNA might be involved in the expression of these pathologically important genes. In the present paper, we have developed a theoretical model on the possible mechanisms/hypothetical proposition of Z-DNA transition and its implications in AD. We developed a model where we try to understand that Z-DNA is formed in the promoter region of the APP, and Presenilin genes and this conformation may absorb the negative supercoils at that region. The decrease in the supercoil density alters the native supercoiling domain and positively regulate gene expression of like APP and Presenilin. We further try to understand that Z-DNA may be involved in the down-regulation of genes involved in Aβ clearance defence mechanisms in AD. The proposed model tries to understand the AD behavioural pathology like emotions, eating behaviour memory loss, and coordination failure (Rao KS thanks SENACYT-Panama for financial support through SNI and all authors thank for the support from Melo Brain Grant-Panama)

Disclosures: **J. Rao:** None. **M.L. Hegde:** None. **J. Kosagisharaf:** None.

Poster

128. Neurodegenerative Disorders and Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 128.24/E25

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIMH R21 R21MH109953
Brain & Behavior Research Foundation NARSAD grant
NIMHD 2G12MD007592

Title: Scully in aging-associated loss of memory and inhibitory control

Authors: *P. R. SABANDAL, A. ARZOLA, C. M. SIERRA, N. M. DELGADO, E. B. SALDES, K.-A. HAN;
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Abstract: Alzheimer's disease and related dementias (ADRD) are characterized by progressive cognitive decline including augmented memory loss and dysfunctional executive function. Although numerous studies have identified several key pathophysiological changes such as

mitochondrial dysfunction, cholinergic neurodegeneration and aggregation of β-amyloid (Aβ), the underlying mechanisms are elusive. This is due to the fact that ADRD involves heterogenous genetic and non-genetic risk factors (e.g. aging, social stress and sleep perturbation). Yet, how these diverse risk factors interplay to cause ADRD is still unclear and our study aims to fill this knowledge gap. To address this, we conducted an unbiased functional genetic screen to identify novel ADRD genes that interact with non-genetic factors, particularly aging and social stress, by measuring premature inhibitory control dysfunction as an endophenotype in the *Drosophila* model. One of the genes that we identified is Scully. It is the fly homolog of 17-β-hydroxysteroid dehydrogenase 10 (HSD17β10), which is a multifunctional mitochondrial enzyme binding to Aβ peptides and thereby being associated with Alzheimer's disease. The Scully-deficient flies exhibit aging-dependent loss of inhibitory control as well as enhanced memory loss compared to control flies. The progress of this study will be presented including Scully's roles in mitochondrial homeostasis, cholinergic neurodegeneration and amyloidogenesis. The findings from this study will advance our understanding of the ADRD pathogenesis mechanisms and possibly uncover unique therapeutic targets.

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Poster

128. Neurodegenerative Disorders and Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 128.25/E26

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: University of Iowa, Aging Mind and Brain Initiative
NINDS T32 NS007421

Title: Mice with progressive communicating hydrocephalus develop urinary incontinence and gait impairment

Authors: *M. M. TISH, J. C. GEERLING;
Neurol., Univ. of Iowa, Iowa City, IA

Abstract: Normal pressure hydrocephalus (NPH) is a chronic form of communicating hydrocephalus, most common in older adults, in which cerebrospinal fluid (CSF) accumulates in the cerebral ventricles. NPH involves a triad of progressive symptoms: urinary incontinence, gait impairment, and cognitive dysfunction. NPH is usually idiopathic, and we lack animal models to investigate the pathophysiologic mechanisms of these disabling symptoms. Previous studies produced acute hydrocephalus by injecting kaolin into the cisterna magna (up to 10 μL and 25%), but this procedure caused high mortality rates, and surviving mice were euthanized in less

than two weeks due to poor health. We sought to study communicating hydrocephalus in a more chronic, survivable model by injecting smaller amounts of kaolin (10 or 15%, 5 μL) or saline into the cisterna magna of adult male mice from a mixed C57B6/J background. Mice were monitored for 10 weeks after surgery with weekly, non-invasive behavioral tests. GaitScan software allowed us to compare pre- and post-operative gait characteristics of each mouse for progressive changes. Urinary continence was tested in open-top and -bottom cages placed atop filter paper, with a thermal camera above. Cognitive impairment was tested using novel object recognition, comparing the tenth week post-surgery with one week before surgery. We performed brain MR imaging once before and every two weeks after surgery to track enlargement of the cerebral ventricles. Progressive enlargement of the cerebral ventricles was accompanied by gait impairment, as well as urinary frequency with incontinent features. We show that it is possible to create a survivable and progressive form of acquired hydrocephalus in adult mice with symptoms mimicking those seen in human NPH patients. This mouse model will help unravel the mechanistic basis of neurologic symptoms in this chronic, disabling syndrome.

Disclosures: M.M. Tish: None. **J.C. Geerling:** None.

Poster

129. Cellular Mechanisms of Parkinson's Disease II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 129.01/E27

Topic: C.03. Parkinson’s Disease

Support: NIEHS T32 Training Grant ES015457
MH114017
DGSOM

Title: Ziram, a pesticide linked to Parkinson’s disease, increases synaptic release and excitability at the *Drosophila* NMJ

Authors: *D. F. BRAMBILA[1], J. HARRIGAN[3], D. E. KRANTZ[2,4], F. E. SCHWEIZER[1,4]; [1]Neurobio., [2]Psychiatry and Biobehavioral Sci., UCLA, David Geffen Sch. of Med., Los Angeles, CA; [3]Mol. Toxicology, [4]Brain Res. Inst., UCLA, Los Angeles, CA

Abstract: Synaptic transmission is the fundamental mode of communication between neurons and its dysregulation has been implicated in diverse neurodegenerative disorders. As previously shown, disruption of the ubiquitination pathway via ziram, an agricultural pesticide linked to increased Parkinson’s disease, leads to increased probability of synaptic vesicle release in mammalian neurons. We expanded these studies to the *Drosophila* NMJ, a well-characterized model synapse. We report that ziram elicits an increase in vesicle release probability, as manifest by an increase in the frequency of spontaneous events (minis) and an increase in the amplitude

evoked responses. These effects are mimicked by inhibitors of the E1 ubiquitin conjugating enzyme, but not by inhibitors of the proteasome or de-ubiquitination inhibitors. Higher doses of ziram, but not E1 inhibitors, also increased the excitability of glutamatergic and aminergic neurons in *Drosophila* (see poster by Harrigan et al.). In a preparation where central octopaminergic input is left intact, the invertebrate equivalent to norepinephrine, we find that ziram increases the frequency of spontaneous end junction potentials compared to preparations where the octopaminergic input is absent. We postulate that ziram acts on octopaminergic neurons in the ventral nerve cord to further drive glutamatergic motor output. Our findings indicate that ziram acts to alter synaptic transmission via at least two mechanisms: increased synaptic vesicle release and excitability. These findings are consistent with the hypothesis that disruption of synaptic transmission via acute ziram exposure may play an important role in neurodegenerative disease pathology.

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Poster

129. Cellular Mechanisms of Parkinson's Disease II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 129.02/E28

Topic: C.03. Parkinson’s Disease

Title: Evaluation of saposin C and acid sphingomyelinase overexpression in a model of GBA-related Parkinson’s disease

Authors: *C. KAYATEKIN, K. JACKSON, J. CLARKE, C. VIEL, A. M. RICHARDS, F. J. RIOS, L. S. SHIHABUDDIN, S. P. SARDI;
Rare and Neurolog. Dis. Res., Sanofi, Framingham, MA

Abstract: Synucleinopathies including Parkinson’s disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy are neurodegenerative diseases characterized by the pathological aggregation of alpha-synuclein. *GBA* mutations are the most common genetic risk factor for the development of PD and DLB. Though clinically indistinguishable from sporadic PD, patients with *GBA* mutations, on average, develop disease earlier, progress faster, and are more likely to develop dementia. *GBA* encodes the lysosomal hydrolase glucocerebrosidase (GCase) which metabolizes glucosylceramide and glucosylsphingosine. Mutations in this enzyme decrease its activity, leading to accumulation of these lipids and perturbations in sphingolipid homeostasis. This loss of activity may be causal in *GBA*-mutant synucleinopathies as restoration of GCase activity or inhibition of the synthesis of the accumulating lipids rescued cognitive phenotypes and reduced pathological aggregates in a mouse model of *GBA*-related synucleinopathy. Here, we evaluated 2 proteins involved in sphingolipid metabolism, saposin C

(SapC) and acid sphingomyelinase (ASMase), as potential therapeutic targets for *GBA*-related synucleinopathies. SapC is a co-factor for GCase and enhances the activity of GCase. ASMase is another enzyme involved in sphingolipid metabolism and mutations in the gene encoding for ASMase, *SMPD1*, was identified as a risk factor for PD. As both ASMase and GCase converge on ceramide in the sphingolipid metabolism pathway, we hypothesized that sphingolipid homeostasis and subsequent pathologies in a *GBA*-related synucleinopathy model could be partially restored by overexpressing ASMase. We administered AAV1-SapC and AAV1-SMPD1 to the hippocampus of $Gba^{D409V/D409V}$ mice to overexpress each of these proteins in a GCase-activity deficient background. This mouse model displays altered sphingolipid homeostasis, cognitive impairments, and progressive accumulation of alpha-synuclein/tau/ubiquitin-positive inclusions. Neither SapC nor ASMase overexpression improved cognitive performance in these mice. Furthermore, SapC overexpression had no effect on tau aggregation, while ASMase overexpression increased tau aggregation in the hippocampus. These results suggest that neither SapC overexpression nor ASMase overexpression are sufficient to rescue phenotypes induced by loss of GCase activity in the context of a D409V mutation.

Disclosures: **C. Kayatekin:** A. Employment/Salary (full or part-time):; Sanofi. **K. Jackson:** A. Employment/Salary (full or part-time):; Sanofi. **J. Clarke:** A. Employment/Salary (full or part-time):; Sanofi. **C. Viel:** A. Employment/Salary (full or part-time):; Sanofi. **A.M. Richards:** A. Employment/Salary (full or part-time):; Sanofi. **F.J. Rios:** A. Employment/Salary (full or part-time):; Sanofi. **L.S. Shihabuddin:** A. Employment/Salary (full or part-time):; Sanofi. **S.P. Sardi:** A. Employment/Salary (full or part-time):; Sanofi.

Poster

129. Cellular Mechanisms of Parkinson's Disease II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 129.03/E29

Topic: C.03. Parkinson’s Disease

Title: Seven murine models of metabolic disease exhibit differences in brain protein aggregation and neuroinflammation

Authors: *J. CLARKE, C. KAYATEKIN, C. VIEL, L. SHIHABUDDIN, P. SARDI; Sanofi, Framingham, MA

Abstract: Mutations in *GBA*, the gene encoding the lysosomal hydrolase glucocerebrosidase (GCase), represent the most common genetic risk factor for the development of Parkinson’s disease and Lewy body dementia. These synucleinopathies progress faster both in motor and cognitive decline when *GBA* mutations are present. Our previous studies showed that the $Gba^{D409V/D409V}$ mouse model had reduced GCase activity, lipid accumulation, and Lewy body-like brain pathology, with progressive neuronal accumulation of proteinase K-resistant alpha-

synuclein, ubiquitin, and tau. In fact, neurological phenotypes have been associated with many different metabolic diseases. Thus, we investigated whether mouse models with mutations affecting other metabolic enzymes exhibit pathologies reminiscent of synucleinopathies. We compared brain tissue from symptomatic Gaucher, Fabry, Sandhoff, Hurler, Niemann-Pick A, Pompe, and Niemann-Pick C mice, immunohistochemically evaluating the presence of Lewy body-like pathology and neuroinflammation. We examined the cortex, hippocampus, cerebellum and brainstem of all mice and observed tau and alpha-synuclein aggregation for Gaucher, Fabry, Hurler, and Pompe mice. This protein aggregation was independent of neuroinflammation. Most mouse models exhibited neuroinflammation, which was distributed more broadly in the brain than the protein aggregates. These results suggest that lysosomal dysregulation can contribute to aggregation of proteins in specific parts of the brain and induce neuroinflammation throughout the brain. Yet, only a few metabolic disease models exhibited phenotypes consistent with synucleinopathies. Thus, increased risk for developing PD and DLB may be restricted to particular lysosomal defects.

Disclosures: **J. Clarke:** A. Employment/Salary (full or part-time):; Sanofi. **C. Kayatekin:** A. Employment/Salary (full or part-time):; Sanofi. **C. Viel:** A. Employment/Salary (full or part-time):; Sanofi. **L. Shihabuddin:** A. Employment/Salary (full or part-time):; Sanofi. **P. Sardi:** A. Employment/Salary (full or part-time):; Sanofi.

Poster

129. Cellular Mechanisms of Parkinson's Disease II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 129.04/E30

Topic: C.03. Parkinson’s Disease

Support: R00 ES024570
P50 AG025688
P30 NS055077

Title: Parkinson's disease alters genome-wide DNA methylation and DNA hydroxymethylation in human brain

Authors: *J. KOCHMANSKI[1], S. E. VANOEVEREN[1], C. SAVONEN[1], M. GEARING[2], A. I. BERNSTEIN[1];
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Abstract: DNA methylation and DNA hydroxymethylation are distinct, stable epigenetic marks that assist in the regulation of gene expression. Research indicates that 5-methylcytosine (5-mC) and its oxidized form, 5-hydroxymethylcytosine (5-hmC), have separate genomic distributions

and regulatory functions in the mammalian brain. In particular, recent data indicate that 5-mC and 5-hmC recruit distinct sets of DNA binding proteins in brain tissue, and that they differ in their regional enrichment during synaptogenesis. Existing work has shown associations between DNA methylation and Parkinson's disease (PD), but little work has been done to examine the potential role for DNA hydroxymethylation in the context of neurodegenerative disease. Here, we utilized human post mortem tissue from control (n=3), mid-stage PD (n=3), and late-stage PD (n=3) patients to determine whether genome-wide levels of DNA methylation and hydroxymethylation differ between control and diseased brains. Genome-wide 5-mC and 5-hmC were measured from both parietal and cingulate cortex for each subject using the Illumina EPIC array paired with bisulfite treatment and oxidative bisulfite treatment (BS/oxBS-EPIC). The BS/oxBS-EPIC array was combined with bioinformatics tools, including the oxBS.MLE function in the *ENmix* R package, to generate beta values for 5-mC and 5-hmC at more than 850,000 CpG sites across the human genome in parietal and cingulate cortex. Beta values for the measured probes were filtered to remove cross-reactive probes, probes at known single-nucleotide polymorphisms, and probes with a detection p-value > 0.01 in more than 10% of samples. Filtered probe beta values were then directly compared between disease and control brains to identify CpG sites where there was a greater than 10% change in beta value by disease status. We identified a large number of CpG probes that showed greater than 10% change in beta values for both DNA methylation and hydroxymethylation (parietal: n=2,962, cingulate: n=3,210) by disease status. We also identified disease-related changes in DNA methylation and hydroxymethylation at candidate genes identified from a previous animal model exposure study, including *DNMT3A* and *GRB10*. To test the significance of the changes identified at these candidate genes, we piloted a statistical approach involving generalized linear mixed effects models to simultaneously analyze paired 5-mC and 5-hmC data as repeated measures. Using this approach, we found significant effects of Parkinson's disease status on the balance between 5-mC and 5-hmC at the base-pair level.

Disclosures: J. Kochmanski: None. **S.E. VanOeveren:** None. **C. Savonen:** None. **M. Gearing:** None. **A.I. Bernstein:** None.

Poster

129. Cellular Mechanisms of Parkinson's Disease II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 129.05/E31

Topic: C.03. Parkinson's Disease

Support: NIH Grant R21ES029205

Title: Developmental dieldrin exposure induces neuroinflammation and male-specific increased susceptibility to α-synuclein pre-formed fibrils

Authors: *A. O. GEZER[1], J. KOCHMANSKI[1], S. O. VANOEVEREN[1], C. J. KEMP[1], J. PATTERSON[1], A. C. STRAUSS[1], S. M. FLEMING[3], K. C. LUK[4], C. E. SORTWELL[2], A. I. BERNSTEIN[2];
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Abstract: Epidemiological studies shows that exposure to organochlorine pesticides, such as dieldrin, increases risk of developing sporadic Parkinson's disease (PD), yet underlying mechanisms are unclear. Mice developmentally exposed to dieldrin show changes in ratio of dopaminergic markers persisting into adulthood and increased susceptibility to MPTP, despite the absence of detectable levels of dieldrin in brain. Glial-mediated neuroinflammation is recognized as an early event in PD etiology, and developmental dieldrin exposure leads to potentiation of glial fibrillary acidic protein by MPTP. In Phase 1 of this study, we screened over 300 markers of neuroinflammation by qPCR and observed dieldrin-induced changes in expression of genes involved in cytokine signaling, T cell development and function, TNF signaling, and HLA class II. In Phase 2 of this study, we assessed if developmental dieldrin exposure increases susceptibility to synucleinopathy induced by the intrastriatal injection of α-syn preformed fibrils (PFFs). α-syn containing Lewy bodies are a defining hallmark of PD, so it's critical to establish if dieldrin exposure alters susceptibility to synucleinopathy in α-syn PFF model. There is a lack of synuclein pathology in the MPTP model, therefore its validity as "second hit" to examine dieldrin-induced PD vulnerability is questionable. α-syn PFF model has greater homology to α-syn pathology and toxicity observed in post-mortem PD brain and allows direct assessment of relationship between α-syn inclusions and resulting nigrostriatal degeneration. Consistent with increased susceptibility to MPTP, male mice developmentally exposed to dieldrin showed increased numbers of nigral neurons containing phosphorylated α-syn aggregates 2 months after PFF injection, as assessed by stereology. No differences were detected in female mice. Furthermore, we did not detect a PFF-induced deficit on rotarod test in vehicle treated mice and found no effect of dieldrin on this outcome. The next set of experiments will assess degeneration of nigral dopamine neurons by stereology, loss of striatal innervation by HPLC and western blot 6 months after PFF injection, and a more sensitive behavioral analysis via beam test. Our current results indicate that dieldrin-induced changes in neuroinflammatory pathways may underlie increased α-synuclein aggregation in response to α-syn PFFs, providing insight into the mechanism by which developmental dieldrin exposure affects neuronal susceptibility and establishing that this exposure is broadly applicable to parkinsonian models.

Disclosures: A.O. Gezer: None. **J. Kochmanski:** None. **S.O. VanOeveren:** None. **C.J. Kemp:** None. **J. Patterson:** None. **A.C. Strauss:** None. **S.M. Fleming:** None. **K.C. Luk:** None. **C.E. Sortwell:** None. **A.I. Bernstein:** None.

Poster

129. Cellular Mechanisms of Parkinson's Disease II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 129.06/E32

Topic: C.03. Parkinson’s Disease

Support: Andrew Mellon Pre-doctoral fellowship

Title: Acquired dysregulation of dopamine homeostasis reproduces features of Parkinson's disease

Authors: *M. L. BUCHER, C. J. MOON, A. D. MORTIMER, C. W. BARRETT, J. T. GREENAMYRE, T. G. HASTINGS;
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Abstract: Dopamine has the potential to act as an endogenous neurotoxin when its vesicular sequestration is impaired and it undergoes oxidation and enzymatic metabolism - two cytosolic processes that generate reactive oxygen species and reactive metabolites. Dysregulation of dopamine has been hypothesized to contribute to the enhanced vulnerability of nigrostriatal dopaminergic neurons to degeneration in Parkinson’s disease. Here, we present findings from a novel *in vivo* rat model of acquired dysregulation of dopamine sequestration in nigrostriatal dopaminergic neurons through viral-mediated small-hairpin RNA interference targeting endogenous vesicular monoamine transporter 2 (VMAT2) expression. Utilizing an adeno-associated (serotype 2) virus (AAV2-shVMAT2), viral-mediated interference of VMAT2 expression resulted in a loss of VMAT2 protein expression in transduced dopaminergic cell bodies in the substantia nigra with a corresponding loss of VMAT2 protein in the striatal terminals, an increase in dopamine metabolism, and deficits in dopamine-mediated behaviors. This model results in nigrostriatal dopaminergic neurodegeneration that can be rescued through reintroduction of exogenous VMAT2, demonstrating that dysregulation in dopamine sequestration via loss of VMAT2 is sufficient to cause neurodegeneration. Analysis of pathogenic mechanisms of degeneration within viral-transduced dopaminergic neurons identified oxidative damage of macromolecules evidenced by a 26.29% increase in 4-hydroxynonenal (paired t-test, $n=9$, $p<0.05$) and a 27.36% increase in 3-nitrotyrosine (paired t-test, $n=9$, $p<0.05$). Proximity ligation assay was used to quantify a 28.35% increase in autophosphorylation of the Parkinson’s disease-associated kinase LRRK2, which indicates increased LRRK2 activation (paired t-test, $n=4$, $p<0.05$). As an additional measure of LRRK2 activity, the amount of phosphorylated Rab10, a substrate of LRRK2, was quantified with a 23.52% increase in phosphorylated Rab10 (paired t-test, $n=4$, $p<0.05$). Although there was no significant increase in total α-synuclein, there was a 42.85% increase in phosphorylated α-synuclein (paired t-test, $n=4$, $p<0.05$) as well as a 90.9% increase in the interaction between α-synuclein and mitochondrial

protein import protein TOM20 (paired t-test, n=3, $p<0.05$), which indicates the formation of aberrant α-synuclein. This model demonstrates that a progressive acquired loss of VMAT2 expression in adulthood is sufficient to induce Parkinson's disease associated pathogenic mechanisms of degeneration and provides a novel model to test therapeutic interventions for Parkinson's disease.

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Poster

129. Cellular Mechanisms of Parkinson's Disease II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 129.07/E33

Topic: C.03. Parkinson's Disease

Support: JSPS KAKENHI Grant Numbers JP 25860112, 17K08444
Shimizu Foundation for Immunology and Neuroscience Grant for 2014

Title: Oxicam-derived non-steroidal anti-inflammatory drugs protect against 1-methyl-4-phenyl pyridinium-induced cell death by suppressing of endoplasmic reticulum stress and mitochondrial dysfunction

Authors: *T. OMURA[1,2], M. SASAOKA[1,3], G. HASHIMOTO[1,3], S. IMAI[1], J. YAMAMOTO[4], Y. SATO[1], S. NAKAGAWA[1], A. YONEZAWA[1,3], T. NAKAGAWA[1], Y. TASAKI[4], I. YANO[1,2], K. MATSUBARA[1];
[1]Dept. of Clin. Pharmacol. and Therapeut., Kyoto Univ. Hosp., Kyoto, Japan; [2]Dept. of Pharm., Kobe Univ. Hosp., Kobe, Japan; [3]Grad. Sch. of Pharmaceut. Sci., Kyoto Univ., Kyoto, Japan; [4]Asahikawa Med. Univ., Department of Hospital Pharmacy & Pharmacology, Japan

Abstract: We previously reported that oxicam-derived non-steroidal anti-inflammatory drugs (oxicam-NSAIDs), including meloxicam, piroxicam and tenoxicam, protect against 1-methyl-4-phenyl pyridinium (MPP^+)-induced neuronal cell death independently of cyclooxygenase (COX) inhibition and demonstrated that oxicam-NSAIDs prevent the decrease in phosphorylation of Akt induced by MPP^+. However, the molecular mechanism by which oxicam-NSAIDs protect cells remains unclear. In the present study, we hypothesized that endoplasmic reticulum (ER) stress and/or mitochondrial dysfunction, causative factors of Parkinson's disease, may be involved in the neuroprotective mechanism of oxicam-NSAIDs. We presented that oxicam-NSAIDs inhibited caspase-3 activation and cell death caused by MPP^+ or ER stress-inducing agent (tunicamycin) in SH-SY5Y cells. Furthermore, they suppressed the increases in the ER stress marker CHOP (apoptosis mediator) caused by MPP^+ or tunicamycin, beside inhibiting eukaryotic initiation factor 2α (eIF2α) phosphorylation and the increase in ATF4 caused by

MPP^+. These results suggest that oxicam-NSAIDs repress the eIF2α-ATF4-CHOP pathway, which is one of the three signaling pathways involved in ER stress response. Oxicam-NSAIDs inhibited the decrease in mitochondrial membrane potential depolarization caused by MPP^+, indicating that they can rescue the cells from mitochondrial dysfunction. The phosphorylation of Akt was suppressed after the incubation with MPP^+, whereas phosphorylation of eIF2α was enhanced. Taken together, our findings suggest that oxicam-NSAIDs can prevent eIF2α phosphorylation and mitochondrial dysfunction through the maintenance of Akt phosphorylation (reduced by MPP^+), thereby inhibiting cell death.

Disclosures: **T. Omura:** None. **M. Sasaoka:** None. **G. Hashimoto:** None. **S. Imai:** None. **J. Yamamoto:** None. **Y. Sato:** None. **S. Nakagawa:** None. **A. Yonezawa:** None. **T. Nakagawa:** None. **Y. Tasaki:** None. **I. Yano:** None. **K. Matsubara:** None.

Poster

129. Cellular Mechanisms of Parkinson's Disease II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 129.08/E34

Topic: C.03. Parkinson’s Disease

Support: NRF-2018R1A2B2003955

Title: ISG15 conjugation of STUB1 positively modulates its ubiquitin E3 ligase activity and inhibits cell growth during IFN1 inflammatory signaling

Authors: L. YOO[1], **C. PARK**[2], *K. C. CHUNG[3];
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Abstract: The STIP1 homology and U-Box containing protein1 (STUB1) acts as a ubiquitin E3 ligase and links between Hsp70/90 and the proteasome system, playing a vital role in maintaining protein homeostasis. STUB1 regulates a number of proteins involved in a myriad of physiological and pathological processes, but the underlying mechanism of action via posttranslational modification has not been extensively explored. In this study, we investigated a novel modulatory mode of STUB1 and its effect on STUB1 enzymatic activity. ISG15, an ubiquitin-like modifier, is induced by type I interferon (IFN1) stimulation and can be conjugated to target proteins (ISGylation). Here we demonstrated that STUB1 may be a novel target of ISGylation in HEK293 cells stimulated with IFN1. We also found that K143/144/145 and K287 in STUB1 are target residues of ISGylation. Moreover, ISGylation promotes the E3 ubiquitin ligase activity of STUB1, causing a decrease in levels of oncogenic c-Myc, one of its ubiquitination targets, in A549 cells and inhibiting A549 cell growth. In conclusion, the present study demonstrates that covalent ISG15 conjugation produces a novel STUB1 regulatory mode

that enhances the tumor-suppressive activity of STUB1, thereby contributing to the antitumor effect of type I IFN.

Disclosures: L. Yoo: None. **C. Park:** None. **K.C. Chung:** None.

Poster

129. Cellular Mechanisms of Parkinson's Disease II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 129.09/E35

Topic: C.03. Parkinson’s Disease

Support: NRF-2018R1A2B2003955

Title: Leucine-rich repeat kinase 2 phosphorylates histone deacetylate 3 and facilitates neuronal cell death through epigenetic histone modification

Authors: K. A. HAN, ***J. H. PARK**, *K. C. CHUNG;
Yonsei Univ., Seoul, Korea, Republic of

Abstract: Parkinson's disease (PD) is characterized by slow, progressive degeneration of dopaminergic neurons in the substantia nigra. The cause of neuronal death in PD is largely unknown, but several genetic loci, including leucine-rich repeat kinase 2 (LRRK2), have been identified. LRRK2 has guanosine triphosphatase (GTPase) and kinase activities, and mutations in LRRK2 are the major cause of autosomal-dominant familial PD. Histone deacetylases (HDACs) remove acetyl groups from lysine residues on histone tails, promoting transcriptional repression via condensation of chromatin. Here, we demonstrate that LRRK2 binds to and directly phosphorylates HDAC3 at Ser-424, thereby stimulating HDAC activity. Specifically, LRRK2 promoted the deacetylation of Lys-5 and Lys-12 on histone H4, causing repression of gene transcription. Moreover, LRRK2 stimulated nuclear translocation of HDAC3 via the phoshorylation of karyopherin subunit α2 and α6. HDAC3phosphorylation and its nuclear translocation were increased in response to 6-hydroxydopamine (6-OHDA) treatment. LRRK2 also inhibited myocyte-specific enhancer factor 2D activity, which is required for neuronal survival. LRRK2 ultimately promoted 6-OHDA-induced cell death via positive modulation of HDAC3. These findings suggest that LRRK2 affects epigenetic histone modification and neuronal survival by facilitating HDAC3 activity and regulating its localization.

Disclosures: K.A. Han: None. **J.H. Park:** None. **K.C. Chung:** None.

Poster

129. Cellular Mechanisms of Parkinson's Disease II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 129.10/E36

Topic: C.03. Parkinson's Disease

Support: Department of Biotechnology, Ministry of Science and Technology, Govt. of India (BT/PR6371/COE/34/19/2013)
Core funds from National Centre for Biological Sciences (NCBS), TIFR
Post-doctoral fellowship from the Department of Biotechnology, Govt. of India
Department of Science and Technology, SERB

Title: Understanding the role of store operated calcium entry in Parkinson's disease using stem cell derived dopaminergic neurons

Authors: *R. GOPURAPPILLY[1], B. DEB[1], P. CHAKRABORTY[1], R. KANNAN[2], G. HASAN[1];
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Abstract: As with many other neurodegenerative disorders, calcium dyshomeostasis is also a hallmark of Parkinson's disease (PD). Importantly, more recently intracellular calcium stores have been implicated in calcium de-regulation in PD through a process known as Store operated calcium entry (SOCE; 1). On depletion of calcium in Endoplasmic Reticulum (ER), STIM (Stromal Interaction Molecule) proteins, the ER calcium sensors, interact with the plasma membrane calcium channel, Orai to replenish the stores. We demonstrate that upon knockdown of ST1M1, human ESC (H9) derived NPCs showed attenuated self-renewal as compared to control cells. Global gene expression patterns were also altered with cell proliferation and DNA replication processes down-regulated through STIM1 knockdown, whereas post-synaptic signaling was identified as an up-regulated process. Phenotypically, this correlated with reduced neurosphere size and number as well as precocious spontaneous differentiation toward the neuronal lineage, as compared to control cells (2). Further we are currently investigating the role of SOCE in altering neuronal gene expression patterns in differentiated neurons in the context of disease pathologies that manifest over several years. Impairment of SOCE and its cellular consequences are being studied in human ESC and iPSC derived dopaminergic neurons with particular emphasis on PLA2G6 (Phospholipase A2 group VI, iPLA2-VIA, PARK-14)-linked PD.

References
1. Zaichick, S. V., McGrath, K. M. & Caraveo, G. The role of calcium signaling in Parkinson's disease. *Disease Model Mechanisms.* 10, 519-535 (2017). 2. Gopurappilly, R., Deb, B. K.,

Chakraborty, P. & Hasan, G. Stable STIM1 Knockdown in Self-Renewing Human Neural Precursors Promotes Premature Neural Differentiation. *Frontiers in Molecular Neuroscience.* 11, 178 (2018).

Disclosures: R. Gopurappilly: None. **B. Deb:** None. **P. Chakraborty:** None. **R. Kannan:** None. **G. Hasan:** None.

Poster

129. Cellular Mechanisms of Parkinson's Disease II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 129.11/E37

Topic: C.03. Parkinson’s Disease

Support: Van Andel Research Institute
NIH R01 NS105432

Title: Proteomic analysis of Parkinson's disease-linked D620N VPS35

Authors: *E. T. WILLIAMS[1], S. ISLAM[2,1], D. MOORE[1];
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Abstract: Parkinson’s disease (PD), the most common neurodegenerative movement disorder, is pathologically characterized by the loss of dopaminergic neurons in the substantia nigra. Mutations in a number genes are known to cause familial forms of PD, including mutations in the *vacuolar protein sorting 35 ortholog* (*VPS35*) gene linked to late-onset, autosomal dominant PD. *VPS35* encodes a core subunit of the retromer complex which functions in endosomal sorting and recycling of transmembrane protein cargo to the *trans*-Golgi network and plasma membrane. It remains unclear how the pathogenic D620N mutation in VPS35 disrupts retromer function to induce neurodegeneration in PD. In this study, we perform proteomic analysis of cellular and rodent models of D620N VPS35 to elucidate whether this mutation causes proteomic alterations at the global or protein interactome level that could underlie D620N VPS35-related toxicity. First, we conducted co-immunoprecipitation (IP) or tandem affinity purification in HEK-293T cells overexpressing VPS35 to determine non-native and native protein interactions of wild-type (WT) and D620N VPS35. Using these cellular models, we show remarkably similar interaction profiles of WT and D620N VPS35, suggesting that the D620N mutation has a subtle effect on VPS35 protein interactions. Since both cellular models are based on VPS35 overexpression, we also conducted proteomic analysis of brain tissue from a D620N VPS35 knock-in (KI) mouse model that expresses VPS35 at endogenous levels. Using IP from hemi-brain and striatal extracts of WT and D620N VPS35 KI mice, we reveal a high degree of similarity between the brain interactomes of WT and D620N VPS35, further suggesting a subtle

effect of the D620N mutation on VPS35 protein interactions. Notably, in both hemi-brain and striatum, we show a decrease in the interaction of TBC1D5 with D620N VPS35. TBC1D5 is a known interactor of the retromer and functions in the endo-lysosomal system as a GTPase-activating protein for Rab7a, however the interaction between TBC1D5 and VPS35 has not been previously reported to be altered by the D620N mutation. A decreased interaction between TBC1D5 and D620N VPS35 could have implications on both retromer and endo-lysosomal function. We also profiled the striatum of WT and KI mice for global proteomic changes induced by the D620N mutation and identify alterations in the levels of several proteins that may represent putative retromer cargo in the brain. Taken together, our study provides a comprehensive evaluation of proteomic alterations induced by the D620N mutation in cells and brain that may provide important insight into the mechanisms of retromer dysfunction in PD.

Disclosures: E.T. Williams: None. **S. Islam:** None. **D. Moore:** None.

Poster

129. Cellular Mechanisms of Parkinson's Disease II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 129.12/E38

Topic: C.03. Parkinson’s Disease

Support: Jim Himelic Foundation
Arizona Biomedical Research Commission

Title: A patient iPSC-based platform for investigating idiopathic Parkinson's disease

Authors: *M. J. CORENBLUM, A. ANNADURAI, K. SHRESTHA, L. MADHAVAN; Univ. of Arizona, Tucson, AZ

Abstract: Human induced pluripotent stem cells (iPSCs) are proving to be a valuable source of patient cells for generating neural phenotypes relevant to Parkinson’s disease. Here we compared iPSC-derived midbrain dopamine (DA) neurons derived from the skin fibroblasts of late-onset idiopathic Parkinson’s disease (PD) subjects and age-matched controls (AMCs). Specifically, we comparatively analyzed several neurodegeneration relevant aspects including DA neuron survival, differentiation, morphology, mitochondrial function, oxidative stress, and autophagy. Our data indicate that the iPSCs from PD subjects had lower viability rates, and a reduced capacity to generate neurons when induced to differentiate via a floorplate dual SMAD inhibition method. At day 42 post-differentiation, the efficiency of tyrosine hydroxylase positive (TH^+) DA neuron generation did not differ between the PD and AMC cultures. However, the morphology of the DA neurons from PD subjects appeared altered in that the cells displayed a smaller soma size, reduced number of neurites, and shorter neurite lengths, compared to AMC cultures. Moreover, PD DA neurons expressed higher levels of reactive oxygen species, and compromised

mitochondrial function. In addition, it was found that autophagy was dysregulated in the PD neurons, and was associated with increased protein levels of alpha-synuclein, as compared to AMC cells. Our current studies are further extending these findings by examining the activity profile (electrophysiological and others) of the iPSC-derived DA neurons. In summary, our study develops an iPSC-based neuronal model that captures a phenotype relevant to the study of idiopathic PD, as well as biomarker and therapeutic testing.

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Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.01/E39

Topic: C.03. Parkinson's Disease

Support: NIH grant NS089622
NIH grant NS100876

Title: Truncation of α-synuclein potentiates pathological inclusion formation and is abundant in human disease

Authors: Z. A. SORRENTINO, N. VIJAYARAGHAVAN, J. CALDWELL, K.-M. GORION, B. I. GIASSON;
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Abstract: Truncation of α-synuclein potentiates pathologic inclusion formation and is abundant in human disease
α-Synuclein (αsyn) aggregates into amyloid fibrils in multiple neurodegenerative diseases where these fibrils form characteristic pathological inclusions such as Lewy bodies (LBs). These fibrils have prion-like properties in that they can induce misfolding of endogenous αsyn into more amyloid fibrils. The mechanisms initiating αsyn aggregation into fibrils are unclear, but ubiquitous post-translational modifications of αsyn present in LBs may play a role. Specific C-terminally (C)-truncated forms of αsyn are present within human pathological inclusions and form under physiological conditions likely in lysosome-associated pathways. Herein, we first biochemically characterized the *in vitro* aggregation propensities, amyloid fibril structures, and prion-like properties for eight of the most common physiological C-truncated forms of αsyn. C-truncated αsyn aggregated into amyloid more readily than full-length (FL) αsyn and formed fibrils with unique morphologies. Furthermore, in cultured human cells C-truncated forms of αsyn similarly formed amyloid inclusions much more readily than FL αsyn, and in primary neuronal cultures co-polymers of C-truncated and FL αsyn were potent prion-like seeds. To

confirm that pathologic significance of αsyn truncation, monoclonal antibodies specific for two truncated forms of αsyn identified to potently form amyloid fibrils were generated and utilized for immunohistochemical staining of tissue from human disease. These truncated forms of αsyn were demonstrated to form prominent inclusions in dementia with Lewy bodies or Lewy body variant Alzheimer's disease but not Alzheimer's disease or healthy controls, suggesting that truncated αsyn aggregation is specific for synucleinopathies and may be important in pathogenesis due to the truncation induced increase in amyloid forming potential from which prion-like spread can follow. Lastly, rAAV constructs engineered to overexpress three truncated forms of αsyn were produced and intra-cerebrally injected into transgenic mice to assess the formation of spontaneous pathologic inclusions.

Disclosures: Z.A. Sorrentino: None. **N. Vijayaraghavan:** None. **J. Caldwell:** None. **K. Gorion:** None. **B.I. Giasson:** None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.02/E40

Topic: C.03. Parkinson's Disease

Support: European Union's Horizon 2020 research and innovation programme under grant agreement No. 721802

Title: Systematic comparison of the molecular mechanisms underlying the spreading of pathology in different neurodegenerative diseases

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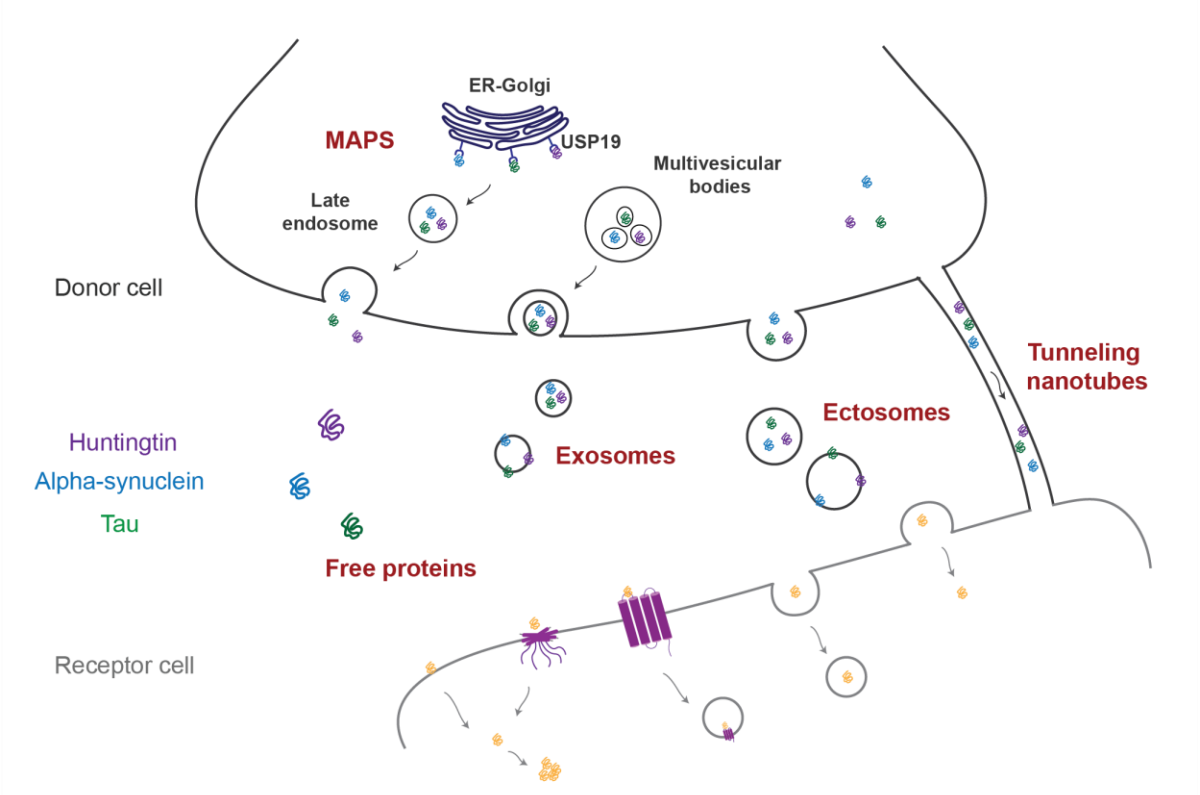
Abstract: The misfolding and aggregation of disease-related proteins is a common hallmark among several neurodegenerative diseases. This includes alpha-synuclein (aSyn) in synucleinopathies, tau in tauopathies, and huntingtin (Htt) in Huntington's disease. Recent studies demonstrated that these proteins can be transferred from cell-to-cell and seed pathology throughout the brain, contributing for disease progression and neurodegeneration.
Several mechanisms have been proposed for the spreading of aSyn, tau and Htt. However, it is not clear what is the relative contribution of each of the possible mechanisms for the spreading of the different proteins. To address this, we are performing a systematic assessment of the release of aSyn, tau and Htt (i) in free form, (ii) via the misfolding-associated protein secretion (MAPS) pathway, that uses USP19 to export aberrant cytosolic proteins, (iii) in extracellular vesicles (EVs), as ectosomes and exosomes, and (iv) via tunneling nanotubes (TNTs).
To evaluate protein secretion, conditioned media of HEK cells stably expressing the different

proteins was collected after 24hours. Purification of EVs was performed by differential centrifugation. To study protein transfer via TNTs, CAD cells expressing the different proteins were imaged after 24hours.
Our results show that aSyn, tau and Htt are secreted to the cell media at different levels. Furthermore, co-expression with USP19 slightly increases their secretion to the cell media. Interestingly, aSyn and tau are present in higher levels in ectosomes than in exosomes, while 25QHtt and 103QHtt are present in identical levels. The EVs can be further internalized in primary neurons. Finally, CAD cells form TNTs to transfer the different proteins to neighbouring cells.
Our study demonstrates that aSyn, tau and Htt can be transferred between cells through similar mechanisms, but reveals that different proteins prefer specific pathways. Therefore, our findings suggest that care must be taken when considering the targeting of spreading of pathology in different neurodegenerative diseases.



Disclosures: **I.C. Brás:** None. **T.F. Outeiro:** None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.03/E41

Topic: C.03. Parkinson's Disease

Support: DFG - CNMPB

Title: Homogenous generation of dopaminergic neurons from multiple hiPSCs lines by transient expression of transcription factors

Authors: S. MAHAJANI, A. RAINA, C. FOKKEN, S. KUEGLER, *M. BAEHR;
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Abstract: A major hallmark of Parkinson´s disease is loss of dopaminergic neurons in the *substantia nigra pars compacta*(SNpc). The pathophysiological mechanisms causing this relatively selective neurodegeneration are poorly understood, and thus experimental systems allowing to study dopaminergic neuron dysfunction are required. Induced pluripotent stem cells (iPSCs) differentiated towards a dopaminergic neuronal phenotype offer a valuable source to generate human dopaminergic neurons. However, currently available protocols result in a highly variable yield of dopaminergic neurons depending on the source of hiPSCs. We have now developed a protocol based on HBA promoter-driven transient expression of transcription factors by means of AAV vectors, that allowed to generate very consistent numbers of dopaminergic neurons from four different hiPSC lines. We also demonstrate that AAV vectors expressing reporter genes from a neuron-specific hSyn1 promoter can serve as surrogate markers for maturation of hiPSC-derived dopaminergic neurons. Dopaminergic neurons differentiated by transient expression of Lmx1a, as compared to differentiated glutamatergic neurons, showed more neurodegeneration through α-synuclein overexpression but were not sensitive to γ-synuclein overexpression, suggesting that they are well suited to study neurodegeneration in the context of Parkinson's disease.

Disclosures: S. Mahajani: None. **A. Raina:** None. **C. Fokken:** None. **S. Kuegler:** None. **M. Baehr:** None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.04/E42

Topic: C.03. Parkinson's Disease

Support: PIP-CONICET 0183
PICT Raices-MINCyT 2015–3205
PIUNT-UNT D644/1
program Investissements d'Avenir [ANR-10-IAIHU-06]
Translational Research Infrastructure for Biotherapies in Neurosciences [ANR-11-INBS-0011-NeurATRIS]

Title: Incyclinide, a non-antibiotic tetracycline, prevents alpha-synuclein aggregation and disrupts fibrillary forms of the protein

Authors: *R. RAISMAN-VOZARI[1], F. GONZÁLEZ-LIZÁRRAGA[2], D. PLOPER[2], S. B. SOCIAS[2], C. L. AVILA[2], P. P. MICHEL[1], R. CHEHIN[2];
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Abstract: Parkinson's disease (PD) is a progressive neurodegenerative illness with age being the main risk factor for its development. The increase in longevity in most Western countries imposes the urgency of finding a disease-modifying approach for its treatment. Several promising molecules targeting PD pathogenic pathways have been proposed but with limited success. Targeted pathways include notably those regulating alpha-synuclein aggregation, oxidative stress and neuroinflammation. Due to the multifactorial characteristics of the disease, a multitarget drug with efficient activity against these processes is required. With this in mind, we previously demonstrated that the tetracycline doxycycline (DOX) reshapes oligomeric species of the PD protein alpha-synuclein reducing their toxicity, seeding capacity and propensity to form toxic fibrillary species. In addition, DOX showed anti-inflammatory and neuroprotective effects in PD models. However, the antibiotic activity of DOX represents a possible hurdle for its repositioning in long-term treatments. Thus, we sought to find a non-antibiotic DOX analog with potent anti-amyloidogenic properties, making this drug an ideal candidate for repurposing to treat PD and conceivably other amyloid-associated disorders. In order to detect putative anti-amyloidogenic ready to use molecules, we used chemicoinformatic methods to extract a novel structural motif capable of interacting with cross-beta structures (Cbeta-IM) and screened a number of pre-existing compounds using this strategy. Incyclinide was selected among tetracyclines because *i*) it contains this motif in a planar structure, *ii*) crosses the BBB, and *iii*) is available for repurposing. Using a combination of biophysical techniques (fluorescence and

infrared spectroscopy, electron microscopy, small-angle X-Ray scattering) together with cell biology approaches, we characterized its impact against alpha-synuclein toxic aggregates. Incyclinide had an exceptional ability to reshape alpha-synuclein oligomers towards less toxic and non-seeding species. Moreover, Incyclinide was able to disrupt mature fibrils and was more efficient than DOX at inhibiting neuroinflammatory processes initiated by microglial cells. The anti-amyloidogenic and anti-inflammatory properties of Incyclinide, together with its ability to cross the BBB, position Incyclinide as an ideal drug to be repurposed in PD and possibly in other amyloid-associated diseases. We also propose the Cbeta-IM as a molecular signature to be exploited for identifying novel drugs of interest for neuroprotection in PD.

Disclosures: R. Raisman-Vozari: None. **F. González-Lizárraga:** None. **D. Ploper:** None. **S.B. Socias:** None. **C.L. Avila:** None. **P.P. Michel:** None. **R. Chehin:** None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.05/E43

Topic: C.03. Parkinson's Disease

Support: Parkinson Canada New Investigator Award
Croucher Foundation

Title: Comparison of recombinant alpha-synuclein fibrils created through continuous shaking or periodic sonication

Authors: *R. W. L. SO, A. LAU, H. H. C. LAU, E. STUART, J. C. WATTS;
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Abstract: Parkinson's disease (PD), multiple system atrophy (MSA), and dementia with Lewy bodies (DLB) are neurodegenerative diseases with very different symptoms and disease progression rates. While they are collectively termed "synucleinopathies" as they all display filamentous aggregates composed of phosphorylated alpha-synuclein in the brain, the afflicted brain regions and cell types are disease-specific. Previous works have shown that, like prions, alpha-synuclein can aggregate as different conformational "strains." We hypothesize that different strains are responsible for producing the variable disease phenotypes in synucleinopathy patients. To investigate this hypothesis, we are polymerizing recombinant alpha-synuclein using a variety of conditions to create a conformationally diverse array of fibrils, then intracerebrally inoculating transgenic mice with these putative strains to determine the diseases they cause. We have found that, under the same protein concentration and buffer conditions, human alpha-synuclein monomers formed different fibril strains depending on

whether they were generated through continuous shaking (thermomixing) for 7 days or through periodic sonication (protein misfolding cyclic amplification, PMCA) for 2 days. These conformational differences were visualized by biochemical assays such as protease resistance and conformational stability. Upon intracerebral inoculation into the right parietal lobe of hemizygous M83 transgenic mice, both thermomixed and PMCA fibrils induced deposition of phosphorylated alpha-synuclein as well as progressive neurological disease. With further in vitro and in vivo characterization of these fibrils, it will be possible to open new avenues for modelling the heterogeneity of human synucleinopathies in mice, perhaps with different recombinant fibril strains inducing different diseases.

Disclosures: R.W.L. So: None. **A. Lau:** None. **H.H.C. Lau:** None. **E. Stuart:** None. **J.C. Watts:** None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.06/E44

Topic: C.03. Parkinson's Disease

Support: AMED 19dk0207046

Title: Systematic analysis on the seeding activity of familial mutant forms of α-synuclein

Authors: *T. TOMITA, N. XU, A. TARUTANI, G. ITO;
The Univ. of Tokyo, Tokyo, Japan

Abstract: Parkinson disease (PD) is one of the most common neurodegenerative diseases. PD is pathologically characterized by the deposition of aggregated α-synuclein proteins as Lewy bodies and Lewy neurites. Six missense mutations in the α-synuclein gene (i.e., A30P, E46K, H50Q, G51D, A53E, and A53T) have been identified in familial PD cases, implicating the pathological importance of α-synuclein. However, it remains unclear how pathogenic mutations in α-synuclein alter the propagation. In this study, we examined seeding and propagation activities of α-synuclein mutants in *in vitro* fibrillization assays, primary cultured neurons and wild-type mouse brain. First, the seeded aggregation with mouse α-synuclein monomer was measured by Thioflavin assay. We found that all the mutants, but not G51D, showed a seeding activity. Next, rat cortical primary neurons were treated with the α-synuclein seeds for 7 days, and all mutants were able to induce phosphorylation of endogenous α-synuclein. Finally, we tested the propagation of α-synuclein pathology in wild-type mouse brains unilaterally injected with the α-synuclein seeds into the striatum. One month after injection, we confirmed the induction and spreading of phosphorylated α-synuclein. These results will elucidate the

difference between wild-type and mutant α-synuclein in terms of the seeding activity both *in vitro* and *in vivo*, which might explain the pathogenicity of the mutations.

Disclosures: **T. Tomita:** None. **N. Xu:** None. **A. Tarutani:** None. **G. Ito:** None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.07/F1

Topic: C.03. Parkinson's Disease

Support: SERB Grant EMR/2018/000336
IIT Gandhinagar
DBT-JRF Fellowship

Title: Dichotomy of carbamylation: A comparison of aggregation propensity of carbamylated alpha synuclein and fibril core peptides

Authors: *J. D. GADHAVI, S. GUPTA;
Indian Inst. of Technol. Gandhinagar, Gandhingar, India

Abstract: Parkinson's Disease (PD) is second most prevalent neurodegenerative disorder after Alzheimer's Disease. Deposition of Lewy bodies comprising of α-synuclein protein aggregates is one of the major pathogenic mechanism for the neuronal damage in the Substantia Niagra region in PD affected brains. Literature reports have pointed at the inverse relationship of smoking with Parkinson's Disease Progression. Though the exact mechanism is unknown, smoking increases the major production of Isocyanic acid (HCNO), a major source of protein carbamylation, which could affect α-synuclein aggregation. While there are several studies reporting carbamylation as a pro-aggregation factor, others hint at a protective role. In the present study, we aim to resolve this ambiguity in the context of α-synuclein. Carbamylation is one of the age-related non-enzymatic post-translational modification which primarily occurs at N^{ε} group of lysines residues. We used full-length α-synuclein protein as well as three core peptide sequences derived from of α-synuclein fibrils (68-78, 71-82, and 64-74) and performed carbamylation by KCNO in a mild acidic environment. Here, fibril core peptides were synthesized by Fmoc based Solid Phase Peptide Synthesis and α-synuclein protein was recombinantly expressed. We studied effect of carbamylation on aggregation kinetics and the characteristics of the resulting fibrils by various biophysical techniques such as ThT assay, ANS assay, Congo red birefringence microscopy, AFM, SEM, and cytotoxicity. We observed that while carbamylation enhances aggregation of fibril core peptides and promoted amyloidogenesis, at the full-length level, the aggregation of α-synuclein was significantly inhibited. Furthermore, acceleration/inhibition was strongly correlated with the degree of carbamylation. These findings suggest that carbamylation may act

as a protective post-translational modification for full-length α-synuclein, however, it could well be one of the major age-dependent initiating factors for fibril assembly.

Disclosures: J.D. Gadhavi: None. **S. Gupta:** None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.08/F2

Topic: C.03. Parkinson's Disease

Title: Effects of aging on inflammatory responses and propagation of alpha-synuclein preformed fibrils in wild type mouse

Authors: *M. IBA[1], R. A. MCDEVITT[2], M. SALLIN[3], C. KIM[1], B. SIEGARS[3], R. ROY[4], S. KWON[1], R. SEN[4], J. M. SEN[3], E. MASLIAH[1];
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Abstract: α-synuclein (α-syn) is a presynaptic protein also found in myeloid cells which progressively accumulates in neuronal and non-neuronal cells in age related neurodegenerative diseases such as Parkinson's disease (PD), Dementia with Lewy bodies (DLB) and Alzheimer's Disease (AD). Current evidence suggests that altered immune responses leading to neuroinflammation might be involved in neurodegeneration in DLB/PD. Recent studies have shown that intracranial injection of α-syn preformed fibrils (pff) into the brains of wild type animals can promote α-syn pathology and behavioral deficits like mimicking DLB/PD (Luk et al., 2012; Henderson et al., 2019). Although protein aggregation and propagation has been extensively investigated in the models, very little is known about the role of aging and innate immune responses in neurodegeneration in models of DLB/PD. We hypothesize that neurodegeneration in the α-syn pff model might be associated with an age dependent increase in cytotoxic T cell responses. For this purpose, α-syn pff or PBS was injected into the striatum of wild type mice at 4 and 18 months of age and analyzed for behavioral deficits, neuropathology and immune responses at 1- and 3-months post injection periods. As expected following intracranial injection of α-syn pff into striatum there was extensive accumulation of α-syn that extended to the amygdala, substantia nigra and neocortex with greater pathology when comparing the 1 vs the 3 months post injection of both mice cohorts, but aged mice cohort showed significantly increased α-syn pathology compare to young mice cohort that was accompanied by infiltration of T cells, microgliosis and astrogliosis. Interestingly, we observed greater increase of T cell numbers detected by CD3 antibody in 1-month post injection of aged mice cohort compared to young mice cohort. To assess motor function, we compared α-syn pff- and PBS-injected young and aged mice on the wire hang test. Although aged mice showed an

overall deterioration of baseline motor function, α-syn pff injections further enhanced this deficit to a degree not seen in the young cohort. Additional studies of analyzing immune cell distributions with flow cytometry and microglia RNA sequence are underway to better understand immune response of intracranial injection of α-syn pff.

Disclosures: M. Iba: None. **R.A. McDevitt:** None. **M. Sallin:** None. **C. Kim:** None. **B. Siegars:** None. **R. Roy:** None. **S. Kwon:** None. **R. Sen:** None. **J.M. Sen:** None. **E. Masliah:** None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.09/F3

Topic: C.03. Parkinson's Disease

Support: NS083054

Title: Induction of adaptive immunity increases the pathogenicity of alpha synuclein and leads to the loss of dopaminergic neurons in the nigra of A53T transgenic mice

Authors: *A. R. ROY[1], K. PAHAN[2];
[2]Neurolog. Sci., [1]Rush Univ. Med. Ctr., Chicago, IL

Abstract: Neuropathological hallmarks of Parkinson's disease (PD) are the presence of intracytoplasmic inclusions containing alpha-synuclein (asyn) and the demise of dopaminergic neurons in the nigra. While A53T asyn transgenic (A53T-Tg) mice are widely used to study asyn pathology, a potential limitation of this model is the absence of dopaminergic neuronal loss in the nigra. Here, we delineate the importance of adaptive immunity in driving nigrostriatal pathology in A53T-Tg mice. While the infiltration of T cells into the nigra of A53T-Tg mice was negligible, twice weekly supplementation of RANTES and eotaxin, chemokines that are involved in T cell trafficking, induced continuous T cell infiltration to the nigra and stimulated the pathogenicity of asyn. Interestingly, supplementation of RANTES and eotaxin drove asyn into nucleus that was absent in untreated A53T-Tg mice. Moreover, RANTES and eotaxin supplementation also led to the death of dopaminergic neurons in the nigra, the loss of neurotransmitters in the striatum and increased motor impairment in A53T-Tg mice. These results suggest that adaptive immunity may be an important component in the regulation of asyn pathogenicity and dopaminergic neuronal loss in the nigra and that targeting this arm of the immune system may halt the disease progression in PD patients.

Disclosures: A.R. Roy: None. **K. Pahan:** None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.10/F4

Topic: C.03. Parkinson's Disease

Support: NINDS/NIA R01 NS078165-08 to JRM
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NIH NINDS R01NS083845-05 to TB
Stanley Fahn Award 2018: Parkinson's Disease Foundation to TB
Michael J. Fox LEAPS Grant 2014 to TB

Title: Effects of a-synuclein isolated from human brain tissue on synaptic vesicle trafficking *in vivo*

Authors: *C. ROMAN-VENDRELL[1], A. T. MEDEIROS[2], J. B. SANDERSON[2], H. JIANG[3], D. J. SELKOE[3], T. BARTELS[4], J. R. MORGAN[1];
[1]Eugene Bell Ctr. for Regenerative Biol. and Tissue Engin., Marine Biol. Lab., Woods Hole, MA; [2]Brown Univ., Providence, RI; [3]Neurol., Brigham & Women's Hosp, Boston, MA; [4]Dementia Res. Inst., Univ. Col. London, London, United Kingdom

Abstract: Synucleinopathies are neurodegenerative diseases characterized by the abnormal aggregation of α-synuclein. These include Parkinson's disease and Dementia with Lewy Bodies (DLB). Under physiological conditions, α-synuclein is localized to presynaptic terminals where it regulates synaptic vesicle (SV) trafficking. Neurons normally express monomeric and multimeric forms of α-synuclein, and evidence suggests that altering the balance between these molecular species leads to aggregation, synaptic dysfunction, and neurotoxicity. However, the precise effects of distinct molecular species of α-synuclein on neuronal function, including SV trafficking, remain unclear. To address these questions, we have acutely introduced different molecular species of α-synuclein to lamprey reticulospinal synapses and assessed the effects on SV trafficking using electron microscopy. Previous studies from our lab showed that introduction of excess recombinant monomeric or dimeric human α-synuclein led to a loss of SVs, expansion of the plasma membrane, and increased numbers of clathrin-coated intermediates, indicating impaired SV endocytosis. Interestingly, monomeric and dimeric α-synuclein inhibited different stages of clathrin-mediated endocytosis (CME). Here, we expand this knowledge by focusing on the effects of physiological α-synuclein isolated from the brains of neuropathologically normal human subjects. Physiological α-synuclein was cross-linked and isolated by size-exclusion chromatography (SEC), and the sample comprised a mixed population of tetrameric and monomeric α-synuclein with more of the former. Unlike recombinant

monomeric or dimeric α-synuclein, exogenous physiological α-synuclein induced a more moderate phenotype suggesting mild defects on intracellular SV trafficking. Specifically, it caused a reduction in the size of the SV cluster, an increase in the number of cisternae (large, atypical vesicles >100 nm in diameter), but no significant effects on the plasma membrane or CME. Confirming the specificity of this effect, introduction of the same SEC fraction immunodepleted of α-synuclein induced no significant changes on synaptic morphology. Going forward, we will determine how α-synuclein isolated from brains of DLB patients, which contain high levels of aggregated α-synuclein, alters SV trafficking. These results provide insight into the cellular mechanisms by which distinct α-synuclein species contribute to synucleinopathy phenotypes.

Disclosures: C. Roman-Vendrell: None. **A.T. Medeiros:** None. **J.B. Sanderson:** None. **H. Jiang:** None. **D.J. Selkoe:** None. **T. Bartels:** None. **J.R. Morgan:** None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.11/F5

Topic: C.03. Parkinson's Disease

Support: National Institutes of Health (R21NS108025)
Department of Veterans Affairs (I01BX003033)

Title: Oral administration of cinnamon mitigates Lewy body pathology in A53T mouse model of Parkinson's disease

Authors: *S. RAHA, D. DUTTA, K. PAHAN;
Neurosci., Rush Univ., Chicago, IL

Abstract: Parkinson's disease (PD) is one of the most common neurodegenerative movement disorder of the CNS that predominately affects dopaminergic neurons in a specific area of the brain called substantia nigra, thereby resulting in a progressive loss of coordination and movement. One of the major pathological hallmarks of PD is the formation of Lewy bodies, which are characterized by the accumulation of α-synuclein (α-syn) in the CNS. Till date, no effective cure is available, although there are different lines of treatment that ameliorate clinical symptoms temporarily. Cinnamon is a widely-used spice and flavoring material for deserts, candies, chocolate, etc. This study highlights the importance of cinnamon in reducing Lewy body pathology in A53T mouse model of PD. Mice were orally administered with ground cinnamon and various regions of the brain including the nigra, cortex, hippocampus, and brain stem were histologically examined as well as quantified for α-syn. Our results showed a significant decrease in the levels of α-syn deposits in cinnamon-fed A53T mice as compared to untreated

A53T mice. In addition, cinnamon-fed A53T mice exhibited improvement in their motor functions. Therefore, cinnamon may find use in lowering Lewy body pathology. Supported by a merit award (I01BX003033) from U.S. Department of Veterans Affairs and a grant from National Institutes of Health (R21NS108025).

Disclosures: **S. Raha:** None. **D. Dutta:** None. **K. Pahan:** None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.12/F6

Topic: C.03. Parkinson's Disease

NS075321
NS097799
Michael J. Fox Foundation Consortium to Develop an Alpha-Synuclein Imaging Agent

Title: Regional distribution of fibrillar alpha-synuclein, amyloid beta and tau in Lewy body dementia

Authors: *D. DHAVALE[1], R. MILLER[1], K. GEHRKING[2], J. O'SHEA[1], J. LIU[1], C. BUDDHALA[2], N. CAIRNS[1], J. CIRRITO[1], M. CAMPBELL[1], J. PERLMUTTER[1], P. KOTZBAUER[1];
[1]Neurol., [2]Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Parkinson's disease (PD) is defined by accumulation of fibrillar alpha-synuclein (Asyn) in Lewy bodies and Lewy neurites. The development of dementia in PD, also known as Lewy Body Dementia (LBD), is associated with pathologic accumulation of Asyn in the neocortex. Amyloid-beta (Ab) plaques and tau-containing neurofibrillary tangles are also observed in some LBD cases. Our previous studies indicate that widespread fibrillar Ab deposition occurs in approximately 60% of LBD cases, and that accumulation of Ab is associated with shorter survival rates in LBD patients. We used sequential extraction methods to isolate fibrillar Asyn, Ab and tau from eight brain regions (caudate, anterior cingulate gyrus (ACG), middle frontal gyrus (MFG), inferior parietal lobule (IPL), precuneus, visual association cortex (VAC), hippocampus (HC) and amygdala) from 15 LBD autopsy cases. For comparison, we also isolated fibrillar protein from neocortical regions in 7 Alzheimer's Disease (AD) and 5 neurologically normal control autopsy cases. We utilized enzyme-linked immunosorbent assays (ELISA) to quantify levels of fibrillar Asyn, phosphorylated Asyn (pAsyn), amyloid-beta (1-42), tau and phosphorylated tau (pTau). We observed widespread fibrillar Asyn accumulation, with the highest levels in amygdala, MFG and caudate. In 8 out of 15 (55 %) LBD cases, we also

detected widespread Ab accumulation, and levels of fibrillar Ab correlated with levels of fibrillar Asyn in the neocortical regions (MFG, IPL, Prec). Only 1 out of 15 (6%) LBD cases had significant tau and ptau accumulation. Levels of fibrillar Ab and tau were substantially higher in AD cases compared to LBD. These results demonstrate distinct patterns of fibrillar protein accumulation in LBD and AD. Abeta deposition occurs in more than 50% of LBD cases and is related to fibrillar Asyn accumulation, but is generally not accompanied by widespread tau accumulation as occurs in AD. Further studies can utilize this quantitative analysis of fibrillar protein accumulation to examine correlations with cognitive phenotypes and disease progression patterns in LBD.

Disclosures: **D. Dhavale:** None. **R. Miller:** None. **K. Gehrking:** None. **J. O'Shea:** None. **J. Liu:** None. **C. Buddhala:** None. **N. Cairns:** None. **J. Cirrito:** None. **M. Campbell:** None. **J. Perlmutter:** None. **P. Kotzbauer:** None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.13/F7

Topic: C.03. Parkinson's Disease

Support: NIH NINDS 5K08NS109287-02

Title: Evaluation of dendritic spine density and turnover using 2-photon *in vivo* imaging before and after viral-mediated overexpression of alpha-synuclein

Authors: S. M. NATHWANI[1], L. M. WAGNER[1], J. E. FLAHERTY[1], Y. M. USACHEV[2], N. S. NARAYANAN[3], *G. M. ALDRIDGE[3];
[1]Univ. of Iowa, Iowa City, IA; [2]Dept Pharmacol, Univ. of Iowa Dept. of Pharmacol., Iowa City, IA; [3]Neurol., Univ. of Iowa Roy J and Lucille A Carver Col. of Med., Iowa City, IA

Abstract: Lewy Body Dementias, including Parkinson's disease and Dementia with Lewy Bodies, are characterized by intracellular alpha-synuclein protein deposits called Lewy bodies, which are prevalent in the cortex of these patients. These diseases cause cognitive changes, including abnormalities in executive function, hallucinations, altered sleep, and fluctuations in consciousness. The cause of these symptoms is still unclear, but it is known that duplication/triplication of alpha-synuclein increases the risk of developing a Lewy Body Dementia. One possibility is that alpha-synuclein aggregates causes synaptic dysfunction in the cortex. The objective of this project is to determine the direct effects of overexpression of alpha-synuclein on cortical neurons by evaluating dendritic spine density and turnover. Dendritic spines are protrusions from the neuron that play a role in excitatory synaptic communication and function. We hypothesized that alpha-synuclein overexpression would lead to a decrease in spine

stability and loss of dendritic spines over time. To test this hypothesis, we first compared mice injected with AAV6 coding for either mCherry or full-length human alpha-synuclein, using rapid Golgi Stain to examine dendritic spines after 2.5 months. Immunohistochemistry showed these injections produced localized overexpression of alpha-synuclein with a punctate pattern suggestive of accumulation at presynaptic sites. Using Golgi staining to evaluate the area of the injection, we found an increase in dendritic spine density after local alpha-synuclein overexpression. Given that these results differ from previously published data from mice with global overexpression of alpha-synuclein, we next decided to evaluate changes *in vivo* to confirm the findings and evaluate their etiology. For this study we are utilizing mice genetically expressing YFP protein in layer V pyramidal neurons (YFP-H line) to evaluate dendritic spine and axonal bouton turnover before and after virally-mediated overexpression of alpha-synuclein. After establishment of a cranial window and initial pre-injection imaging, half of the mice are randomized to injection with AAV6 coding for human alpha-synuclein + mCherry vs. mCherry alone. *In vivo* 2-photon imaging is then conducted in awake animals in one-week increments, in order to observe dynamic changes in these structures, including formation and elimination rates, and the proportion of surviving spines and boutons. These data will help determine the effect of overexpression of alpha-synuclein on cortical neurons overtime in a living, aging system.

Disclosures: S.M. Nathwani: None. **L.M. Wagner:** None. **J.E. Flaherty:** None. **Y.M. Usachev:** None. **N.S. Narayanan:** None. **G.M. Aldridge:** None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.14/F8

Topic: C.03. Parkinson’s Disease

Title: Impairment of lysosomal activity contributes to synucleinopathy and neurodegeneration in *in vitro* models of Parkinson’s disease

Authors: *A. HENRIQUES, M. COMBES, P. POINDRON, N. CALLIZOT;
Neuro-Sys, Gardanne, France

Abstract: Motor symptoms in Parkinson’s disease are caused by the degeneration of the dopaminergic signal in the *substantia nigra*. The loss of dopaminergic neurons is due to mitochondrial impairments and alpha-synuclein aggregation, which are pathological hallmarks in Parkinson’s disease.
Multiple lines of evidence suggest that lysosomes are involved in the degradation of toxic alpha-synuclein in dopaminergic neurons. Mutations on the gene coding for the lysosomal protein GBA1 is a strong risk factor for Parkinson’s disease.
Here, we have investigated lysosomal function in *in vitro* models of Parkinson’s disease. In a

second step, we addressed whether an impairment of the lysosomal activity can directly lead to the loss of dopaminergic neurons.
Mesencephalic neurons were intoxicated with oligomers of alpha-synuclein (250 nM, up to 96 hours), mitochondrial toxins (MPP+, 4 µM, 48 hours) or with the lysosomal toxin CBE (from 50 to 400 µM, 48 hours). Immunocytochemistry was applied to study the survival and neurite network of dopaminergic neurons (positive for tyrosine hydroxylase) and lysosomal function (positive for Lamp1/Lamp2).
Our results showed that mitochondrial stress induced a clear enlargement of lysosomes which is linked to an accumulation of alpha-synuclein in dopaminergic neurons. The direct application of alpha-synuclein oligomers on dopaminergic neurons, cultured in absence of microglial cells, induced a chronic toxicity that led also to lysosomal enlargement.
The application of CBE was toxic for dopaminergic neurons and triggered a dose-dependent lysosomal pathology. Altogether, our results indicate that lysosomal dysfunctions are tightly associated with the alpha-synucleinopathy and the loss of dopaminergic neurons, in *in vitro* models of Parkinson's disease.

Disclosures: A. Henriques: None. **M. Combes:** None. **P. Poindron:** None. **N. Callizot:** None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.15/F9

Topic: C.03. Parkinson's Disease

Support: T32GM008076
Michael J. Fox Foundation

Title: The role of Poly(ADP-ribose) (PAR) in seeding intracellular alpha-synuclein aggregation

Authors: *L. N. PUENTES[1], Z. LENGYEL[2], R. H. MACH[2];
[1]Pharmacol., [2]Radiology, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Introduction: Alpha-synuclein (α-syn) is a protein that has been implicated in Parkinson's disease pathogenesis. Recent research has shown that α-syn preformed fibrils (PFF) activate nuclear protein Poly(ADP-ribose) (PAR) Polymerase-1 (PARP-1) resulting in increased intracellular accumulation of PAR (Kam et al., Science, 2018). The non-covalent interactions between PAR and α-syn result in the formation of PAR-bound α-syn PFF; these PAR-bound fibrils display faster fibrillization kinetics and are more neurotoxic than α-syn PFF alone. Furthermore, *in vitro* studies have shown that PAR seeds and accelerates the aggregation of α-syn. However, the mechanism by which PAR seeds this aggregation and the role that it plays in enhancing α-syn PFF neurotoxicity is largely unknown. In this study, we sought to investigate

the mechanisms by which PAR seeds α-syn aggregation *in vitro* and elucidate the amino acids involved in PAR-binding. **Hypothesis:** We hypothesize that PAR helps nucleate intracellular α-syn aggregation by binding to the N-terminal region of the soluble α-syn protein. **Methods:** Thioflavin T (THT) assays were used to measure the aggregation kinetics of PAR-bound and non-PAR bound α-syn PFF. *In vitro* toxicity assays were performed on neuroblastoma cell lines using either PAR-bound α-syn-PFF, α-syn-PFF or PAR-only. Immunofluorescence (IF) microscopy was used to image PAR and α-syn expression *in vitro* and *ex vivo*. Biochemical methods were used to quantify PAR and α-syn levels on A53T transgenic and nontransgenic mouse brain tissue. Proximity ligation assays (PLA) were performed on cells and on brain tissue samples to investigate interactions between PAR and α-syn. Bioinformatic analysis was performed on the full-length α-syn protein to determine the amino acids involved in PAR binding. **Results:** PAR-bound α-syn PFF fibrillize faster in the presence of PAR. Furthermore, the results from our toxicity assay showed increased toxicity in the PAR-bound α-syn PFF. IF imaging of cells treated with DNA damaging agents showed PARP-1 activation, as well as, PAR and α-syn co-localization. Tissue staining and PLA assays showed areas of co-localization between PAR and α-syn. Based on bioinformatic analysis, it was determined that α-syn has two possible PAR binding sites in the N-terminal region of the protein. **Discussion:** The presence of PAR increases the rate of α-syn fibrillization, PAR-bound α-syn fibrils are more neurotoxic than α-syn fibrils with no PAR. Based on our results, we show that PAR co-localizes with α-syn *in vitro* and *ex vivo*. Furthermore, we show that upon PARP-1 activation, α-syn aggregates and co-localizes with intracellular PAR.

Disclosures: L.N. Puentes: None. **Z. Lengyel:** None. **R.H. Mach:** None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.16/F10

Topic: C.03. Parkinson’s Disease

Support: NTU
The Ministry of Science and Technology

Title: Human alpha-synuclein regulates the export of exosomes

Authors: *P.-C. CHEN[1], C.-T. WANG[1,2,3,4];
[1]Inst. of Mol. and Cell. Biol., [2]Dept. of Life Sci., [3]Neurobio. and Cognitive Sci. Ctr., Natl. Taiwan Univ., Taipei, Taiwan; [4]Genome and Systems Biol. Program, Natl. Taiwan Univ. and Academia Sinica, Taipei, Taiwan

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disease, affecting motor control due to the death of dopamine (DA) neurons in substantia nigra pars compacta (SNc). In past several decades, human α-synuclein (αSyn) aggregation has been found in Lewy bodies and implicated in the pathogenesis of both familial and sporadic PD. In the patients of dementia with Lewy bodies, human αSyn aggregates can been found in brain-derived exosomes, which are thought as one type of extracellular vesicles (EVs) that can be released from donor cells and uptaken by recipient cells. Recent studies showed that EVs can be released from PC12 cells in a calcium-dependent manner. However, it remains unclear how αSyn may regulate the export of different subtypes of EVs. In this study, by adopting PC12 cells as the model, we determined the effects on the EV export by overexpressing human αSyn, the human pathogenic αSyn mutant (A53T), or rat αSyn. The transfected cells were treated by the high-KCl solution for 1 min to stimulate the release of EVs. The KCl supernatant was harvested to collect the different subtypes of EVs, by performing centrifugation at 10,000 g (10K pellets as microvesicles) and followed by 100,000 g (100K pellets as exosomes). These different subtypes of EVs were further detected by the exosome marker CD63 or CD9 using western analysis. Our data showed that the export of the CD63-containing microvesicles was not altered by overexpressing human αSyn, the human pathogenic αSyn mutant (A53T), or rat αSyn. In contrast, the export of the CD63-containing exosomes was increased by overexpressing human αSyn or the human pathogenic αSyn mutant (A53T), but not by overexpressing rat αSyn. Together, our results suggested that the human αSyn may specifically regulate the export of exosomes.

Disclosures: P. Chen: None. **C. Wang:** None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.17/F11

Topic: C.03. Parkinson's Disease

Support: The Danish Innovation Foundation grant 5189- 00051B

Title: Investigating alpha-synuclein pathology in primary mouse neurons

Authors: *H. BORLAND[1], M. AMBJØRN[1], N. DAMSGAARD[1], F. VILHARDT[2], J. PEDERSEN[1], K. FOG[1];
[1]H. Lundbeck A/S, Valby, Denmark; [2]Univ. of Copenhagen, Copenhagen, Denmark

Abstract: Aggregation of α-synuclein has been implicated in several neurodegenerative diseases, e.g. Parkinson's disease, where aggregates propagate in the CNS in a topological and temporal manner akin to spreading. However, not much is known about how endogenous and

exogenous α-synuclein is handled by different cell types in the brain, information which will be valuable in the development of new therapeutic targets. Using primary neurons from α-synuclein-overexpressing mice (F28 strain), we have established a seeding model whereby pre-formed α-synuclein fibrils can be added exogenously and induce intracellular aggregation and phosphorylation, thus modelling one of the disease hallmarks of Parkinson's disease. α-synuclein aggregation and phosphorylation was evaluated by homogenous time-resolved FRET, high-content image screening and western blot. To verify that the aggregation and phosphorylation signals indeed come from endogenous α-synuclein and not the seed material itself, we knocked down the endogenous α-synuclein by siRNA, thus dramatically reducing seeding. For α-synuclein pathology to spread in the CNS, α-synuclein must be taken up, engage endogenous α-synuclein and be released again. We find that protease inhibition leads to α-synuclein release into the extracellular medium, from where it will be available for recipient cells. The effect of protease inhibition on cellular degradation was evaluated by western blot of LC3b, p62 and α-synuclein, and quantified by MSD human α-synuclein kits. Combined, these studies suggest that α-synuclein pathology can be modelled in F28 primary mouse neurons, and that inhibition of cellular degradation may affect the spreading of pathological α-synuclein between cells.

Disclosures: **H. Borland:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **M. Ambjørn:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **N. Damsgaard:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **F. Vilhardt:** None. **J. Pedersen:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **K. Fog:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.18/F12

Topic: C.03. Parkinson's Disease

Support: NIH-COBRE 2P20GM-10365306

Title: The neuroprotective effects of SUMOylation in Parkinson's disease *in vitro* and mouse models

Authors: ***D. K. VERMA**[1], D. WILLIAMS[1], E. CARTIER[1], S. NAM[1], M. MOORE[2], Y. H. KIM[1];
[1]Biol. Deparment, [2]OSCAR, Delaware State Univ., Dover, DE

Abstract: The Small Ubiquitin-like Modifier (SUMO) is a form of post-translational modification that may regulate protein stability and function. The SUMO ligase, Ubc9 conjugates SUMO to its target proteins, however, the role of SUMOylation in dopaminergic

neurons in brain remains unclear for Parkinson's disease (PD) pathology. We hypothesize that the overexpression of Ubc9 protects dopaminergic neurons against oxidative stress. In cell viability (MTT) and cytotoxicity (LDH) assay, the Ubc9-EGFP overexpression protected N27 rat dopaminergic cells against H_2O_2 or MPP+ induced toxicities, compared to EGFP overexpressing control cells. Using CellROX® Deep Red Reagent (Thermo, C10422), we found that cellular reactive oxygen species (ROS) level was significantly lower in Ubc9-EGFP cells than that in EGFP cells, after the exposure of 640 μM MPP+ for 24 hrs. Thereafter, we applied the Ubc9 up- or down-regulation to *in vitro* and *in vivo* models for validating SUMOylation as a potential regulatory target in PD pathological models. In N27 cells, the higher protein level of alpha-synuclein was identified in Ubc9-EGFP cells than in EGFP only cells. Ubc9 knock-down by RNAi in N27 parental cells showed a decrease in alpha-synuclein protein level, suggesting that SUMOylated alpha-synuclein has higher protein stability than non-SUMOylated form.
In immunohistochemistry using confocal microscopy, we identified that dopaminergic neurons in the striatum from Ubc9 overexpressing transgenic mice are more resistant to MPTP toxicities than those from WT siblings. In addition, we found that higher number of dopaminergic cell bodies in the SN from Ubc9-Tg than that from WT siblings. In MPTP-lesioned mice, we also confirmed that ROS level was significantly lower in the striatum of Ubc9 transgenic mice than that of WT siblings after chronic MPTP injection. These results indicate that Ubc9 overexpression significantly reduced the MPTP toxicity in the striatum and SN. Currently we are confirming the findings using Western blots for quantifying the levels of tyrosine hydroxylase (TH) as a dopaminergic marker and of synaptophysin as a synaptic marker, from the tissues of the striatum and brain stem. The most intriguing observation is that chronic MPTP injection strips off the SUMO1 from alpha-synuclein by SUMO proteases (SENPs) in the striatum after immuno-precipitation, which can be a detrimental process in reducing alpha-synuclein protein stability. Our studies suggest that SENPs can be regulatory targets to protect dopaminergic neurons against oxidative stress, implicating that high level of SUMOylation in dopaminergic neurons can slow PD pathology.
Funding: NIH-COBRE 2P20GM-10365306

Disclosures: D.K. Verma: None. **D. Williams:** None. **E. Cartier:** None. **S. Nam:** None. **M. Moore:** None. **Y.H. Kim:** None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.19/F13

Topic: C.03. Parkinson's Disease

Title: Acute and chronic dopaminergic neurotoxicity of alpha-synuclein oligomers: Two distinct mechanisms

Authors: *N. CALLIZOT[1], A. HENRIQUES[2], M. COMBES[1], C. FARRUGIA[1], P. POINDRON[1];
[1]Neuro-Sys, Gardanne, France; [2]Pharmacol., Neuro-Sys, GARDANNE, France

Abstract: The loss of dopaminergic neurons is caused by mitochondrial impairments and alpha-synuclein (a-syn) aggregation, which are pathological hallmarks in Parkinson's disease. It is currently admitted that alpha-synuclein oligomers (a-synO) are one of the key toxic factors triggering the TH expressing cell death and contribute to the progression of symptoms in Parkinson's disease. Multiple lines of evidence suggest that microglial cells are involved in the pathophysiology of this disease. The uptake of released alpha-synuclein by microglia has been evidenced in Parkinson's disease and interpreted as a defensive mechanism at early disease stage. On the other hand, activated microglia cause neuroinflammation and neuronal cells death in the later stages of the disease.We studied the acute and late toxicity of a-synO on TH-expressing neurons and the role of microglial cells in the process of neuronal death. Neurons expressing mesencephalic TH were cultured, in the presence or absence of microglial cells, and injured by a-syn solution (250 nM) from 24 h to 7 days. The survival of TH positive neurons, neurite network and a-syn aggregation were evaluated by immunocytochemistry, microglia activation and cytokine release were also studied. We showed that a-synO toxicity involved 2 distinct mechanisms: a) an acute rapid and indirect mechanism mediated by activated microglial cells (activated by protofibril formations) and cytokine production, and b) a slow and late process (starting 4 days after a-synO application) involving mitochondrial stress and a-syn aggregation in TH neuron cytoplasm. This slow and discrete direct mechanism led to late neuronal death of TH neurons. Altogether, these results show that the acute toxicity of a-syn is mainly supported by microglia activation, whereas the direct neurotoxicity of a-synO is dependent on a slow process leading to TH positive neuron death after several days of contact.

Disclosures: N. Callizot: None. **A. Henriques:** None. **M. Combes:** None. **C. Farrugia:** None. **P. Poindron:** None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.20/F14

Topic: C.03. Parkinson's Disease

Support: Jane and Aatos Erkko Foundation
Academy of Finland
The Finnish Parkinson Foundation

Title: Role for syndecan-3 in alpha-synuclein aggregate internalization to neurons

Authors: *A. PANHELAINEN[1], A. SINGH[1], H. RAUVALA[2], M. SAARMA[1];
[1]Inst. of Biotechnology, Univ. of Helsinki, Helsinki, Finland; [2]Neurosci. Center, Univ. of Helsinki, Helsinki, Finland

Abstract: Neuronal Lewy bodies are the general pathological feature in Parkinson´s disease patient brain. Lewy bodies consist of a common neuronal protein called alpha-synuclein (aSyn) in its pathological aggregated forms. Our data suggest that fibrillar aSyn aggregates bind to heparin sulfate (HS) side chains of certain proteoglycans (PGs) on the neuron surface that triggers their internalization to neurons. A variety of ligands can interact with cell surface HSPG receptors and trigger ligand-receptor internalization. However, from the large variety of different HSPGs existing in extracellular matrix, the identity of the HSPG critical for aSyn aggregate internalization is not known. In the brain, the membrane-associated HSPGs are mainly the transmembrane syndecan-3 and syndecan-2 (Sdc3 and Sdc2) or the Glycosylphosphatidylinositol (GPI)-anchored glypican-1 (Gpc1). Our data suggests that Sdc3 plays a role in aSyn aggregate pathology spreading in the brain.

Disclosures: **A. Panhelainen:** None. **A. Singh:** None. **H. Rauvala:** None. **M. Saarma:** None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.21/F15

Topic: C.03. Parkinson’s Disease

Support: Branfman Family Foundation
CTSI Eli-Lilly Stark Predoctoral Fellowship

Title: Inhibition of alpha-synuclein aggregation and prion-like propagation as intervention strategies to slow the progression of Parkinson’s disease

Authors: *S. DUTTA[1], D. YSSELSTEIN[2], R. V. STAHELIN[1], R. SENGUPTA[1], J.-C. ROCHET[1];
[1]Purdue Univ., West Lafayette, IN; [2]Neurol., Northwestern Univ., Chicago, IL

Abstract: A practical therapeutic goal for Parkinson’s disease (PD) should be slow the progression of the pathology by (i) preventing the formation of new aggregates in existing live dopaminergic neuronal cells; and (ii) inhibiting propagation of the disease by interfering with cell-to-cell spreading of aggregated alpha-synuclein (aSyn), which is thought to be the major toxic species. aSyn has been shown to bind to anionic phospholipid vesicles, and recent studies by our lab and other groups suggest that aSyn-membrane interactions can catalyze the protein’s aggregation at the membrane surface, a process that leads to vesicle permeabilization. We have

identified four heptapeptides that interact with membrane-bound aSyn and inhibit membrane-induced aggregation and vesicle permeabilization. These peptides have been shown to exhibit neuroprotection in a primary midbrain neuronal culture model of PD. Interestingly, NMR studies and other biophysical experiments indicate that the interaction does not involve direct perturbation of aSyn-lipid binding. Current efforts are focused on understanding the detailed mechanism of interaction and testing these peptides and their derivatives for neuroprotective effects in other cellular and *in vivo* models of PD.
In parallel, to understand molecular mechanisms behind the prion-like progression of aSyn aggregates, we have developed a cellular model that enables us to examine the internalization of aSyn PFFs and the fate of the fibrillar seed in the recipient cell. With the use of a pH-dependent fluorophore conjugated aSyn which fluoresce only in the acidic environment, we have been able to monitor the dynamics of sonicated fibril uptake and gain an understanding of the lifetime of aggregates in the endo-lysosomal compartment of recipient neurons. Current studies are focused on elucidating the mechanism of endosomal escape in primary neurons and glial cells via correlative light and electron microscopy (CLEM) and super-resolution microscopy.
Together, these studies will yield insights into the molecular underpinnings of aSyn neuropathology in PD and other synucleinopathy disorders and set the stage for developing therapeutic strategies to slow disease progression.

Disclosures: S. Dutta: None. **D. Ysselstein:** None. **R.V. Stahelin:** None. **R. Sengupta:** None. **J. Rochet:** None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.22/F16

Topic: C.03. Parkinson’s Disease

Support: NIH/NINDS Udall Grant NS53488
Jeff and Anne Keefer Fund
Neurodegenerative Disease Research Fund

Title: Amyloid-beta (aβ) plaques promote seeding and spreading of alpha-synuclein and tau in a mouse model of lewy body disorders with aβ pathology

Authors: *S. P. PATTABHIRAMAN, F. BASSIL, H. J. BROWN, J. IWASYK, E. MEYMAND, T. COX, C. MAGHAMES, D. M. RIDDLE, B. ZHANG, J. Q. TROJANOWSKI, V. M. Y. LEE;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Aβ plaques, tau tangles and, α-synuclein (α-syn) rich Lewy bodies (LBs) are major neuropathological hallmarks of Alzheimer's disease (AD) and Parkinson's disease (PD) with dementia (PDD). Post-mortem studies have shown an overlap of both pathologies in the brains of about 75% of PDD and at least 50% of AD patients, with increased Aβ, tau and α-syn pathological burden correlating with increased severity of both cognitive and motor symptoms. Despite observed co-pathology and the concomitance of motor and cognitive phenotypes, the consequence of the primary amyloidogenic proteins on the secondary pathologies remain poorly understood. To better define the relationship between α-syn burden and Aβ plaque density, we injected α-syn mouse preformed fibrils into transgenic mice with abundant Aβ plaque pathology and found that the presence of Aβ deposits dramatically accelerated α-syn pathogenesis and spread throughout the brains of Aβ baring mice compared to wild-type (WT) littermates while also leading to more abundant Aβ deposition. Remarkably, hyperphosphorylated tau (p-tau) was induced in α-syn mpff-injected Aβ bearing mice. Finally, α-syn mpff-injected mice showed neuron loss, the onset and severity of which correlated with the progressive decline of cognitive and motor performance. Our findings suggest a "feed-forward" mechanism whereby Aβ plaques enhance endogenous α-syn seeding and spreading over time post-injection with mpffs.

Disclosures: S.P. Pattabhiraman: None. **F. Bassil:** None. **H.J. Brown:** None. **J. Iwasyk:** None. **E. Meymand:** None. **T. Cox:** None. **C. Maghames:** None. **D.M. Riddle:** None. **B. Zhang:** None. **J.Q. Trojanowski:** None. **V.M.Y. Lee:** None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.23/F17

Topic: C.03. Parkinson's Disease

Support: NIH/NINDS NS088322

Title: Evolution of alpha-synuclein pathology following a unifocal site of origin

Authors: A. CAPUTO[1], A. LO[2], Y. LIANG[2], E. LUNA[4], B. ZHANG[3], ***K. C. LUK**[2]; [1]Pathology and Lab. Med., Perelman Sch. of Med. at UPenn, Philadelphia, PA; [3]Pathol Lab. Med, Sch. of Med., [2]Univ. of Pennsylvania, Philadelphia, PA; [4]Perelman Sch. of Med. at the Univ. of P, Philadelphia, PA

Abstract: Lewy bodies (LBs) and Lewy neurites (LNs) containing alpha-synuclein (aSyn) characterize Parkinson's disease. Although an association between Lewy pathology accumulation and clinical symptoms is well established, how these deposits originate and target vulnerable cell populations is unknown. We and others have previously shown that aSyn pathology can be experimentally initiated in animals and that misfolded conformations of

recombinant aSyn fibrils induce LB- and LN-like inclusions through a self-propagating mechanism. Here, we compare aSyn pathology patterns at various time points 0.5 to 24 months after fibril-injection into one of three circuits (striatal, hippocampal, and limbic) of C57/Bl6 mice. We report that seeded LBs and LNs in animal models spread to multiple connected nuclei in a predictable pattern consistent with neuroanatomical connectivity, recapitulating a phenomenon observed during human disease progression. The rate of pathology accumulation, as detected by immunohistochemistry, correlates with the known retrograde and anterograde connectivity strength to the site of injection, although inclusions in regions lacking direct afferent or efferent projections to the injection site were also observed indicating trans-cellular transmission of pathology. Furthermore, afflicted regions also exhibited time-dependent increases and decreases in aSyn pathology consistent with the dysfunction and degeneration of inclusion bearing neurons that was supported by Nanostring transcriptomic analysis. Collectively, these data provide new perspectives on the dynamics of synucleinopathy *in vivo* and how aSyn and other misfolded proteins may contribute to neurodegeneration over the duration of disease.

Disclosures: **A. Caputo:** None. **A. Lo:** None. **Y. Liang:** None. **E. Luna:** None. **B. Zhang:** None. **K.C. Luk:** None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.24/F18

Topic: C.03. Parkinson's Disease

Support: Michael J. Fox Foundation Grant
Branfman Family Foundation Grant

Title: Effects of the alpha-synuclein-interacting protein endosulfine-alpha on PD-related neuropathology

Authors: C. CHANDRASEKARAN[1,3], J. HENSEL[1,3], S. DUTTA[1,3], P. MONTENEGRO[1,3], E. FAGGIANI[4], B. DEHAY[4], E. BEZARD[4], J. R. CANNON[2,3], ***J.-C. ROCHET**[1,3];
[1]MCMP, [2]Hlth. Sci., Purdue Univ., West Lafayette, IN; [3]Purdue Inst. for Integrative Neurosci., West Lafayette, IN; [4]Inst. of Neurodegenerative Dis., Bordeaux, France

Abstract: The aggregation of the presynaptic protein alpha-synuclein (aSyn) plays a major role in neurotoxicity in Parkinson's disease (PD) and other synucleinopathy disorders. aSyn undergoes accelerated aggregation in the presence of phospholipid membranes by adopting an exposed alpha-helical structure, a state that favors membrane-induced self-assembly. In turn, aSyn aggregation at membrane surfaces may cause a disruption of dopamine vesicles, leading to

preferential dopaminergic neuronal death in the substantia nigra (SN), a pathological hallmark of PD. Endosulfine-alpha (ENSA), a cAMP-regulated phosphoprotein expressed in the CNS, has been reported to interact with membrane-bound aSyn. We have found that WT ENSA (but not a phosphomimic mutant that fails to interact with membrane-associated aSyn) interferes with aSyn aggregation at the membrane surface and alleviates aSyn-mediated dopaminergic cell death and neurite retraction in primary midbrain cultures. ENSA is downregulated in the frontal cortex of patients with dementia with Lewy bodies (DLB) and shows a trend towards being downregulated in the SN region of PD brains. Collectively, these data provide a strong premise for the hypothesis that ENSA alleviates aSyn neurotoxicity by inhibiting aSyn aggregation at the surface of phospholipid membranes. Currently, we are testing this hypothesis in rodent *in vivo* models by determining the effects of co-expressing ENSA on aSyn neurotoxicity assessed using behavioral and immunohistochemical endpoints. The results of these studies will provide new insights into the molecular underpinnings of aSyn neurotoxicity and ENSA-mediated neuroprotection and set the stage for designing therapies to slow nigrostriatal degeneration in PD patients.

Disclosures: **J. Rochet:** None. **C. Chandrasekaran:** None. **J. Hensel:** None. **S. Dutta:** None. **P. Montenegro:** None. **E. Faggiani:** None. **B. Dehay:** None. **E. Bezard:** None. **J.R. Cannon:** None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.25/F19

Topic: C.03. Parkinson’s Disease

Support: NSERC
MJFF

Title: The role of sirtuin 3 in the pre-formed fibril α-synuclein rat model of Parkinson’s disease

Authors: *J. E. NASH;
Biol. Sci., Univ. of Toronto, Toronto, ON, Canada

Abstract: Parkinson’s disease (PD) is the second most common neurodegenerative disorder, affecting 2% of the population over 65 years of age. Central to the pathological progression of PD is mitochondrial dysfunction, which highlights the importance of studying mitochondrial regulatory proteins in PD. Sirtuin 3 (SIRT3), an NAD+-dependent protein, is the main mitochondrial deactylase and a potential disease-modifying agent. We utilised the recently developed pre-formed fibril (PFF) α-synuclein model of PD, which causes dopaminergic cell death, Lewy body aggregation, and spreading of misfolded αsynuclein through interconnected brain regions when stereotaxically administered into the substantia nigra pars compacta (SNc) of

rats. Unilateral administration of PFF or monomeric α-synuclein three weeks following rAAV overexpression of empty vector, SIRT3-H248Y-myc, and SIRT3-myc allowed for the evaluation of SIRT3's disease modifying potential. In PFF-treated rats, SIRT3 over-expression showed a significant improvement in forelimb asymmetry (46.17 ± 5.046%) compared to control (mono+EV) groups. Unbiased stereological analysis of dopaminergic neuron populations in the SNc revealed that PFFs caused bilateral cell death in the presence of SIRT3 (ipsilateral: 1512 ± 398.4, contralateral: 2966 ± 320.2). This study suggests that SIRT3 may be affecting the pathological progression of Parkinsonism in the PFF rat model of PD.

Disclosures: J.E. Nash: None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.26/F20

Topic: C.03. Parkinson's Disease

Title: Alpha synuclein interacts with calcium modulated proteins in dopaminergic cells

Authors: *D. ERDENGIZ, K. MAGUIRE-ZEISS;
Georgetown Univ., Washington D.C., DC

Abstract: Sporadic and genetic forms of synucleinopathies like Parkinson's disease share invariant pathological hallmarks such as loss of dopaminergic neurons in the substantia nigra pars compacta and intracytoplasmic, eosinophilic proteinaceous inclusions enriched in alpha-synuclein (αSyn) called Lewy bodies and Lewy neurites. αSyn is a 140 amino acid protein that misfolds and aggregates into oligomeric species, which are considered pathogenic. αSyn overexpression has been linked to changes in calcium homeostasis, which could lead to neuronal dysfunction. Here we asked whether synuclein alters calcium-modulated proteins important for dopamine neuron function, such as calmodulin and Cav1.2, the channel pore-forming subunit of L-type VGCCs. Employing a dopaminergic doxycycline-inducible (DOX) αSyn overexpressing cell line (MN9Dsyn) we show that calmodulin in significantly reduced ($p<0.0007$) when synuclein is overexpressed. Gene expression for calmodulin remains unchanged in the presence of αSyn, suggesting that this pathogenic protein affects calmodulin protein degradation. Furthermore, using a proximity ligation assay we show that αSyn and calmodulin interact ($p<0.0001$). In contrast, Cav1.2 levels remain unchanged even though αSyn interacts with this VGCC ($p<0.0001$). We hypothesize that the interactions of αSyn with these specific proteins will lead to a disruption of calcium homeostasis. Next steps will investigate the mechanism underlying the αSyn-mediated decrease in calmodulin expression and whether the interaction of these calcium-modulated proteins with αSyn alters calcium homeostasis.

Disclosures: **D. Erdengiz:** None. **K. Maguire-Zeiss:** None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.27/F21

Topic: C.03. Parkinson's Disease

Support: MJFF
R01

Title: Defining alpha-synuclein species responsible for Parkinson's disease phenotypes in mice

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Abstract: Parkinson Disease (PD) is a neurodegenerative disorder characterized by fibrillar neuronal inclusions composed of α-synuclein. α-Synuclein exists in multiple structural forms including disordered, non-amyloid oligomers, ordered amyloid oligomers, and fibrils. It is critical to understand which conformers contribute to specific PD phenotypes. In this study, we utilized a mouse model to explore the effects of stable amyloid β-sheet oligomers compared to fibrillar α-synuclein. These species were characterized biophysically using transmission electron microscopy, atomic force microscopy, circular dichroism spectroscopy, Fourier transform-infrared spectroscopy, and thioflavin T assays. The different forms of α-synuclein were then unilaterally injected into the striatum to determine their ability to induce PD-related phenotypes. We show that β-sheet oligomers produce a small but significant loss of dopamine neurons in the substantia nigra pars compacta (SNc). Injection of small β-sheet fibril fragments, however, produces the most robust phenotypes; significantly reduction of striatal dopamine terminals, SNc loss of dopamine neurons, and appearance of motor behavior defects were detected. Thus, although the β-sheet oligomers cause some toxicity, the potent effects of the short fibrillar fragments can be attributed to their ability to spread and replicate *in vivo* and hence to the development of PD-related phenotypes. These results suggest that strategies to reduce the formation and propagation of β-sheet fibrillar species could be an important route for therapeutic intervention in PD and related disorders.

Disclosures: **J.M. Froula:** None. **M. Castellana-Cruz:** None. **N.M. Anabtawi:** None. **J. Camino:** None. **S. Chen:** None. **D.R. Thrasher:** None. **J. Freire:** None. **A.A. Yazdi:** None. **S.**

Fleming: None. **C. Dobson:** None. **J. Kumita:** None. **N. Cremades:** None. **L.A. Volpicelli-Daley:** None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.28/F22

Topic: C.03. Parkinson's Disease

Support: NIH / NINDS R01NS092823
NIH / NINDS R21NS107770

Title: Persistence of a-synuclein aggregates occurs by disrupting stress-induced cellular clearance in patient-derived iPSC midbrain neurons

Authors: L. K. CUDDY, W. W. WANI, M. L. MORELLA, K. TSUTSUMI, C. PITCAIRN, N. R. BELUR, F. ZUNKE, K. FREDRIKSEN, ***J. R. MAZZULLI**;
Northwestern Univ., Chicago, IL

Abstract: Synucleinopathies including Parkinson's disease (PD) and dementia with Lewy bodies (DLB) are characterized by a slow and persistent accumulation of insoluble a-synuclein (a-syn) aggregates. Previous studies have indicated that a-syn perturbs lysosomal function, however the mechanisms are not completely understood. We find that a-syn disrupts the physiological response to lysosomal stress by impeding SNARE protein ykt6 that is involved in regulated protein trafficking of lysosomal hydrolases. Overexpression or pharmacological activation of ykt6 can activate lysosomal function and reduce pathological a-syn aggregates in PD patient derived iPSC midbrain models. Our study indicates that a-syn creates a permissive environment for aggregate persistence by inhibiting stress-induced cellular clearance, and provide a novel therapeutic strategy to restore lysosomal function and reduce protein aggregation by augmenting protein trafficking.

Disclosures: L.K. Cuddy: None. **W.W. Wani:** None. **J.R. Mazzulli:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lysosomal Therapeutics, Inc, Lysosomal Therapeutics, Inc. **M.L. Morella:** None. **K. Tsutsumi:** None. **C. Pitcairn:** None. **N.R. Belur:** None. **F. Zunke:** None. **K. Fredriksen:** None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 130.29/F23

Topic: C.03. Parkinson's Disease

Title: Phenotypic characterization of synuclein pathology in an intracranial seeding A30P synuclein mouse model of Parkinson's disease

Authors: *D. TOOLAN, R. C. GENTZEL, S. JINN, K. TANIS, J. SCHACHTER, S. M. SMITH, J. N. MARCUS;
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Abstract: Substantial evidence links α-Synuclein (αSyn) protein to both familial and sporadic Parkinson's disease (PD). In human PD brain, α-Syn accumulates in Lewy bodies/neurites and is aggregated, detergent insoluble and phosphorylated at serine 129 (pS129). The accumulation and spread of aggregated αSyn is thought to be toxic leading to disease progression. Development of animal models to study αSyn aggregation and recapitulate pathological features of PD would enable evaluation of mechanisms and therapeutics to prevent αSyn pathology. To this end, we have characterized and describe the histological, biochemical, and transcriptional brain phenotypes of 3, 6, 12 and 16 month A30P heterozygous and homozygous mice and an αSyn preformed fibril (PFF) seeded A30P mouse model. The A30P mouse expresses human A30P synuclein, a known genetic mutation in familial PD, under the control of the Thy-1 promoter. PFFs were introduced to this mouse line to further induce the formation and spread of αSyn pathology. Our data show that human A30P αSyn was robustly overexpressed throughout the rodent brain whereas the pS129 αSyn was only observed in a few restricted brain structures. Further, administration of αSyn PFFs 30 and 90 days post injection resulted in the spread of αSyn aggregates to anatomically interconnected brain regions. Interestingly, we observed a morphological conversion of endogenous pS129 signal into Lewy neurite-like structures. Injection of αSyn PFFs into A30P also resulted in an elevation of Iba-1 staining, indicating a role for activated microglia and a reduction in dopamine containing fibers, suggesting neurodegeneration. Biochemical evaluation confirmed αSyn was aggregated, phosphorylated and detergent insoluble similar to human PD brain. Additionally, we assessed the transcriptional profile of striatal gene expression by RNA sequencing to determine the nature of microglial involvement. Transcriptional genomics further informed the relationship between synuclein pathology, inflammation and neurodegeneration in this model. Taken together our data demonstrate that this animal model of αSyn aggregation recapitulates many features that are pathologically similar to PD. This model can be used to expand our understanding of disease pathogenesis and investigate therapeutics targeting α-Syn mediated propagation and neurodegeneration in PD.

Disclosures: **D. Toolan:** None. **R.C. Gentzel:** None. **S. Jinn:** None. **K. Tanis:** None. **J. Schachter:** None. **S.M. Smith:** None. **J.N. Marcus:** None.

Poster

130. Alpha-Synuclein: Mechanisms and Transmission

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Program #/Poster #: 130.30/F24

Topic: C.03. Parkinson's Disease

Support: FONDECYT 1180186
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Michael J. Fox Foundation for Parkinson´s Research – Target Validation grant 12473
Millennium Institute P09-015-F
FONDAP program 15150012

Title: A new model to study cell-to-cell transfer of αSynuclein *in vivo*

Authors: *A. MARTINEZ, G. MERCADO, N. LOPEZ, C. HETZ;
Univ. of Chile, Santiago, Chile

Abstract: Parkinson's disease (PD) is characterized by the accumulation of Lewy bodies, large protein inclusions containing aggregated αSynuclein. The progression of PD involves the spreading of misfolded αSynuclein through the brain, where the protein is transferred between cells. We hypothesized that αSynuclein internalization is a dynamic process and its aggregation is different from producing cells.We studied the dynamic and pattern of αSynuclein aggregates *in vitro* using fluorescent microscopy. We developed a new tool to monitor cell-to-cell transfer of αSynuclein *in vivo,* using an adeno-associated viral vector (AVV) expressing αSynuclein fused to a red fluorescent protein in addition to soluble EGFP to label donor cells. In *in vitro* experiments, using HEK293 cells we validated the cellular uptake of extracellular αSynuclein and showed that its subcellular localization is dynamic over time. We observed that αSynuclein distributed into a puncta pattern resembling vesicles or inclusion-like structures that were surprisingly mostly present in the recipient cell and not cells synthetizing the protein. In brains, after intra-nigral delivery of these constructs in mice, we followed histological analyses to determine αSynuclein incorporation in brain. This method allowed the visualization of αSynuclein into surrounding neurons differentially from donor cells.Thus, we developed a new tool to study αSyn cell-to-cell transfer and may open new opportunities to study PD pathogenesis.

Disclosures: **A. Martinez:** None. **G. Mercado:** None. **N. Lopez:** None. **C. Hetz:** None.

Poster

131. Parkinson's Disease Progression

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Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 131.01/F25

Topic: C.03. Parkinson's Disease

Support: Thomas Hartman Center for Parkinson's Research
IMSD MERGE Fellowship to MC

Title: Dramatic effects of combining gonadectomy and the neostriatal 6-OHDA lesion model of Parkinson's disease on episodic-like-memory in adult male rats

Authors: *M. R. CONNER[1], S. GUPTA[2], A. GURBA[2], B. ANDERSON[3], M. F. KRITZER[4]; [1]Grad. Program in Neurosci., [2]Neurobio. and Behavior, [3]Psychology, [4]Dept. of Neurobio. and Behavior, Stony Brook Univ., Stony Brook, NY

Abstract: These studies use a preclinical model of Parkinson's disease (PD) to investigate predicted links between circulating hormones, PD-related dopamine depletion, and episodic memory (EM) in adult male rats. Episodic memory is the ability to recall past experiences context of time and place [Tulving, Org of Memory, 1972]. Deficits in EM are also among the earliest signs of cognitive decline in PD [see Dujardin & Laurent, Curr Opin Neuro, 2003]. Like other aspects of PD, mnemonic symptoms are most prevalent in males [see Watson et al., Alzheim & Demen, 2013] suggesting important influences of gonadal hormones and perhaps androgens in particular on these at-risk behaviors. Using the What-Where-When (WWW) task [Dere et al., Brain Res Protocol, 2005] we recently confirmed that there are indeed strong effects of gonadectomy (GDX) and hormone replacement on episodic-like memory (ELM) in adult male rats. Specifically, discriminations of "What", "Where", and "When" were all impaired by GDX in manners that were attenuated by estrogen and androgen ("What"); androgen but not estrogen ("Where"); and estrogen but not androgen ("When"), respectively. Based on our previous studies using the Barnes Maze task showing that cognitive deficits induced by GDX [Locklear et al., Horm Behav, 2014] and by partial bilateral neostriatal 6-OHDA lesion model of PD are fully attenuated when the two manipulations are combined [Betancourt et al., Neuro, 2016], we asked whether similar interactions between 6-OHDA lesions and GDX extend to behaviors measured in the WWW ELM task. As a first step, we defined the effects of 6-OHDA lesions on this task. Like GDX, we found that 6-OHDA lesioned rats were unable to discriminate between sets of objects (What), object location (Where), and order of presentation (When). Next, in investigating the effects of combined GDX and 6-OHDA manipulations, we found that GDX/6-OHDA rats showed improved discrimination of "What", full restoration of "Where" discrimination, but no improvement in discrimination of "When". Recalling that the effects of GDX on "What" discriminations are attenuated by estrogen and androgen, that "Where" discriminations are

attenuated by androgen replacement, and that “When” discrimination is sensitive to estrogen alone, the WWW task not only provides a second example of cognitive rescue induced by GDX in a preclinical rat model of PD, but further shows that this rescue mainly targets androgen sensitive
behaviors. Because the cognitive deficits of PD are largely resistant to available therapeutics, it may be important to identify the neural mechanisms by which androgens positively and negatively impact cognitive function in health and PD.

Disclosures: M.R. Conner: None. **S. Gupta:** None. **A. Gurba:** None. **B. Anderson:** None. **M.F. Kritzer:** None.

Poster

131. Parkinson's Disease Progression

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 131.02/F26

Topic: C.03. Parkinson’s Disease

Title: Early screening of 6-OHDA striatal lesion in rat model of Parkinson’s disease using MRI and PET imaging

Authors: *J. RYTKÖNEN[1], K. LEHTIMÄKI[1], R. O. PUSSINEN[1], P. POUTIAINEN[2], T. HUHTALA[1], A. J. NURMI[1], D. MISZCZUK[1];
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Abstract: Well established unilateral 6-hydroxy dopamine (6-OHDA) lesion in nigrostriatal pathway is widely used rodent model for Parkinson’s Disease (PD). The destruction of dopaminergic cells accounts for robust and permanent changes in behavioral and biomarker readouts changes e.g. asymmetric amphetamine induced rotations and depletion of total striatal dopamine that reflect the motor deficits seen in PD. However, in striatal 6-OHDA injections the lesion size and its specific location has a major effect of the dopaminergic cell loss. This variability between the animals causes variation which drives the use of higher group sizes for efficacy testing.
In this study, male CD rats underwent 6-OHDA-induced unilateral striatal injury (20 µg/rat). Lesion volume and location as well as tissue viability was assessed with MRI T_2-weighted imaging on D1. Asymmetric rotation behavioral tests, namely amphetamine induced rotation and cylinder test, were performed to detect early functional deficits at 2 days, 2 weeks and 4 weeks after 6-OHDA. Behavioral outcome has been correlated with MRI findings.. The dopaminergic dysfunction was also imaged with 3,4-dihydroxy-6-(18)F-fluoro-l-phenylalanine (^{18}F-FDOPA) at 3 weeks after 6-OHDA lesion. At 5 weeks the animals underwent in vivo ^{1}H-magnetic resonance spectroscopy (MRS), followed by sampling for HPLC measurement for total striatal levels of

dopamine and its metabolites.
Early detection of striatal 6-OHDA lesions was captured using MRI T_2-weighted imaging. The lesion size, behavioral readouts, PET and MRS readouts were examined and studied whether they correlated with total striatal dopamine levels. As conclusion, early screening of 6-OHDA lesion size reduces the model variability and improves the data quality for efficacy testing in drug development for PD.

Disclosures: J. Rytkönen: None. **K. Lehtimäki:** None. **R.O. Pussinen:** None. **P. Poutiainen:** None. **T. Huhtala:** None. **A.J. Nurmi:** None. **D. Miszczuk:** None.

Poster

131. Parkinson's Disease Progression

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Topic: C.03. Parkinson's Disease

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Title: Differential changes in the forelimb selectivity of IT and PT projection neurons of motor cortex in hemiparkinsonian rats

Authors: *A. RÍOS[1], S. SOMA[5], S. NONOMURA[2], J. YOSHIDA[6], M. KAWABATA[1], Y. SAKAI[3], Y. ISOMURA[4];
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Abstract: In the parkinsonian state, the motor cortex and basal ganglia undergo dynamic remodeling of movement representation. One such change is the loss of the normal contralateral forelimb selectivity. These changes may cause motor problems, including impaired balance, reduced bimanual coordination, and abnormal mirror movements. However, it remains unknown how individual types of motor cortical neurons organize this reconstruction. To explore the effect of dopamine depletion on lateralized activity in the parkinsonian state, we used a partial hemiparkinsonian model (6-hydroxydopamine lesion) in Long-Evans rats performing unilateral movements in a right-left pedal task, while recording from primary (M1) and secondary motor cortex (M2). The lesion decreased contralateral preferred activity in both M1 and M2. In addition, this change differed among identified intratelencephalic (IT) and pyramidal tract (PT)

cortical projection neurons, depending on the cortical area. We detected a decrease in lateralized activity only in PT neurons in M1, whereas in M2, this change was observed in IT neurons, with no change in the PT population. Our results suggest a differential effect of dopamine depletion in the lateralized activity of the motor cortex, and suggest possible compensatory changes in the contralateral hemisphere.

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Poster

131. Parkinson's Disease Progression

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 131.04/F28

Topic: C.03. Parkinson's Disease

Title: Characterization of a humanized A53T alpha-synuclein (aSyn A53T KI) and alpha-synuclein KO (aSyn KO) rat models

Authors: *T. PARKKARI[1], J. T. PUOLIVÄLI[1], L. RAUHALA[1], T.-K. STENIUS[1], L. KOISTINEN[1], T. BRAGGE[1], A. OKSMAN[1], K. M. A. PALDANIUS[1], Y. CHEN[2], O. S. MABROUK[2], K. E. GLAJCH[2], W. D. HIRST[2], M. PERKINTON[3], N. K. POLINSKI[4]; [1]Charles River Discovery, Kuopio, Finland; [2]Biogen, Cambridge, MA; [3]AstraZeneca, Cambridge, United Kingdom; [4]The Michael J. Fox Fndn. for Parkinson's Res., New York, NY

Abstract: Mutations and multiplications in the *SNCA* gene encoding the alpha-synuclein protein are linked to an autosomal dominant form of Parkinson's disease (PD). One such mutation is the alanine-to-threonine substitution at amino acid 53, leading to early onset PD, possibly through an increased propensity for the A53T alpha-synuclein to aggregate. To better understand the biology of this mutation and develop a model for PD, The Michael J. Fox Foundation for Parkinson's Research developed a rat model in which CRISPR/Cas9 genome targeting strategies were used to insert humanized amino acids for the region spanning amino acids 53-122 in the rat *SNCA* gene to create a humanized A53T alpha-synuclein rat model with a non-functional rat *SNCA* gene (aSyn A53T KI). A rat *SNCA* KO model (aSyn KO) was also developed using CRISPR/Cas9 through the insertion of a single base pair to read a premature stop codon. Here, we describe phenotypic characteristics of the aSyn A53T KI rat at 4, 8, and 12 months of age as well as biochemical characterization of aSyn KO rat at 6 and 12 months of age. aSyn A53T KI rats and their wildtype littermates (20 rats/genotype/timepoint, mixed gender) were subjected to a battery of behavior tests, including fine motor kinematic analysis, home cage motor activity, open field, tapered beam balance, nest building and GI motility. After the behavioral assessment, various tissue samples were collected from each age cohort for subsequent histological and biochemical analyses. *Ex vivo* outcome measures included

expression of *SNCA* gene on different brain regions, immunohistochemical analysis of pS129 aSyn and total aSyn in the gut, and immunohistochemical analysis of the brains for proteinase K resistant aSyn, GFAP, Iba-1, AT8 pS202/pT205 Tau, pS129 aSyn, total aSyn, and tyrosine hydroxylase.

Disclosures: **T. Parkkari:** None. **J.T. Puoliväli:** None. **L. Rauhala:** None. **T. Stenius:** None. **L. Koistinen:** None. **T. Bragge:** None. **A. Oksman:** None. **K.M.A. Paldanius:** None. **Y. Chen:** None. **O.S. Mabrouk:** None. **K.E. Glajch:** None. **W.D. Hirst:** None. **M. Perkinton:** None. **N.K. Polinski:** None.

Poster

131. Parkinson's Disease Progression

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 131.05/F29

Topic: C.03. Parkinson’s Disease

Title: Structural and electrophysiological changes occurring in the olfactory pathway of a rat model of pre-motor Parkinson's disease are partially prevented by exendin-4 treatment

Authors: *M. SANCANDI, A. CONSTANTI, A. MERCER;
Pharmacol., UCL Sch. of Pharm., London, United Kingdom

Abstract: The symptomatology of Parkinson’s disease consists of motor and non-motor symptoms (NMSs). The latter arise several years before the appearance of motor symptoms, and are not ameliorated by conventional dopaminergic agonist/replacement treatments, suggesting the involvement of other neurotransmitter systems in their aetiology. Indeed, NMSs have been shown to be modulated by treatments other than the dopaminergic ones, such as the glucagon-like peptide-1 (GLP-1) receptor agonist exendin-4 (EX-4). Nevertheless, the aetiology of NMSs, alongside their potential treatments, has yet to be fully investigated. Recently, using injections of the neurotoxins N-ethyl-2-bromobenzylamine (DSP-4) and 6-hydroxydopamine (6-OHDA), a rat model of pre-motor PD, that displayed NMSs in the absence of motor symptoms was developed. In this study, taking advantage of this model, the effect of partial noradrenergic and dopaminergic denervation in several brain regions, such as the primary olfactory cortex, the olfactory bulbs, and the prefrontal cortex, was investigated using immunohistochemical and electrophysiological recording techniques. Surprisingly, the combined partial denervation led to a significant reduction in the expression of interneuronal calcium binding proteins and triggered neuroinflammation in the olfactory cortex, whilst in contrast, the number of dopaminergic interneurones in the olfactory bulbs was found to be increased. Electrophysiologically, neurones recorded intracellularly in layer II-III of the piriform cortex in brain slices prepared from treated animals, exhibited abnormal prolonged epileptiform-like depolarizing postsynaptic potentials on local electrical stimulation of lateral olfactory tract (LOT) afferent fibres, suggestive of a

deficiency in local interneuronal inhibition. Both structural and electrophysiological changes induced by the neurotoxins, were partially prevented following treatment with EX-4. This rat model of pre-motor PD may offer a useful means for research into the early diagnosis as well as early intervention of PD, possibly resulting in a delay of disease progression together with an improved quality life for patients.

Disclosures: M. Sancandi: None. **A. Constanti:** None. **A. Mercer:** None.

Poster

131. Parkinson's Disease Progression

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Program #/Poster #: 131.06/F30

Topic: C.03. Parkinson's Disease

Support: VIEP Grant 2018 (IDLPL)
VIEP Grant 2019 (IDLPL)

Title: Intrapallidal injection effect of cannabidiol and lysophosphatidylinositol on motor behavior in hemiparkinsonian rats

Authors: *F. PATRICIO-MARTÍNEZ, N. ARANA-DEL CARMEN, D. LIMÓN; Neuropharmacology's Lab, Fac. of Chem. Sci., Meritorious Autonomous Univ. of Puebla, Benemérina Universidad Autónoma de Puebla, Mexico

Abstract: Cannabidiol (CBD), a compound phytocannabinoid, has been shown to be a CB_1/CB_2 receptor agonist and likewise having an inverse agonism of the GPR55 receptor. The role of lysophosphatidylinositol (LPI), endogenous ligand of the GPR55 receptor, on the GABAergic system of the striatal-pallid pathway and its effects on motor behavior is unknown. The aim in this work was to study the activation or inhibition of the GPR55 receptor with the administration of LPI and CDB into external globus pallidus (GPe) on the motor behavior in hemiparkinsonian rats. Male Wistar rats were used weighing approximately 250-300g were used next groups (Ascorbic Acid+Vehicle, Ascorbic Acid+CBD, Ascorbic Acid+LPI, 6-OHDA+Vehicle, 6-OHDA+CBD; 6-OHDA+LPI). The groups lesioned they were intracranially administered with 6-OHDA [16 µg/2µL]in the nigrostriatal route. Fourteen days post-surgery, amphetamine was administered at a dose of 5 mg/kg subcutaneously to determine the degree of dopaminergic lesion. Once the animals that rotated above 15 turns per minute were selected for a second stereotaxic surgery was performed to implantate of a guide cannula in the GPe. Seven days after cannulation in the GPe, [5µg/µl] of CBD and [21µg/µl] of LPI were administered for three consecutive days (28, 29- and 30-days post-injury). The results showed a statistically significant decrease in the ipsilateral turns to the lesion of the group 6-OHDA vs 6-OHDA+LPI ($p < 0.5$).

The findings suggest that LPI agonism with the GPR55 receptor restore behavioral motor probably when activated in the GABAergic neurons of the denervated striatopallidal pathway.

Disclosures: F. Patricio-Martínez: None. **N. Arana-del Carmen:** None. **D. Limón:** None.

Poster

131. Parkinson's Disease Progression

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 131.07/F31

Topic: C.03. Parkinson's Disease

Title: The role of dopamine D_3 receptor on the development of L-dopa-induced dyskinesia in 6-OHDA-lesioned mice

Authors: Y.-K. CHEN[1], ***P.-K. CHANG**[3], J.-C. CHEN[2];
[1]Chang-Gung Univ., Taoyuan, Taiwan; [2]Physiol. and Pharmacol., Chang-Gung Univ., Tao-Yuan, Taiwan; [3]Chang-Gung University/ Grad. Insititute of Biomed. Sci., Taoyuan, Taiwan

Abstract: Parkinson's disease (PD), a well-known neurodegenerative disease, characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNc). Though akathisia could be improved initially by L-DOPA treatment, most patients develop dyskinesia after prolong medication. Until now, the underlying mechanism of L-DOPA-induced dyskinesia (LID) remained unclear, but numerous reports indicated dopamine D1 receptor supersensitivity accounts as a major factor that contributes to occurrence of LID. Due to dopamine D3 receptor functions synergistically with D1 receptor in various aspects, previously studies have found expression of D3 receptor altered in the striatum of LID animals. However, both D3 agonist and antagonist were reported to reduce the severity of LID, hence this study intent to delineate the role of D3 receptor on the development of LID. Male C57BL/6 and D3R knock-out mice lesioned with unilateral 6-OHDA in the medial forebrain bundle developed typical PD symptom and axial and limb abnormal involuntary movement (AIM) after 2 weeks L-dopa (10 mg/kg) treatment. Comparing the degree of AIMs between WT and D3RKO mice, the total scores of AIMs in the D3RKO group was significantly decreased as compared to WT group. Similar results were also observed when 6-OHDA-lesioned WT mice pretreated with or without D3R antagonist, FAUC365 (3 mg/kg) concomitantly with daily L-dopa administration. In addition, LID mice exhibited enhancement of phospho-ERK1/2 levels in the striatum as compared to PD animals, whiles this enhancement of phospho-ERK1/2
signal was not observed in the D3RKO mice exhibited LID. Overall, current experimental results support a negative role of dopamine D3 receptor on the development of LID in 6-OHDA-lesioned PD animals.

Disclosures: Y. Chen: None. **P. Chang:** None. **J. Chen:** None.

Poster

131. Parkinson's Disease Progression

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 131.08/F32

Topic: C.03. Parkinson’s Disease

Title: Stereological and histopathological characterization of the neuronal and inflammatory changes in the MPTP mouse model of Parkinson's disease

Authors: M. STAUP[1], J. BAILY[2], ***T.-K. STENIUS**[3], R. O. PUSSINEN[3], T. HUHTALA[3]; [1]Charles River Safety Services, Durham, NC; [2]Charles River Safety Services, Tranent, United Kingdom; [3]Charles River Discovery, Kuopio, Finland

Abstract: Parkinson’s disease (PD) is a neurodegenerative disorder characterized by motor and non-motor symptoms, including, rigidity, difficulty of movement, and impaired with walking and gait. The main pathological feature of PD is the cell death of dopaminergic neurons located in the substantia nigra pars compacta (SNc) accompanied by substantial loss of dopamine (DA) and its metabolites with reduced dopamine active transport (DAT) activity in the striatum. The toxin-induced PD mouse model, in which 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration results in catecholamine depletion in the SNc, is commonly used to test novel therapeutics. This model reproduces many of the behavioral and physiological characteristics of the disease, including the selective vulnerability of dopaminergic DA cells of the SNc, while DA cells of the neighboring ventral tegmental area (VTA) remain comparatively protected from the neurotoxin. In addition to this well-established mechanism, there is growing evidence that a link between neuroinflammation and PD-like neurodegeneration exists. Combining these features and pathologies, the MPTP model is widely used in PD research to evaluate whether novel compounds may protect from neuronal loss..
This study combines advanced stereological analysis with pathological evaluation to comprehensively evaluate the neuronal and inflammatory changes in the MPTP mouse model. The tyrosine kinase inhibitor, Nilotinib, was used as an agent of recovery. Concentration of DA, 3,4-Dihydroxyphenylacetic acid (DOPAC) and Homovanillic acid (HVA) in the striatum were measured by HPLC. Immunohistochemical (IHC) methods were used to stain for tyrosine hydroxylase (TH) in the SNc and the VTA. Activation of resident glia and infiltration of peripheral immune cells into the SNc and VTA were also assessed *in situ*. Two unbiased stereological methods of counting (fractionator and proportionator techniques) were used to estimate catecholamine cell loss and changes in the expression level of TH in MPTP-treated mice. The ability of these methods to detect the effect of the tyrosine kinase inhibitor, Nilotinib, a known agent that protects against MPTP-induced cell loss, was compared to standard morphometry.
As a summary, advanced stereological analysis with pathological evaluation to comprehensively

evaluate the neuronal and inflammatory changes in the MPTP mouse model were studied. The increased sensitivity and specificity in histopathological analysis enables also to capture possible drug effect more efficiently compared to traditional approach.

Disclosures: M. Staup: None. **J. Baily:** None. **T. Stenius:** None. **R.O. Pussinen:** None. **T. Huhtala:** None.

Poster

131. Parkinson's Disease Progression

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 131.09/F33

Topic: C.03. Parkinson's Disease

Title: Comparison of the effects of clozapine-nitric-oxide (CNO) and clozapine to activate hM4Di containing striatal dopaminergic grafts in hemiparkinsonian rats

Authors: *K. JARDINE[1], K. M. LE[2], K. VENKITESWARAN[3], T. SUBRAMANIAN[1]; [2]Neurol., [3]Neurol. and NBS, [1]Penn State Col. of Med., Hershey, PA

Abstract: Parkinson disease (PD) is a chronic, progressive neurodegenerative disorder associated with loss of dopamine-secreting neurons in the substantia nigra. One treatment method that is being explored is the use of dopaminergic grafts, such as Fetal Ventral Mesencephalic (FVM) grafts. However, previous studies have shown that FVM grafts are associated with complications such as draft-induced dyskinesia. One answer to this issue is the use of Designer Receptors Exclusively Activated by Designer Drugs (DREADD) and Cre-Lox system as a mechanism to control the graft. In the past, Clozapine-Nitric Oxide (CNO), a biologically inert designer drug, was widely used as a ligand to activate DREADDs but a recent study showed that CNO is first converted to clozapine before crossing the blood-brain barrier. This raises the question as to whether, the effects of CNO or its metabolite Clozapine is specific to the DREADD. We tested the hypothesis that FVM cells that contain hM4Di and Cre will have similar reactions to both CNO and clozapine at equivalent doses and that control animals will have no effects from the equivalent treatments with CNO and Clozapine. Each rat underwent unilateral 6-hydroxydopamine (6-OHDA) injections and received transplants of FVM grafts from E13.5 pregnant mice. The experimental group received injections of WGA-Cre into the striatum prior to FVM transplantation. FVM grafts were transfected with hM4Di (DREADD receptor) prior to transplant. There were also two control groups: one group with FVM + WGA-Cre, and one with FVM + hM4Di. Animals then underwent behavioral testing using Rodent Battery of Behavioral Testing (RBBT) after receiving CNO 10 mg/kg and various doses of clozapine. Our results showed that at lower doses (0.1 mg/kg or 1 mg/kg), clozapine did not effectively inhibit the graft (less than 50% reduction of functions). However, at higher doses, clozapine appeared to also have sedative side effects. In addition to CNO and Clozapine, we also

compared the efficacy of a novel DREADD ligand, JHU37160. Like CNO, JHU37160 is biologically inert, but was able to effectively inhibit the graft (>75% reduction) using much smaller doses (0.1 mg/kg compared to 10 mg/kg with CNO). Striatal microdialysis showed near complete diminution of dopamine levels that was temporarily correlated with DREADD activation. These findings suggest that CNO is more effective than clozapine for chemogenetic control of the FVM grafts, as clozapine did not effectively inhibit the graft at lower doses but caused sedative effects at higher doses. These findings also support that JHU37160 is more specific to the DREADD receptor, since a smaller dose was able to inhibit the graft.

Disclosures: K. Jardine: None. **K.M. Le:** None. **K. Venkiteswaran:** None. **T. Subramanian:** None.

Poster

131. Parkinson's Disease Progression

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Program #/Poster #: 131.10/F34

Topic: C.03. Parkinson's Disease

Support: William N. & Bernice E. Bumpus Foundation
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Title: Levodopa-induced dyskinesia triggers bidirectional changes in striatal connectivity and excitability

Authors: *S. ZHAI[1], J. R. CRITTENDEN[2], D. WOKOSIN[1], M. A. CENCI[3], A. M. GRAYBIEL[2], D. SURMEIER[1];
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Abstract: The cardinal motor symptoms of Parkinson's disease (PD) are caused by progressive degeneration of dopaminergic neurons in the substantia nigra. In the early stages of the disease, these symptoms of PD are effectively alleviated by dopamine replacement therapy. However, as the disease progresses, the administration of levodopa at the high doses required to achieve symptomatic relief leads to debilitating hyperkinetic movements, so termed levodopa-induced dyskinesia (LID). Dyskinetic movements are induced in the 'on-state' and typically followed by a profound hypokinetic 'off-state', making life for patients a difficult 'roller-coaster ride'.

Although previous work has shown that there are homeostatic adaptations in the striatum that accompany chronic levodopa administration, it is unclear how the striatum changes during this ride. To date, the assessment of corticostriatal connectivity and intrinsic excitability in LID mice has been performed exclusively in the 'off-state' —hours after the last injection of L-DOPA. Thus it remains unclear how intrinsic excitability and synaptic connection would change in the 'on-state'—within hours of L-DOPA injection, when the behavioral manifestations of LID are the strongest. Here, using two-photon imaging and slice electrophysiology, we show that LID on- and off-states are associated with bidirectional changes in synaptic connectivity and intrinsic excitability of principal striatal spiny projection neurons (SPNs). Specifically, the onset of dyskinesia is associated with a decrease in spine density in indirect (i)-pathway SPNs and an increase in mushroom spine density in direct (d)-pathway SPNs. Moreover, the on-state is accompanied by a decrease in somatodendritic excitability in iSPNs and an increase in somatodendritic excitability in dSPNs. Finally, we show that knockout of CalDAG-GEFI, a guanine nucleotide exchange factor for the small GTPases Rap1/2 that is downregulated in rat models of LID, prevents some of the on-state associated changes and dampens the development of abnormal involuntary movements of LID. Together, these findings suggest that the onset of LID is associated with immediate alterations in corticostriatal connectivity and intrinsic excitability of SPNs that can be therapeutically targeted to alleviate LID.

Disclosures: S. Zhai: None. **J.R. Crittenden:** None. **D. Wokosin:** None. **M.A. Cenci:** None. **A.M. Graybiel:** None. **D. Surmeier:** None.

Poster

131. Parkinson's Disease Progression

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 131.11/F35

Topic: C.03. Parkinson's Disease

Support: Capes
Cnpq
FINEP (IBN-Net #01.06.0842-00)

Title: The intranasal MPTP administration in rats: An animal model to study nociceptive alterations in the early stages of Parkinson's disease

Authors: *K. ROVERSI[1], R. TONELLO[2], S. TALBOT[3], J. FERREIRA[1], A. LATINI[1], R. PREDIGER[1];
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Abstract: Pain is a non-motor alteration present in a large proportion of Parkinson's disease (PD) patients and has a significant negative impact on their quality of life. Although this symptom occurs secondarily to the motor alterations of PD, about 40% of PD patients experience pain in the early stages of PD. Considering that the pathophysiology is not well understood and there is not appropriate management for this symptom, it is important to define these alterations in rodent models of PD. Therefore, our aim was evaluated the nociceptive alterations followed the intranasal administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a model of the early stages of Parkinson's disease.
For this, 32 male Wistar rats (90 days) were treated with a single i.n. infusion of MPTP (1 mg/nostril) or saline. At 7, 14 and 21 days later 20 rats (10 per group) were evaluated in von Frey and hot plate tests, and 12 rats (6 per group) were evaluated in acetone and tail flick tests. In another experiment, 24 rats (6 animals per group) also received i.n. MPTP or saline, and 14 days later received a single intraplantar administration of capsaicin (20 µL, 5 mM, right hind paw) or vehicle (0.15% ethanol in saline) and was measured the time spent licking the injected paw. In order to evaluate the motor alterations, 48 rats (8 per group) animals received MPTP or saline and were independently evaluated at 7, 14 and 21 days later in the pole test and cylinder test. The evaluators were blinded with regard to the experimental groups. (CEUA 1454/2017)
Our results indicated that the MPTP induces mechanical and hot hyperalgesia at 14 and 21 days after i.n. infusion, and also increases the nociceptive responses followed the intraplantar capsaicin 14 days later. The MPTP administration did not modify the nociceptive responses followed the intraplantar acetone test and increase the latency of response to the tail flick test 14 days later. In relation to motor evaluations, the MPTP did not modify the turn time in the pole test and the rearing behavior in the cylinder. This study demonstrates that intranasal MPTP administration, an experimental model of early PD, induced nociception alterations in rats earlier than motor disabilities.
CNPq, CAPES, FAPESC (PRONEX - Project NENASC)**, FINEP** (IBN-Net #01.06.0842-00), **UFSC, CEUA** 1454/2017.

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Poster

131. Parkinson's Disease Progression

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 131.12/F36

Topic: C.03. Parkinson's Disease

Title: Oscillatory signatures of L-DOPA-induced dyskinesia are dependent on the LID induction protocol and L-DOPA dose

Authors: *T. YE[1], M. J. BARTLETT[1,2], M. SEXAUER[3], S. J. SHERMAN[1], T. FALK[1,2], S. L. COWEN[4,5];
[1]Neurol., [2]Pharmacol., [3]Neurosci., [4]Psychology, [5]Evelyn F. McKnight Brain Inst., Univ. of Arizona, Tucson, AZ

Abstract: The gold-standard treatment for Parkinson's disease (PD) is dopamine (DA) replacement therapy with the DA precursor L-DOPA; however, long-term L-DOPA treatment results in debilitating L-DOPA-induced dyskinesias (LID). Hyper-synchronous oscillatory activity is thought to contribute to LID, and a few groups have identified high-frequency oscillatory activity in preclinical rodent models of LID. For example, narrow-band 80 Hz high-gamma oscillations in motor cortex (MX) have been observed in a rodent model of LID induced using a 7-day (12 mg/kg) L-DOPA priming regimen (Dupre et al., 2016; Halje et al., 2012). Work by our group using the more commonly used model of LID (21-day priming using 7 mg/kg L-DOPA; Lundblad et al., 2002) did not result in narrow-band 80 Hz activity, but, instead, resulted in wide-band gamma activity (35 - 80 Hz). We conducted a set of experiments to determine if these different outcomes were due to the duration or differences in the dosage used for LID induction. Separate groups of unilaterally 6-OHDA-lesioned male PD rats were primed for LID using different protocols, and local-field activity was recorded from electrodes placed in MX, dorsolateral striatum (DLS), dorsomedial striatum (DMS), and the nucleus accumbens (NAc). Six rats were primed using 12 mg/kg (*s.c.*) of L-DOPA for 7 days, followed by an additional 3 days at 7 mg/kg (*s.c.*). Consistent with other groups, 12 mg/kg of L-DOPA triggered significant narrow-band 80 Hz oscillations (ANOVA, p=0.004) in MX (p=0.0002, Tukey-corrected) during the 7 days of priming. This signature was also found in the DMS and NAc (all $p < 0.05$, Tukey-corrected). A lower dose of L-DOPA (7 mg/kg) administered on the following 3 days did not trigger narrow-band 80 Hz oscillations. Despite the lack of narrow-band 80 Hz after 7 mg/kg L-DOPA, dyskinesia was clearly present. In comparison to our previous findings, 6-OHDA-lesioned animals primed with the lower dose of L-DOPA (7 mg/kg) daily for 21 days did not trigger wide-band nor narrow-band 80 Hz gamma oscillations in MX, while showing strong dyskinetic behavior. This indicates that despite the presence of dyskinesia, the oscillatory signature of LID is dose-dependent, while the actual dyskinetic behavior can occur during either wide- or narrow-band gamma. It is conceivable that influxes of DA produced by L-DOPA engages different gamma-generating networks in the corticostriatal circuit in a dose-dependent manner. Furthermore, these observations suggest that narrow-band 80 Hz in MX is not a necessary condition for the expression of LID.

Disclosures: T. Ye: None. **M.J. Bartlett:** None. **M. Sexauer:** None. **T. Falk:** None. **S.L. Cowen:** None. **S.J. Sherman:** None.

Poster

131. Parkinson's Disease Progression

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Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 131.13/F37

Topic: C.03. Parkinson's Disease

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Title: Efficacy and specificity of Cre-Lox system in the TH-Cre, Dat-Cre and wildtype Sprague Dawley rat models with the expression of inhibitory chemogenetic or optogenetic viral vectors in the substantia nigra to cause Parkinsonism

Authors: *K. M. LE[1], S. CHINNIAH[1], V. IYER[2], C. LIEU[1], P. MICHAEL[1], E. HANDLEY[1], A. ZENOROWITZ[1], S. SAVALIYA[1], M. SUBRAMANIAN[1], K. VENKITESWARAN[1], T. SUBRAMANIAN[1];
[1]Neurol., Penn State Col. of Med., Hershey, PA; [2]Prog. in Neurosci. and Dept. of Psychological and Brain Sci., Indiana Univ., Bloomington, IN

Abstract: The Cre-Lox system is widely used as a method to achieve specificity in the expression of optogenetic (AAV5-Ef1a-DIO-eNpHR3.0-EYFP) or chemogenetic (AAV8-hSynDIO-hM4Di-mCherry) viral vectors. Using this paradigm, there is an inherent need for the neurons that were transfected with either vectors previously mentioned to receive Cre Recombinase in order to express the genes. Our preliminary studies show that wildtype rats that receive AAV2-Ef1a-mCherry-IRES-WGA-Cre (a variant of the usual Cre construct that allow for retrograde transport) in the left striatum and either eNpHR3.0(n=7) or hM4Di (n=8) in the ipsilateral substantia nigra(SN) will express the G-protein channels (chemogenetics) or chloride channels (optogenetics) that is responsive to either the specific ligand or optical stimulation and will cause the animals to temporarily become hemiparkinsonian. Animals were repeatedly tested for onset of hemiparkinsonian(RHP) state while under the inhibitory effects of the specific ligand or optical stimulation. Additionally, animals were tested for the reversal of the RHP state post experiment. The eNpHR3.0 treated animals served as controls for the hM4Di treated animals and vice versa. In both the the chemogenetic and optogenetic cohort, animals consistently developed RHP as characterized by mean unilateral reduction of vibrissae-evoked forelimb test scores of

>75%($p<0.01$). Similar results were observed in the TH-Cre animals for both the optogenetic (n=5) and chemogenetics cohort (n=8). Striatal microdialysis in the chemogenetic animals showed a complete diminution of dopamine at the drug's peak effect. Additionally, animals were shown to be levodopa (LD) responsive but did not develop LD-induced dyskinesias which suggest that plastic changes might play an important role in developing dyskinesias. Immunohistochemistry showed great expression of EYFP or mCherry in the SN of the wildtype animals whose striatum were transfected with WGA-Cre. The transgenic TH-Cre animals however, showed even more robust expression of EYFP or mCherry in the SN. These findings show that the system is highly specific and while the TH-Cre model showed more robust expression of the eNpHR3.0 or hM4Di virus, it did not cause a difference in causing parkinsonian symptoms. Following these results, we will similarly compare the DAT-Cre rat model to the two types that were previously used.

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Poster

131. Parkinson's Disease Progression

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 131.14/F38

Topic: C.03. Parkinson's Disease

Support: NKFIH FK 124188

Title: Urocortin 1 neurons of the centrally projecting Edinger-Westphal nucleus contribute to non-motor symptoms of Parkinson's disease in the rat

Authors: *B. GASZNER[1], N. FUREDI[1], T. GASZNER[1], L. A. KOVACS[1], J. FARKAS[1], A. KECSKES[2], V. KORMOS[2], A. HUNYADY[2], B. UJVARI[1];
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Abstract: According to the self-assessment of patients, the non-motor symptoms of Parkinson's disease (PD) such as depression and anxiety deteriorate the quality of their life in a greater extent, than the well-known disabilities in motor control including, rigor tremor and bradykinesia. However the neuropathological background of motor symptoms is relatively well understood, little is known about neurohistological changes underlying neuropsychiatric anomalies associated to PD. For more than thirty years is already known, that besides Lewy body accumulation and neuron loss in the substantia nigra (SN), the Edinger-Wespthal nucleus (EW) also shows similar neuropathology. As the pupillomotor functions and the lens accommodation are not compromised in PD, and because the urocortin1 expressing centrally projecting division

of the EW nucleus (EWcp) is involved in mood control, we hypothesized that the urocortin1 expressing neurons of the EW may be affected by neurodegeneration contributing to altered mood status and anxiety level. Six weeks subcutaneous rotenone treatment was used as a toxic model for PD in the rat. Open field (OFT), sucrose preference (SPT) and rotarod tests were used to assess the mood status and motor performance. Histological changes were assessed in the SN and EWcp. Rotenone treated rats in OFT spent more time at the peripheral part the arena, and showed reduced locomotor activity. In SPT, they consumed less sweetened water than vehicle injected control rats. Rotenone treatment resulted in a drastic deterioration of motor performance. Parkinsonian rats showed increased relative adrenal weight, and decreased thymus weight. Rotenone treatment resulted in significant reduction of Ucn1 cell counts, but increased Ucn1 content of the neurons in the EWcp which correlated with the neuron loss in the dopaminergic SN. In Parkinsonian rats, increased microglial activity was observed in the EWcp, and occasionally, degenerating Ucn1 immunoreactive neurons were found being removed by phagocytotic microglia. Increased Ucn1 content was found in the EWcp upon rotenone treatment. Both the dopaminergic SN and the Ucn1-EWcp cells showed marked cytoplasmic alpha-synuclein expression upon rotenone exposure. Parkinsonian rats show serious hypokinesia, associated with increased anxiety level, and increased anhedonia. Organ weight data suggest the long-term activation of hypothalamus-pituitary-adrenal axis. These findings suggest that motor symptoms, anxiety and depression-like phenotype also occurs in rotenone treated rats and the loss of Ucn1 neurons in the EWcp may contribute to the non-motor symptoms of PD.

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Poster

131. Parkinson's Disease Progression

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 131.15/F39

Topic: C.03. Parkinson’s Disease

Title: Robust chemically induced animal model of Gaucher disease for preclinical study

Authors: *S. RAMBOZ, A. PENNINGTON, K. COX, M. HALL, W. ARIAS, K. KAYSER, J. GUTERL, M. BANSAL, D. HAVAS, K. CIRILLO;
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Abstract: Parkinson’s disease (PD) and synucleinophathies are neurodegenerative disorders defined by α-synuclein (α-syn) accumulation. Mutations in the α-syn gene have been demonstrated *in vitro* and *in vivo* to accelerate the aggregation and formation of α-syn fibrils, a disease marker. Presence of α-syn positive Lewy bodies were identified in neuropathological analyses of a group of Gaucher disease type I [GD] patients [Sidransky, 2005]. Further human

genetic studies have also linked glucocerebrosidase (GCase) gene GBA1 mutations to PD making this mutation the highest genetic risk factor to PD [Sidransky, 2009]. Due to the strong clinical correlation between PD and GD diseases, animal models that demonstrate relevant and robust phenotypes [behavior, mRNA and protein profiling, IHC, ⋯] are of necessity. Genetically modified animals models carrying point mutations in GCase mimicking type 2 and type 3 have been generated targeting neuronal cells [Enquist et al. 2007; Liu et al. 1998]. In the present study, we focus on a chemically induced model consisting in daily injection of the irreversible GCase inhibitor conduritol B-epoxide (CBE) [Vardi et al., 2016). C57Bl6 mice were dosed daily via intraperitoneal injection at two different doses starting at post-natal day 8 to 22. Each pup was monitored closely and assess to motor, coordination and gait functions weekly from 3 weeks of age until study completion. Gait measures were assessed using PsychoGenics proprietary high through put gait platform, NeuroCube®. Preliminary assessment of data demonstrate progressive and CBE dose-dependent phenotypic deterioration of responses during assessments. Thus, our data demonstrate the progressive and robust phenotype of the chemically-induced GD model as an alternative to genetically modified animal models.

Disclosures: **S. Ramboz:** A. Employment/Salary (full or part-time):; PsychoGenics. **A. Pennington:** A. Employment/Salary (full or part-time):; PsychoGenics. **K. Cox:** A. Employment/Salary (full or part-time):; PsychoGenics. **M. Hall:** A. Employment/Salary (full or part-time):; PsychoGenics. **W. Arias:** A. Employment/Salary (full or part-time):; PsychoGenics. **K. Kayser:** A. Employment/Salary (full or part-time):; PsychoGenics. **J. Guterl:** A. Employment/Salary (full or part-time):; PsychoGenics. **M. Bansal:** A. Employment/Salary (full or part-time):; PsychoGenics. **D. Havas:** A. Employment/Salary (full or part-time):; PsychoGenics. **K. Cirillo:** A. Employment/Salary (full or part-time):; PsychoGenics.

Poster

131. Parkinson's Disease Progression

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 131.16/F40

Topic: C.03. Parkinson’s Disease

Support: Rush University Cohn Fellowship

Title: Chronic amphetamine exposure during adolescence in a rat model of ADHD leads to Parkinson's disease-like behavioral pathology as adults

Authors: *A. L. PERSONS, I. D. CALMA, B. D. BRADARIC, T. C. NAPIER;
Rush Univ. Med. Ctr., Chicago, IL

Abstract: The etiology of Parkinson’s disease (PD) is unclear, but both genetic and environmental factors are involved. Thus, the identification of risk factors for developing PD is

of great interest. A recent epidemiological study revealed that individuals with attention-deficit/hyperactivity disorder (ADHD) have a two-fold risk for developing a basal ganglia or cerebellar disorder (including PD) later in life; this risk increases to six-fold if ADHD was treated with stimulants (Curtin *et al. Neuropsychopharmacology*, 2018). In 2016, 6.1 million children between the ages of 2 and 17 had a diagnosis of ADHD, and 3.7 million received ADHD medication (Danielson *et al. J Clin Child Adolesc Psychol*, 2018). There is a chance that the prevalence of PD may increase as these individuals age. To model this potential scenario, we used the spontaneously hypertensive (SH) rat, a validated model of ADHD. We hypothesized that SH rats chronically treated with amphetamine during adolescence will develop behavioral and brain pathology as adults that mirror early stages of PD. Juvenile male SH rats (aged 4 weeks; n=16) and age-matched controls (WKY; n=16) were treated with escalating doses of *d*-amphetamine (A; 0.5 – 2mg/kg, sc) or vehicle (V; 1mL/kg, sc) for four weeks. As a behavioral index of PD, we assessed two common rat motor homologs of early stage motor symptoms, postural instability and akinesia, once per week for five weeks after treatment ended. While initially normal, a time-dependent worsening of motor symptoms emerged in treated rats. By week five, there was a significant main effect of strain and treatment, as well as a strain x treatment interaction. SH/V and WKY/A rats demonstrated significant postural instability (compared to WKY/V rats), and this effect was exacerbated in SH/A rats (two-way ANOVA; $p<0.05$). Only SH/A rats developed akinesia by week five (two-way ANOVA; $p<0.05$). To determine if PD-like behaviors were driven by PD-like brain pathology, we are assessing optical density of tyrosine hydroxylase (TH) in PD-relevant brain regions. The dorsolateral striatum did not demonstrate a treatment effect. Studies are ongoing to assess changes in other brain regions and with other biomarkers of PD.

Disclosures: A.L. Persons: None. **I.D. Calma:** None. **B.D. Bradaric:** None. **T.C. Napier:** None.

Poster

131. Parkinson's Disease Progression

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 131.17/F41

Topic: C.03. Parkinson’s Disease

Support: NIEHS Grant ES029344

Title: SLC39A14 knockout mice-A genetic model of manganese-induced Parkinsonism: Preliminary studies on behavioral and neurochemical phenotype

Authors: A. N. RODICHKIN, *J. L. MCGLOTHAN DZIEDZIC, D. R. BROOKS, A. G. SANCHEZ, T. R. GUILARTE;
Envrn. Hlth. Sci., Florida Intl. Univ., Miami, FL

Abstract: Manganese (Mn) is an essential nutrient but excess accumulation in the brain from occupational exposures results in a form of Parkinsonism (PNM) with dystonia that is not responsive to L-dopa, the standard therapeutic treatment for idiopathic Parkinson's disease (iPD). Genetic mutations of *SLC39A14* (a Mn influx transporter) have been recently described with a clinical presentation of PNM with dystonia and increased blood and brain Mn (Tuschl et al. 2016). Humans with mutations in the *SLC39A14* gene exhibit PNM that is not responsive to L-dopa therapy. Here, we present preliminary studies using *SLC39A14* knock-out (KO) mice on the behavioral and neurochemistry phenotype resulting from increased systemic and brain Mn concentrations. Assessment of Mn concentrations in blood and striatum of post-natal day (PN) 60 *SLC39A14-KO* male mice, indicates a highly significant increase in Mn concentrations when compared to wild-type (WT) controls (Blood: 46.9 ± 10.7µg/L in WT vs 975.7 ± 70.9µg/L in KO; Brain: 2.64 ± 0.158 µg/g in WT vs 12.3 ± 1.4µg/g in KO). Behavioral characterization of the PN60 male mice showed significant locomotor impairment, which manifests itself as a decrease in distance travelled and rearing behavior. Furthermore, preliminary data from the pole descend test indicates that there is a significant decrease in the ability to descend in KO mice, when compared to WT. HPLC with electrochemical detection of striatal dopamine (DA) concentrations and its metabolites resulted in no significant difference in the concentrations of striatal DA, DOPAC and HVA in PN60 *SLC39A14-KO* mice. Tyrosine hydroxylase immunohistochemistry in the striatum of PN140-160 animals indicates no significant differences between *SLC39A14-KO* mice and WT, supporting the lack of nigrostriatal dopaminergic neuron terminal degeneration in the presence of significant locomotor impairment. These preliminary findings are consistent with non-human primate studies from our laboratory indicating a dysfunctional but intact nigrostriatal dopaminergic system. Collectively, these preliminary results indicate that the neuropathological changes resulting from Mn overexposure are different from those observed in iPD. Ongoing studies aim to perform unbiased stereological cell counting of dopaminergic neurons in the *Substantia Nigra pars compacta* and assess striatal dopamine release using in-vivo micro dialysis. Our results further suggest that other neuronal systems besides dopamine are involved in Mn-induced PNM with dystonia.

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Poster

131. Parkinson's Disease Progression

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Program #/Poster #: 131.18/F42

Topic: C.03. Parkinson's Disease

Support: Arizona Biomedical Research Commission (ABRC)

Title: Region-dependent cross-frequency interactions in a preclinical model of L-DOPA-induced dyskinesia after low-dose ketamine

Authors: *T. FALK[1,2], T. YE[1], M. J. BARTLETT[1,2], S. J. SHERMAN[1], S. L. COWEN[3]; [1]Neurol., [2]Pharmacol., Univ. of Arizona Col. of Med., Tucson, AZ; [3]Dept. of Psychology, Univ. of Arizona, Tucson, AZ

Abstract: Mounting evidence suggests that ketamine can provide lasting relief for disorders such as chronic pain and treatment-resistant depression. Our group has recently shown that 10-hr exposure to low-dose ketamine also results in a weeks-to-month long reduction in L-DOPA-induced dyskinesias (LID) associated with Parkinson's disease (PD) treatment (Bartlett et al., 2016). A common feature of LID, depression, and chronic pain is the presence of hypersynchronous oscillatory activity. We previously reported that low-dose ketamine triggers high-frequency oscillations (>135 Hz; HFOs) and enhances cross-frequency coupling (CFC) between HFOs and lower frequencies (Ye et al., 2018) in naïve rats. CFC is believed to facilitate communication between brain regions and support neural plasticity. Little is known about the potential contribution of CFC to PD or LID. We hypothesized that ketamine would disrupt oscillatory activity and CFC associated with LID. To address this, we measured oscillatory activity in the motor cortex (MX) in a rodent LID model, induced by treating unilateral 6-OHDA-lesioned male rats (N=7) with L-DOPA (7 mg/kg, *i.p.*) daily for 21 days. Dyskinetic rats with abnormal involuntary movement (AIMs) scores of 33.6 ± 6.6 (mean ± SD) were implanted with electrode arrays in MX, dorsolateral striatum (DLS), dorsomedial striatum (DMS), and nucleus accumbens (NAc). Neural recordings were acquired continuously for 11 hrs during ketamine (20 mg/kg, *i.p.*) or saline treatments (5 injections total). The 5th injection on each session was paired with L-DOPA (7 mg/kg) to induce LID. We observed that L-DOPA injection induced LID and increased theta to high-gamma phase-amplitude CFC (ANOVA, p=0.001) in the MX of LID rats. In addition, LID was significantly decoupled when L-DOPA was co-injected with ketamine (all p<0.05, post-hoc tests were Tukey-corrected). In the DLS, co-administration of ketamine + L-DOPA significantly enhanced theta-HFO coupling (ANOVA, p=0.000002) compared to administration of either drug alone (all p<0.01). We observed the same coupling in the DMS (all p<0.01). Ketamine also decoupled L-DOPA-induced theta to high-gamma oscillations in the DMS and NAc (ANOVA, all p<0.01). Other groups have found decreased theta to high-gamma (80 Hz) CFC after L-DOPA injection (12 mg/kg, 7 days priming; Belić et al., 2016). We, however, observed increased CFC with a wider range of high-gamma (60 - 80 Hz) using a lower L-DOPA dose (7 mg/kg, 21 days priming), suggesting a dose-dependent priming effect. The observation that ketamine decouples oscillatory activity associated with LID movements suggests that ketamine disrupts neural networks involved in LID.

Disclosures: T. Falk: None. **T. Ye:** None. **M.J. Bartlett:** None. **S.J. Sherman:** None. **S.L. Cowen:** None.

Poster

131. Parkinson's Disease Progression

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 131.19/F43

Topic: C.03. Parkinson's Disease

Support: Veteran Affairs Merit Award I01BX002477
NIH Grant AG048205
NIH Grant NS073670

Title: Glia maturation factor dependent activation of mast cells and microglial calpain 1 synergize dopaminergic neuronal loss and behavioral deficits in MPTP induced mouse model of Parkinson's disease

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Abstract: Parkinson's disease (PD) is the second most common age associated neurodegenerative disease. However, the molecular mechanism mediating nigrostriatal dopaminergic neuron degeneration is not yet fully understood. In this study, methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced nigrostriatal neurodegeneration and astro-glial activations were determined by western blot and immunofluorescence in wild type (WT) mice, mast cell deficient (MC-KO) mice and GMF-deficient (GMF-KO) mice, with or without mast cell reconstitution before MPTP administration. We show that GMF-KO in the mast cells reduces the synergistic effects of mast cell and Calpain1 (calcium-activated cysteine protease enzyme)-dependent dopaminergic neuronal loss that reduces the motor behavioral impairments in MPTP-treated mouse. We found that MPTP administered WT mice exhibit oxidative stress due to significant increases in the levels of malondialdehyde, superoxide dismutase and reduced the levels of reduced glutathione and glutathione peroxidase activity when compared with both MC-KO and GMF-KO mice. The number of TH-positive neurons in the ventral tegmental area, substantia nigra and the fibers in the striatum were significantly reduced while granulocyte macrophage colony-stimulating factor (GM-CSF), mast cell-Tryptase, GFAP, IBA1, Calpain1 and intracellular adhesion molecule 1 expression were significantly increased in WT mice. Similarly, protein expressions of tyrosine hydroxylase, dopamine transporters and vesicular monoamine transporters 2 were significantly reduced in the SN of MPTP treated WT mice. The motor behavior as analyzed by rotarod and hang test was significantly reduced in WT mice as

compared with both the MC-KO and GMF-KO mice. We conclude that GMF-dependent mast cell activation enhances the detrimental effect of astro-glial activation-mediated oxidative stress and neuroinflammation in the midbrain, and propose that its inhibition may slowdown the progression of PD.

Disclosures: S. Govindhasamy pushpavathi: None. **A. Mohammad ejaz:** None. **K. Duraisamy:** None. **R. Thangavel:** None. **I. Dubova:** None. **S. Mentor:** None. **A. Dhaliwal:** None. **H. Zahoor:** None. **S. Zaheer:** None. **S. Raikwar:** None. **S. S iyer:** None. **A. Zaheer:** None.

Poster

131. Parkinson's Disease Progression

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Program #/Poster #: 131.20/F44

Topic: C.03. Parkinson’s Disease

Support: USPHS Grant R01ES022614

Title: Forward genetic assessment of paraquat toxicity in ventral midbrain and cerebellum in mice

Authors: *B. C. JONES[1], C. TORRES-ROJAS[1], P. JIMENEZ-CARRION[2], W. ZHAO[1], L. LU[3], M. K. MULLIGAN[1], J. P. O'CALLAGHAN[4];
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Abstract: The herbicide, paraquat (PQ), has been associated with risk for several ailments, including pulmonary and neurological diseases. For the latter, PQ has been implicated as a risk factor for sporadic Parkinson’s disease (sPD); however, apparently, not everyone exposed to paraquat is equally susceptible. The exact mechanism by which paraquat produces PD is not well known, but there is evidence for genetic contribution to susceptibility in humans and for PQ-related neurodegeneration in mice. Previously, we showed that PQ altered iron regulation in 2 inbred mouse strains. Here, we show that PQ produces individual differences in iron regulation in the ventral midbrain across 28 BXD recombinant inbred strains and the parental C57BL/6J and DBA/2J strains. We nominate a candidate gene, *Bag5*, that underlies the individual differences among the strains. *Bag5* codes for a protein that inhibits Parkin and promotes degeneration of dopamine neurons. We also show that PQ produces individual differences among the 30 strains in expression of the proinflammatory cytokine genes *Il1b* and *Lif* in cerebellum. Finally, we observed large strain-related differences in PQ concentration in cerebellum at each of three doses, 1, 5, and 10 mg/kg administered i.p.

Disclosures: **B.C. Jones:** None. **C. Torres-Rojas:** None. **P. Jimenez-Carrion:** None. **W. Zhao:** None. **L. Lu:** None. **M.K. Mulligan:** None. **J.P. O'Callaghan:** None.

Poster

131. Parkinson's Disease Progression

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 131.21/F45

Topic: C.03. Parkinson's Disease

Title: Allosteric modulation of nicotinic receptors reduces L-DOPA induced dyskinesias in Parkinsonian mice

Authors: *A. GOMEZ[1], M. PALOMERO-RIVERO[2], D. A. MILLÁN[3], R. DRUCKER-COLIN[4], J. BARGAS[5];
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Abstract: Dopamine (DA) precursor L-3,4-Dihydroxyphenylalanine (L-DOPA) remains the most effective symptomatic treatment of Parkinson's disease (PD), which is primarily due to dysfunction of the nigrostriatal dopaminergic pathway. However, long-term administration of L-DOPA induces the development of abnormal involuntary movements known as L-DOPA induced dyskinesia (LID), which can be quite incapacitating and are a major challenge in PD management. Mechanisms underlying LID are not fully understood and its medical treatment is generally unsatisfactory. Neuroprotective effects of nicotine and its amelioration of LID in different parkinsonian animal models have been reported. Nicotine exerts its effect primarily through nicotinic receptors (nAChRs), of which those that contain the α7 or the β2 receptor subunit are the most abundant in the brain. In this study, we sought to determine whether nicotine may also reduce LID in a mouse model of PD and whether this response can be potentiated by the positive allosteric modulation of the α7 and the α4β2 nAChRs. Adult male CD1 mice were stereotaxically injected with 6-OHDA in the forebrain bundle (unilaterally) to induce PD-like symptoms. These mice were then exposed to nicotine via drinking water (30 mg/l) for 3 weeks after which they were treated daily for 4 weeks with L-DOPA (12 mg/kg i.p). Nicotine concentration in the water was maintained for the entire period in these animals. Controls were treated identically, except no nicotine was present in the water. Once the animals were dyskinetic, two groups were formed, the first one was administered with PNU-120596 (allosteric modulator of the α7 nAChRs) and the second one was administered with NS9283 (allosteric modulator of α4β2 nAChRs). Afterwards, the dyskinesias were evaluated. Our

findings indicate that animals exposed to nicotine plus the allosteric modulators had significant abatement of dyskinesia, even better than the nicotine treatment by itself. Together, our results provide further support for therapeutic potential of nicotine in LID and suggest that the allosteric modulators PNU-120596 and NS9283 may also be useful to reduce it.

Disclosures: **A. Gomez:** None. **M. Palomero-Rivero:** None. **D.A. Millán:** None. **R. Drucker-Colin:** None. **J. Bargas:** None.

Poster

131. Parkinson's Disease Progression

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Program #/Poster #: 131.22/F46

Topic: C.03. Parkinson's Disease

Support: CAPES
CNPq

Title: Effects of monoamines reduction in depressive-like behavior and short-term memory in the rotenone model of Parkinson's disease

Authors: *D. C. RAMOS[1], J. C. F. VIEIRA[2], R. ANDREATINI[1], M. A. B. F. VITAL[1]; [1]Pharmacol., Univ. Federal Do Paraná, Curitiba, Brazil; [2]Neurosci. and Behavioral Sci., Univ. de São Paulo, Ribeirão Preto, Brazil

Abstract: The dopaminergic neurodegeneration is a hallmark of Parkinson's disease (PD), however the noradrenergic and serotonergic systems are also altered. According to the Braak staging of PD, neuropathological alterations in neurons of the raphe nuclei, locus coeruleus arise from stage 2, which precede the alterations in substantia nigra compacta during the stage 3. Studies have suggested that non-motor disturbances are a consequence of dopamine dysfunction concomitant with noradrenaline and/or serotonin alterations, indicating that the neurodegeneration is beyond the dopaminergic transmission in PD. We aimed to investigate the involvement of monoamines in brain areas related to depressive- and anxiety-like behaviors, and short-term memory in an animal model of PD. Male Wistar rats underwent a 10-day protocol used to induce nigrostriatal lesion. One group received rotenone 2.5 mg/kg systemically, and the other received vehicle (1 mL/kg). Anxiety-like behavior and short-term memory were evaluated on 22 and 25 days after the last rotenone injection, respectively. The animals were also tested in the modified forced swim test 29 days, in order to assess the depressive-like behavior. At the end of the last test, the rats were decapitated, and brains dissected for neurochemical analysis. Data were analyzed using repeated measures ANOVA followed by Bonferroni post hoc test and Student's t test ($p \leq 0.05$). The social recognition test demonstrated that the rotenone group showed a significant increase in the ratio of investigation ($p=0.01$) compared with the control

group, indicating an impairment in the short-term memory. In the modified swim test, a significant increase in immobility time was observed in the rotenone group compared with the control group ($p=0.027$). Anxiety-like behavior evaluation, assessed by elevated plus maze test, did not indicate a significant difference between the groups, neither in the percentage of open arms entries nor in the percentage of time in open arms. Dopamine levels were found significantly reduced in striatum, prefrontal cortex and amygdala ($p<0.01$). Moreover, dopamine metabolites, DOPAC and HVA were decreased in those structures ($p<0.01$). Noradrenaline and serotonin were decreased significantly in prefrontal cortex and striatum ($p<0.01$). The analysis of these monoamines showed that only noradrenaline was significantly decreased in amygdala of rotenone group compared with the control group ($p<0.01$). The results indicate that the depressive-like behavior and short-term memory impairment observed may be related to alterations in monoamines content in striatum and prefrontal cortex.

Disclosures: D.C. Ramos: None. **M.A.B.F. Vital:** None. **R. Andreatini:** None. **J.C.F. Vieira:** None.

Poster

131. Parkinson's Disease Progression

Location: Hall A

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Program #/Poster #: 131.23/G1

Topic: C.03. Parkinson’s Disease

Support: NIH NS101737
Thomas Hartman Center for Parkinson's Disease Research at Stony Brook University

Title: Time course underlying changes in inspiratory motor output variability in the 6-OHDA SN-lesioned Parkinson's disease rat model

Authors: *R. WADOLOWSKI, I. C. SOLOMON;
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Abstract: Impairment of central respiratory control is a common non-motor symptom (NMS) in Parkinson’s disease (PD). Ongoing work in our laboratory has focused on characterizing the respiratory phenotype of PD, including the time course of respiratory deficits, in 6-hydroxydopamine (6 OHDA) neurotoxin-induced unilateral substantia nigra (SN)-lesioned rat PD model. While the loss of dopaminergic neurons is the primary pathology of PD, it is also becoming better appreciated that serotonergic (5-HT) dysfunction may also play a role in the development of motor and NMS and complications in PD. To this end, recent studies addressing the role of 5-HT in PD symptoms have revealed that activation or antagonism of specific 5-HT receptor subtypes may have therapeutic benefit. 5-HT neurotransmission is also known to play a

critical role in control of breathing; thus, respiratory abnormalities in PD may be further complicated by the progressive 5-HT dysfunction in PD. Here, we examine the time course underlying changes in short- and long-term breath-to-breath variability in inspiratory motor (diaphragm EMG) activity during basal breathing and in response to acute administration of the 5-HT_{1A} receptor agonist 8-OH DPAT in spontaneously breathing urethane-anesthetized adult female rats at 1-4 weeks after SN 6-OHDA injections; control rats received vehicle injections. Using coefficient of variation (CV) and Poincaré plot analyses, we found that 6-OHDA lesioned rats generally had slightly lower short- and long- term variability in basal burst frequency compared to vehicle controls, with the magnitude of variability being greatest at 1-week post injection. In both 6 OHDA- and vehicle-injected rats, acute 8-OH-DPAT injection increased both burst frequency and amplitude, which was accompanied by an increase in short- and long-term frequency variability that was influenced by time post injection, and a decrease or negligible effect in burst amplitude variability that appeared to be unaffected by time post injection. While these preliminary observations suggest that (compared to control rats) SN- lesioned rats exhibit differences in basal and 5-HT_{1A} receptor activation-induced amplitude and frequency burst-to-burst variability, additional experiments are needed to identify specific mechanisms underlying these differences. Additionally, it remains to be determined whether the ventilatory variability impairments noted in this PD rat model persist beyond the 4-week time point studied here.

Disclosures: R. Wadolowski: None. **I.C. Solomon:** None.

Poster

131. Parkinson's Disease Progression

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Program #/Poster #: 131.24/G2

Topic: C.03. Parkinson’s Disease

Support: NIH R01

Title: Behavioral defects associated with amygdala and cortical dysfunction in mice with seeded alpha-synuclein inclusions

Authors: *M. VISWANATHAN[1], L. STOYKA[2], L. VOLPICELLI-DALEY[1];
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Abstract: Parkinson’s disease (PD) is characterized by loss of dopaminergic neurons within the substantia nigra pars compacta and intracellular inclusions composed mostly of the protein alpha-synuclein (a-syn). Individuals living with PD experience a variety of motor and nonmotor symptoms, with up to 80% of patients eventually developing cognitive changes. A strong negative correlation between the density of a-syn pathology and the mini-mental state examination (MMSE) suggests a possible causal role of a-syn pathology and cognitive deficits.

This project expanded knowledge on modeling non-motor symptoms of synucleinopathies in an animal model through the use of a-syn fibril injection and further characterized the resulting pathology. We show that bilateral injections of fibrils into the striatum results in robust a-syn inclusion formation in the cortex and amygdala. Fibril injected mice show deficits in a social dominance behavioral task associated with prefrontal cortex function. They also show deficits in fear conditioning, which is associated with amygdala function. Inclusions in the amygdala and prefrontal cortex were abundant and primarily localized to excitatory neurons. Together, these observations suggest fibril exposure can be an important technique in studying the nonmotor deficits of PD.

Disclosures: M. Viswanathan: None. **L. Stoyka:** None. **L. Volpicelli-Daley:** None.

Poster

131. Parkinson's Disease Progression

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Program #/Poster #: 131.25/G3

Topic: C.03. Parkinson’s Disease

Support: Spanish Ministry of Education through the National Program Juan de la Cierva (FJCI-2015-25095)
Spanish Ministry of Education through the National Program. SAF2015-67239-P
BBVA foundation grant

Title: Motor onset of Parkinson’s disease: Is there a somatotopic pattern?

Authors: M. H. G. MONJE[1,2], Á. SÁNCHEZ-FERRO[1], J. A. PINEDA-PARDO[1], *J. A. OBESO[1];
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Abstract: The vulnerability of the nigrostriatal dopaminergic system in Parkinson’s disease (PD) seems to follow a topography pattern, with the earliest and highest dopamine depletion occurring in the posterodorsal area of the putamen. From previous work, this area appears to correspond with the representation of the foot. Thus, the motor signs of PD should be more evident in this area of the body and subsequently affect the upper extremity and face. However, early motor features of PD seem to begin most intensely in the upper limbs. We aim to study the clinical onset and progression of motor manifestations in different body regions in early unmedicated PD patients. Twenty-one drug naïve PD patients were included in a prospective longitudinal study. The eligibility criteria included unilateral motor manifestations and less than 18 months since diagnosis. Twenty-one age and gender-matched healthy subjects (HS) were also enrolled. The most affected body region was determined using various standards including the patient and

neurologist reports; validated clinical MDS-UPDRS scale and objective quantitative metrics of motor function with inertial measurement units (Kinesia system and Xens). Non-parametric tests were used to compare motor assessment of the most affected side (MAS), less affected side (LAS) in PD patients, the dominant side (DS) and non-dominant side (NDS) in HS. Mean disease duration since diagnosis was 12 months. No differences were found in sociodemographic features between PD patients and controls. The large majority of patients (90.5%) reported upper limb impairment as the most affected region at disease onset. Upper limb predominant involvement was confirmed by both neurological examination (91%) and the MDS-UPDRS score (86%). The kinematic analyses reflected that all PD patients have significant (i.e. reduced) differences in acceleration and rotation amplitude in the tasks of the upper limbs, even for those fewer patients who showed predominantly affected the lower limbs (14%). The quantitative measurement showed that upper limbs performance was more affected than the lower limbs in PD patients ($p<0.05$). Agreement among the different measured standards (81%), confirmed upper limb predominant motor impairment. These findings showed in a more objective evaluation that PD patients first exhibit upper limb motor dysfunction. The apparent discrepancy with data suggesting a dorsal putaminal denervation preference at onset needs further exploration. We hypothesize that degree of putaminal dopamine lost require for developing motor signs in the lower limb is higher than the upper limb, thus compensating the initial dorsal predominant reduction.

Disclosures: M.H.G. Monje: None. **Á. Sánchez-Ferro:** None. **J.A. Pineda-Pardo:** None. **J.A. Obeso:** None.

Poster

131. Parkinson's Disease Progression

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Topic: C.03. Parkinson’s Disease

Support: Spanish Ministry of Education through the National Program Juan de la Cierva (FJCI-2015-25095)
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BBVA foundation grant

Title: Somatotopic related topography of dopaminergic denervation in early *de-novo* Parkinson's disease patients

Authors: M. H. G. MONJE[1,2], J. A. PINEDA-PARDO[1], Á. SÁNCHEZ-FERRO[1], *J. BLESA[1], J. A. OBESO[1];
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Abstract: Dopamine loss in Parkinson's disease (PD) affects most intensely the mesostriatal system. Early in the evolution, the topography of dopaminergic striatal denervation follows a posterior-anterior gradient, being the caudal (posterior) putamen the earliest and most depleted area. This area receives direct projections from motor cortices and have a somatotopic gradient with a dorsal-to-ventral, legs-hand-face orientation. As not all patients present the earliest clinical motor manifestations in the same body parts different denervation patterns might be observed along this axis. Our aim is to clarify how dopaminergic denervation occurs along the dorsoventral axis of the posterior putamen in the earliest stages of PD.
Early drug-naïve PD patients (n=23) and healthy subjects (HS) (n=19) underwent 6-[^{18}F]-fluoro-L-dopa (FD) examination in a hybrid PET-MR system (mMR-Biograph 3T Siemens). FD uptake rate (*Kocc*) was estimated using Patlak graphical method. Region-of-interest (ROIs) analysis was made by segmenting the putamen along the dorsoventral axis. We used a post-commissural superior-inferior division with respect to the anterior commissure; and a task fMRI-based subdivision for foot and hand putaminal motor activations from the Human Connectome Project dataset. Comparisons were made using unpaired 2-sample t-test between the uptake rates for the ROIs corresponding to the more affected side in PD patients (MAS) and the dominant side for HS (DS), and between the less affected side (LAS) and the non-dominant side for HS (NDS). Paired t-test comparison was used within group.
FD uptake differed between HS and PD patients for all studied ROIs in both hemispheres ($P < .001$). The dorsal part of the posterior putamen was more depleted than the ventral part in both MAS and LAS hemispheres. However, the percentage of depletion for the hand putaminal region was significantly higher than that of the foot in both MAS and LAS hemispheres.

ROI	DS	NDS	MAS	LAS	P-value	MAS (%)	LAS (%)
Dorsal Posterior Putamen	12.86 (1.66)	12.57 (1.61)	3.67 (0.95)	6.17 (1.39)	5.89E-15	71.4 (7.4)	50.9 (11.1)
Ventral Posterior Putamen	11.31 (1.66)	11.10 (1.73)	5.56 (1.06)	7.62 (0.98)	4.31E-14	50.8 (9.3)	31.4 (8.8)
Foot HCP t-fMRI	14.97 (1.71)	15.06 (1.67)	5.86 (1.33)	8.44 (1.64)	1.25E-14	60.9 (8.9)	43.9 (10.9)
Hand HCP t-fMRI	15.36 (1.56)	15.16 (1.59)	4.85 (1.11)	7.73 (1.57)	6.27E-15	68.4 (7.2)	49.0 (10.3)

Table 1. Regional average FD uptake rates and P-values showing non-parametric Kruskal-Wallis statistical comparison (1-factor; 4-conditions). The last two columns show the percentual difference between the PD uptake rates and the HS group.

The dorsal posterior putamen is the most affected dopamine-depleted region, but this larger depletion effect seems more specific for the functional representation of upper limbs. These results might indicate that there is a specific dopaminergic vulnerability for the upper-limbs striatal representation in the early stages of PD.

Disclosures: **M.H.G. Monje:** None. **J.A. Pineda-Pardo:** None. **Á. Sánchez-Ferro:** None. **J. Blesa:** None. **J.A. Obeso:** None.

Poster

131. Parkinson's Disease Progression

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Program #/Poster #: 131.27/G5

Topic: C.03. Parkinson's Disease

Support: Spanish Ministry of Education through the National Program Juan de la Cierva (FJCI-2015-25095)

Title: Reorganization of cortical motor fMRI activations in early de-novo Parkinson's disease

Authors: *J. A. PINEDA-PARDO[1], I. OBESO[2], M. H. G. MONJE[1,4], A. SANCHEZ-FERRO[1], J. A. OBESO[3];
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Abstract: Striatal dopaminergic deficit in patients with Parkinson's disease (PD) is associated with a myriad of basal ganglia and cortical changes, including compensatory mechanisms. fMRI studies in-vivo have demonstrated that cortical motor activation is abnormally boost in order to perform simple and complex self-initiated hand movements, while basal ganglia activation behave oppositely. However, no study has characterized how both cortical and striatal functional somatotopy is rearranged in PD, in response to upper- and lower-limbs movements in the same group of patients. This study aimed to describe the somatotopic functional organization in the cortex and striatum in early unmedicated PD patients.
Drug-naïve PD patients (n=17 [7w]; age=53.0; HY=1) and healthy subjects (HS) (n=23 [10w]; age = 49.3) performed a self-paced motor task in an MR system (mMR-Biograph 3T Siemens). The task had 4 conditions (right/left finger tapping and foot flexion-extension), 6 blocks of rest and movement per condition. GLM analysis was performed to extract first-level subject-wise activations. Subject's contrasts images were L-R flipped to match symptoms laterality between patients. Second-level random-effects analysis was performed to extract group main effects and differences between groups. Statistical significance was established for $P<0.001$, FWE-corrected at the cluster-level. Group activation maps showed clear somatotopic representation in both groups in the cortex, the cerebellum and the putamen. Cortical activations extended over the sensorimotor and premotor cortices in both hand and foot movements. Striatal activations were restricted to the mid-to posterior putamen. PD patients showed increased cortical activation in the sensorimotor cortex and bilateral ventral premotor cortex. Cerebellar motor cortex ipsilateral

to the movement side was hyperactive in PD patients. Ventral putamen showed decreased activation for the more affected hemibody. The coordinates for the more affected hand were shifted towards the anterior putamen (P=0.0045) while the less affected hand showed a marginal statistical trend for a shift in the same direction (P=0.0745). No differences were found for the foot peak activation.Cortical motor areas were found hyperactivate in early PD while performing self-paced movements with either hand/foot. Striatal hypoactivation and topography (i.e. somatotopy representation) re-organization was only found for the more affected hemibody. These results might suggest that cortical compensatory mechanisms concur with striatal function re-organization that could be directly related with degree of dopaminergic striatal depletion .

Disclosures: J.A. Pineda-Pardo: None. **I. Obeso:** None. **M.H.G. Monje:** None. **A. Sanchez-Ferro:** None. **J.A. Obeso:** None.

Poster

131. Parkinson's Disease Progression

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Topic: C.03. Parkinson’s Disease

Support: Branfman Family Foundation

Title: Gait analysis in male PINK1 knock-out and hemiparkinsonian 6-OHDA rats

Authors: *V. M. DEANGELO, G. C. MCCONNELL;
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Abstract: Introduction: Parkinson’s disease (PD) motor symptoms include tremor, bradykinesia, rigidy, and gait and postural disturbances. The hemiparkinsonian 6-hydroxydopamine (6-OHDA) rodent model of PD is widely used to investigate neural mechanisms underlying Parkinsonian gait. A possible alternative to the 6-OHDA model is the PINK1 knock-out (PINK1 KO) in which the PINK1 (PTEN-induced putative kinase 1) gene is deleted. We hypothesized that PINK1 KO rats more closely model the onset and progression of gait disturbances seen in humans with PD than 6-OHDA rats. Methods: A total of twelve male rats were used in this study: four 6-OHDA (lesioned at age 6 months), four PINK1 KO, and four wild-type. Multiple gait parameters were measured using a gait-tracking runway system at 5, 7, and 8 months of age. Results: Only one out of the four PINK1 KO rats displayed significant changes in gait from age 5 months to 8 months when compared to age-matched wild-type rats. This PINK1 KO rat had significantly decreased stride length, swing time, and rear-track width, all of which are consistent with clinical presentations of PD. An increase in stance time was found in contrast to what is typically discussed in the literature. Overall, PINK1 KO rats displayed similar stride length and significantly decreased stance time, stride time, rear-track width, and swing time in comparison

to 6-OHDA rats. Conclusion: Humans with PD typically experience a decrease in base of support, stride length, and swing time, along with an increase in stance time and no significant change in stride time. Our results suggest that certain features of clinical gait disturbances more closely parallel the PINK1 KO model while others more closely parallel the 6-OHDA model. Future studies should account for the high variability in gait disturbances observed in PINK1 KO rats.

Disclosures: V.M. DeAngelo: None. **G.C. McConnell:** None.

Poster

131. Parkinson's Disease Progression

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 131.29/G7

Topic: C.03. Parkinson's Disease

Support: The Branfman Family Foundation

Title: Mild gait deficits in aged female PINK1 knock-out rats without concomitant loss of dopamine neurons

Authors: *B. O'NEILL, G. MCCONNELL;
Dept of Biomed. Engin., Stevens Inst. of Technol., Hoboken, NJ

Abstract: Progress toward understanding the neural mechanisms of gait and postural dysfunction in Parkinson's disease (PD) is hampered by a lack of spontaneously progressive PD animal models. We characterized gait and posture in *Park6* knock-out rats (lacking the PINK1 protein) as an approach to determine the suitability of this model to 1) investigate neural mechanisms and 2) test novel treatments for gait and postural dysfunction in PD. Using six female knock-out and six conspecific wild-type rats, we monitored behavior in a gait-tracking runway, a treadmill, and on a posture-monitoring force plate. We subsequently performed a prolonged upright posture task, followed by IHC analysis of markers of activity in motor areas (c-FOS, cytochrome c oxidase) and dopamine neuron loss (tyrosine hydroxylase). The runway task was conducted across aging - at 5, 7, 8, and 12 months of age. The treadmill and force plate were conducted between 11 and 13 months of age.
PINK1 knock-out rats at 11 months displayed decreased stride length relative to wild-type controls during treadmill walking (speed = 9 cm/s). They also displayed increased rear track width and increased rear limb duty factor during this task - trends consistent in persons with PD. Results in the runway task differed from these treadmill results, with increased stride length at 8 months, and no change (relative to wild-type rats) in stride length or rear track width at 12 months. No significant differences between genotypes were observed in force-plate measurements. These results suggest that gait and postural dysfunction in female PINK1 knock-

out rats is mild - even around 1 year of age - and may be task specific. No significant loss of dopaminergic neurons was detected in PINK1 knock-out rats. Studies are ongoing to determine the extent to which gait deficits depend on loss of cholinergic neurons in the pedunculopontine tegmental nucleus.

Disclosures: B. O'Neill: None. **G. McConnell:** None.

Poster

132. Parkinson?s Disease Therapeutic Strategies: Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 132.01/G8

Topic: C.03. Parkinson’s Disease

Support: NIH/NIAAA R03AA022479
NIA/NIH 1R25AG047843-01

Title: Butyrate protects against salsolinol-induced toxicity in SH-SY5Y cells: Implication for Parkinson's disease

Authors: B. GETACHEW[1], A. B. CSOKA[2], R. L. COPELAND[1], ***Y. TIZABI**[1];
[1]Pharmacol., [2]Anat., Howard Univ. Col. of Med., Washington, DC

Abstract: Parkinson's disease(PD) is a progressive neurodegenerative disorder associated with the destruction of dopamine neurons in the substantia nigra (SN) and the formation of Lewy bodies in basal ganglia. Risk factors for PD include aging, as well as environmental and genetic factors. Recent converging reports suggest a role for epigenetic mechanisms in the onset and/or progression of PD. Of particular relevance and potential therapeutic targets in this regard, are histone deacetylases (HDACs), enzymes that are involved in chromatin remodeling. Butyrate, a short-chain fatty acid produced in the gut, is a well-known HDAC inhibitor that plays an important role in maintaining the gut’s normal function. It also serves as the primary energy source for the colonocyte, inducing epithelium proliferation and regulating gene expression. Thus, butyrate may be of significant importance in gut-brain axis and regulation of critical brain functions. In this study, we sought to determine whether butyrate may have protective effects against salsolionl (SALS)-induced toxicity in SH-SY5Y cells. SALS, an endogenous product of aldehyde and dopamine condensation, may be selectively toxic to dopaminergic neurons. SH-SY5Y cells, derived from human neuroblastoma cells are used as a model of these neurons. Exposure of SH-SY5Y cells for 24 h to 400 μM SALS resulted in approximately 50% cell death. Pretreatment with butyrate dose-dependently prevented SALS-induced toxicity. Maximal (100%) protection was observed with 20 μM butyrate pretreatment. Although the mechanism by which butyrate confers this neuroprotection is not evident, our findings do suggest therapeutic

potential of butyrate in PD. Supported by: NIH/NIAAA R03AA022479 (YT), NIA/NIH 1R25AG047843-01 (ABC)

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Poster

132. Parkinson?s Disease Therapeutic Strategies: Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 132.02/G9

Topic: C.03. Parkinson's Disease

Support: NINDS T32 NS086749
CDMRP, DoD, US Army PD160107

Title: Transient elevated dopamine pretreatment alleviates motor impairments in a 6-OHDA model of Parkinson's disease

Authors: *J. C. BRAGUE, R. P. SEAL;
Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Parkinson's disease (PD) is one of the most prevalent neurological diseases, producing striking motor impairments that greatly impact patients' quality of life and place a significant burden on families, the healthcare system, and society as a whole. This debilitating neurodegenerative disorder results from the loss of substantia nigra pars compacta (SNpC) dopamine (DA) neurons and few treatment strategies exist to either slow or halt the progression of the disease or to ameliorate the debilitating motor symptoms. L-3,4-dihydroxyphenylalanine (L-dopa) therapy increases striatal DA content and is the most effective therapy for treating symptoms, but its persistent use often results in a loss of efficacy and L-dopa induced dyskinesias (LIDs), which are themselves debilitating. Thus, novel strategies to treat the motor impairments of PD are still needed. Our finding that vesicular glutamate transporter 3 knockout (KO) mice do not develop motor impairments in a 6-OHDA mouse model of PD provides a unique opportunity to investigate the mechanistic basis for motor symptoms that normally occur in the disease as well as potential new strategies to ameliorate them. Specifically, the work pointed to a previously unappreciated mechanism of striatal MSN plasticity that restores synaptic connectivity and motor function in Parkinsonian mice. KO mice show a circadian-dependent hyperdopaminergia, hyper locomotion, and upregulation of immature dendritic spines on dopamine 1 receptor containing medium spiny neurons (D1R MSNs). Unexpectedly, DA depleted KO mice exhibit an increase of mature D1R MSN spines and do not show motor impairments. I hypothesize that transient elevated dopamine (TED) and subsequent DA depletion impart the spine and normalized behavioral phenotypes we see in DA depleted KO mice. Here I present preliminary data using an excitatory designer receptor exclusively activated by designer

drugs (eDREADD) approach that supports this hypothesis. The data indicate that TED mice show upregulated immature spines on D1R MSNs (n=11, $p<0.05$) and hyperlocomotive behavior (n=7, $P<0.01$) prior to depletion. Following depletion, TED mice exhibit an upregulation of mature spines on D1R MSNs (n=11, $p<0.01$) and normalized motor function throughout the circadian cycle (n=13, $p<0.001$), when compared to controls. These data suggest that dopamine upregulation, prior to depletion, provides a potential therapeutic effect in PD by altering dendritic spines on D1R MSNs. Understanding how striatal dopamine levels affect spine plasticity within basal ganglia motor circuits will help the development of more effective treatment strategies for patients suffering from PD.

Disclosures: J.C. Brague: None. **R.P. Seal:** None.

Poster

132. Parkinson?s Disease Therapeutic Strategies: Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 132.03/G10

Topic: C.03. Parkinson's Disease

Support: Postdoctoral scholarship by DGAPA-UNAM

Title: Characterization of the physiological effect induced by SiO2 nano-matrices in differentiated SH-SY5Y cells

Authors: *A. J. ESPADAS-ALVAREZ, Jr[1], P. VERGARA-ARAGON[2], R. GARCIA-VILLEGAS[3], L. TEXCO-MARTINEZ[4], H. G. ESPADAS-ALVAREZ[5], D. MEDINA-BUENO[6]; [1]Fisiologia, Univ. Nacional Autónoma De México (UNAM), Mexico, Mexico; [2]Fisiología, Facultad De Medicina, Mexico DF, Mexico; [3]Fisiologia Celular, [4]Fisiología, [5]Fisiología Celular, CINVESTAV-IPN, Mexico, Mexico; [6]CICATA-LEGARIA-IPN, Mexico, Mexico

Abstract: We are interested in developing new and better biotechnological strategies for the treatment of diseases of the central nervous system. Silicon dioxide (SiO_2) has been used to build nanomatrices (NMs) capable of releasing and transporting dopamine (DA) to treat Parkinson's disease in animal model. We build, characterize SiO_2 NMs empty or DA-containing and evaluate the physiological effect of these on the differentiated or undifferentiated SH-SY5Y human cells. Surprisingly, we observed that the SiO_2 NMs empty increased the characteristics of the dopaminergic phenotype in both differentiated and undifferentiated SH-SY5Y cells. Both results are comparables with dopaminergic neurons *in vivo*, evaluate by their morphology as by the high levels of expression of tyrosine hydroxylase. In addition, the SiO_2 NMs empty did not produced nitrosative stress in cultured cells.

Disclosures: **A.J. Espadas-Alvarez:** None. **P. Vergara-Aragon:** None. **R. Garcia-Villegas:** None. **L. Texco-Martinez:** None. **H.G. Espadas-Alvarez:** None. **D. Medina-Bueno:** None.

Poster

132. Parkinson?s Disease Therapeutic Strategies: Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 132.04/G11

Topic: C.03. Parkinson's Disease

Title: Effect of docosahexaenoic acid (DHA) at the enteric level in a synucleinopathy mouse model

Authors: *J. LAMONTAGNE-PROULX[1,2], K. COULOMBE[1], C. GUYAZ[1,2], M. CÔTÉ[1], C. TREMBLAY[1], F. CALON[1,2,3], D. SOULET[1,2];
[1]Crchuq-Université Laval, Québec, QC, Canada; [2]Fac. of Pharmacy, Univ. Laval, Québec, QC, Canada; [3]Assn. des Laboratoires OptiNutriBrain Intl., NutriNeuro France -INAF Canada, QC, Canada

Abstract: The main neuropathological feature of Parkinson's disease (PD) is the aggregation of α-synuclein protein (α-syn). The hypothesis has been made that PD could start with the deposition of α-syn in the enteric nervous system (ENS) resulting in gut dysfunction. Recent studies suggest that the pathology could spread from the gut to the brain. Furthermore, it has already been reported that a diet enriched with docosahexaenoic acid (DHA) acts as a neuroprotective agent in the brain in models of PD and may have an impact on the aggregation of α-syn. Thus, we want to study the effect of DHA supplementation on the α-syn protein at the peripheral level. We believe that a diet rich in DHA would reduce the progression of the disease by targeting dopaminergic (DA) neurons in the intestine.
To verify our hypothesis, Thy1-aSyn mice, which overexpressed human α-syn, were fed *ad libitum* for 10 months with either control, low or high DHA diets. Animals were sacrificed at 12 months, and guts were collected to assess the effect of the various diets on the intestine of this PD mouse model via different immunohistochemistry analyses.
Our data show a lower level of DA neurons in the ENS of Thy1-aSyn mice with control diet compared to wild-type animals. This decline in DA neurons was prevented when Thy1-a-syn mice were fed with a DHA-rich diet compared to control and low DHA diet. Interestingly, a DHA receptor, GPR120, was highly expressed in myenteric neurons, suggesting that this receptor could mediate the neuroprotective effects of DHA. Moreover, results show an increase of glucagon-like peptide-1 (GLP-1) in the group fed with DHA compared to the control and low DHA groups. Of interest, the GLP-1 receptor can be found in myenteric neurons. Our results appear to be consistent with studies showing neuroprotective effects related to the treatment with GLP-1 in PD models and patients.

In conclusion, DHA acts as a neuroprotective agent preventing the loss of DA neurons in the myenteric plexus. We believe that this effect can be mediated through the DHA by the presence of the DHA receptor and/or the increase in GLP-1 expression. These results have great translational value for nutraceutical strategies since GLP-1 is known to be protective in PD patients.

Disclosures: J. Lamontagne-Proulx: None. **K. Coulombe:** None. **C. Guyaz:** None. **M. Côté:** None. **C. Tremblay:** None. **F. Calon:** None. **D. Soulet:** None.

Poster

132. Parkinson?s Disease Therapeutic Strategies: Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 132.05/G12

Topic: C.03. Parkinson's Disease

Support: State of Connecticut
Private donor

Title: Generation of pluripotent stem cells using SCNT and iPS cell technologies in African green monkeys

Authors: Y. G. CHUNG[1,2,3], M. SEAY[2], J. D. ELSWORTH[2], T. J. COLLIER[4], *D. E. REDMOND, Jr[1];
[1]Axion Res. Fndn., Hamden, CT; [2]Yale Univ., New Haven, CT; [3]Enolc, Inc., Farmington, CT; [4]Neurosci., Michigan State Univ., Grand Rapids, MI

Abstract: Few pluripotent stem cell lines have been derived from monkeys, limiting studies of possible autologous cell replacement therapy for Parkinson's disease (PD). Therefore, our goal was to establish and compare stem cell lines using somatic cell nuclear transfer (SCNT) and induced pluripotent somatic cell (iPS) techniques. As we have validated the MPTP model in African green monkey and used it extensively to study cell therapies from human & monkey fetal brain tissue, neural stem, iPS, and parthenogenic cells, we chose this species to generate pluripotent stem cells to carry out studies of autologous cells differentiated to dopamine (DA) neurons. Using 24 female monkeys as egg donors and 18 adult somatic nuclear (fibroblasts) donors, we derived 11 SCNT ES cell lines and 2 iPS cell lines. Surprisingly, the generation of stem cell lines using the SCNT method was significantly more efficient than using the iPS counterpart (11/24 vs 2/120 attempts). They all expressed the typical stemness marker genes including OCT-4, NANOG, SSEA-1, and TRA1-60, and formed all 3 germ layer derivatives in the teratomas in immune deficient mice, proving pluripotency. All the cell lines kept intact chromosome numbers (60, XX or XY) even after extensive cell passages (> 30) in serum free and feeder cell free culture systems. Furthermore, all the stem cell lines were differentiated into

DA neurons *in vitro* expressing TH when an A-9 DA cell differentiation protocol was applied. There was no difference in the efficiency of DA neuron differentiation between SCNT ES cell lines and iPS cell lines. We also injected green fluorescent protein labeled DA cells with varying degrees of differentiation (14 to 28 days) derived from 6 different SCNT cell lines into the nigrostriatal systems of 6 male monkeys. After 3 months in vivo 5 out of the 6 cell lines survived but did not express TH. Histologically two of the cell lines exhibited no nestin but stained positive for Pitx3, a transcription factor implicated in DA neuron differentiation. One monkey developed a large GFP labeled teratoma. In conclusion, we have developed an effective SCNT derivation protocol which culminated in multiple pluripotent stem cell lines in the African green monkey. Improving the differentiation and maintenance of the A9 DA phenotype and eliminating the teratoma risk should allow more systematic comparative studies in the MPTP model of Parkinson's disease in the future.

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Poster

132. Parkinson?s Disease Therapeutic Strategies: Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 132.06/G13

Topic: C.03. Parkinson's Disease

Support: NIH/NINDS NS 086604
NS096282

Title: Chemical activation of nigrally grafted human dopaminergic neurons accelerates their axonal growth and re-innervation into the host striatum

Authors: *Y. TAO[1], Y. CHEN[2], M. XIONG[2], S.-C. ZHANG[3];
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[3]Waisman Ctr., Univ. Of Wisconsin - Madison, Madison, WI

Abstract: Human neural progenitors, following transplantation into the adult mammalian brain, can mature and grow axons, often specifically to their target brain regions. Such a behavior raises hopes for reconstructing a neural circuit through cell transplantation. Nevertheless, the growth rate is rather stereotypic, roughly 1 mm/month. Such a growth rate may become a barrier for application in humans in which the distance is long. We hypothesize that activation of the immature neurons may accelerate the axonal growth, thus shortening the period for reconstructing the neural circuit. By transplanting midbrain dopamine neuron progenitors, derived from DREADD-expressing human pluripotent stem cells (hPSC), into the substantia nigra of Parkinson's disease model mice, we found that the axons reach the dorsal-lateral

striatum with concomitant motor behavioral recovery in 5-6 month post-transplant. This process was shortened to 3.5 months when the animals were fed with the DREADD ligand CNO. Histological analysis showed a similar axonal innervation in the striatum between animals with or without CNO treatment, suggesting that the accelerated axonal growth does not change the targeting. These results suggest that potentiation of grafted neuronal activities speeds up the axonal growth and their re-innervation into the host brain. This finding opens a possibility for reconstructing a neural circuit in the human brain.

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Poster

132. Parkinson?s Disease Therapeutic Strategies: Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 132.07/G14

Topic: C.03. Parkinson's Disease

Support: Instituto de Salud Carlos III grant - Fund. Inv. Biomed. Hospital Universitario La Princesa

Title: New family of multitarget compounds for the treatment of Parkinson's disease: Design, synthesis, biological and computational evaluation

Authors: ***P. DUARTE**[1,2], P. MICHALSKA[1,2], I. BUENDIA[1,2], J. FRANCO-GONZÁLEZ[1], E. CRISMAN[2], A. CUADRADO[3], R. LEÓN[1,2];
[1]Pharmacol., Inst. Teófilo Hernando, Univ. Autónoma de Madrid, Madrid, Spain; [2]Inst. de Investigación Sanitaria Hosp. Universitario La Princesa, Madrid, Spain; [3]Inst. de Investigaciones Biomédicas, Madrid, Spain

Abstract: Parkinson's disease (PD) is the second most prevalent neurodegenerative disease. One of the major complications about PD is the lack of effective treatments able to stop the progression of the disease, so finding new therapies is an important challenge for this century. Physiopathological features such as dopamine depletion and selective destruction of dopaminergic neurons, along with the presence of α-synuclein protein deposits, known as Lewy bodies, are considered as main events in PD. Apart from this, oxidative stress has appeared playing an important role in the disease progression and could be a good pharmacological target. It is related with deregulation of protein processing, mitochondrial damage and neuroinflammation and these factors, in turn, lead to the increase of oxidative stress generating a loop that accelerates the neurodegeneration process. Regarding the high complexity and multifactorial nature of PD we think about multitarget strategy as a good approach. In this sense, we considered two main targets to develop new treatments. On the one hand, NF-E2-related factor 2 (NRF2) is the master regulator of antioxidant defence in cells, among other general

pathways. On the other hand, inhibitors of monoamine oxidase B (MAO-B) help controlling oxidative stress, regarding dopamine metabolism, and they have been used for years in clinic to treat PD symptoms. Hence, we have designed and synthesized a small chemical library of new multitarget compounds combining several activities: (i) NRF2 inducing capacity; (ii) MAO-B potent and selective inhibition; (iii) Neuroprotective ability in oxidative stress related *in vitro* models: 6-hydroxydopamine (6-OHDA) and rotenone/oligomycin in neuroblastoma cell line SH-SY5Y. In addition, we performed a more complex model of adult rat striatal slices and acute treatments with rotenone and 6-OHDA as toxics. Selected compounds were protective in terms of cell death and oxidative stress (measured with fluorescent probes) and showed a good profile in the expression modulation of several proteins related with neurodegeneration. Our study also includes an *in silico* program to go deeper into the inhibition of MAO-B and to improve this property for future families of compounds. To this end, we implemented docking and molecular dynamics studies with computational calculations of binding energies. To conclude, we have obtained hit compounds with a good pharmacological profile for PD and the present work leads us to consider further structural modifications to enhance the desired properties.

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Poster

132. Parkinson?s Disease Therapeutic Strategies: Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 132.08/G15

Topic: C.03. Parkinson’s Disease

Support: NIH Grant R21-NS109505-01

Title: Potent inhibitors of toxic alpha-synuclein oligomers identified via novel cellular time-resolved FRET biosensor

Authors: ***A. R. BRAUN**[1], C. LO[1], H. ZAHID[2], J. JOHNSON[2], M. HORVATH[4], M. C. YOUNG[1], K. C. LUK[4], W. POMERANTZ[2], D. D. THOMAS[3], J. N. SACHS[1];
[1]Biomed. Engin., [2]Chem., [3]Biochemistry, Mol. Biology, and Biophysics, Univ. of Minnesota TC, Minneapolis, MN; [4]Dept of Pathology and Lab. Med., Univ. Pennsylvania, Philadelphia, PA

Abstract: Preventing or reversing the pathological misfolding and self-association of alpha-synuclein (aSyn) can rescue a broad spectrum of pathological cellular insults that manifest in Parkinson’s disease (PD), Dementia with Lewy bodies (DLB), and other alpha-synucleinopathies. We have developed a high-throughput, FRET-based drug discovery platform that combines high-resolution protein structural detection in living cells with an array of functional and biophysical assays to identify novel lead compounds that protect cells and

neurons from aSyn induced cytotoxicity, pathology, as well as inhibiting seeded aSyn aggregation, even at nanomolar concentrations.
Our combination of cellular and cell-free assays allow us to distinguish between direct aSyn binding or indirect mechanisms of action (MOA). We focus on targeting oligomers with the requisite sensitivity to detect subtle protein structural changes that may lead to effective therapeutic discoveries for PD, DLB, and other alpha-synucleinopathies. Pilot high-throughput screens (HTS) using our aSyn cellular FRET biosensors has led to the discovery of the first nanomolar-affinity small molecules that disrupt toxic aSyn oligomers in cells and inhibit cell death. Primary neuron assays of aSyn pathology (e.g. phosphorylation of mouse aSyn PFF in neurons) show rescue of pathology for two of our tested compounds, similar to that of control compound Baicalin. Subsequent biophysical assays using CPMG NMR and seeded aSyn aggregation demonstrate these compounds act directly on aSyn. Other hit compounds identified in our pilot HTS are known to modulate oxidative stress, autophagy, and ER stress, providing validation our biosensor is sensitive to indirect MOA as well.

Disclosures: **A.R. Braun:** None. **C. Lo:** None. **H. Zahid:** None. **J. Johnson:** None. **M. Horvath:** None. **M.C. Young:** None. **K.C. Luk:** None. **W. Pomerantz:** None. **D.D. Thomas:** A. Employment/Salary (full or part-time):; Photonic Pharma LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Photonic Pharma LLC. **J.N. Sachs:** None.

Poster

132. Parkinson?s Disease Therapeutic Strategies: Cellular and Animal Models

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Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 132.09/G16

Topic: C.03. Parkinson’s Disease

Support: NIGMS Grant T32 GM 65841
The Grainger Foundation

Title: Effect of high frequency stimulation on neurological non-motor symptoms in a rat model of premotor-stage Parkinson's disease

Authors: ***S. G. HILLAN**[1], A. J. ASP[1], S. BOSCHEN DE SOUZA[2], J. L. LUJAN[3];
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[2]Neurologic Surgery, [3]Neurologic Surgery and Physiol. and Biomed. Engin., Mayo Clin., Rochester, MN

Abstract: Parkinson’s disease (PD) is a neurodegenerative disease caused by progressive loss of dopaminergic neurons in the substania nigra. Although primarily associated with movement symptoms such as tremor and muscle rigidity, most PD patients also experience severe non-

motor symptoms including anxiety, depression, sleep problems, cognitive decline, and autonomic dysfunction [1].
A well-established treatment for the motor symptoms of PD is high frequency deep brain stimulation (DBS) of the subthalamic nucleus (STN). Unfortunately, the impact of STN DBS on the non-motor symptoms of PD is yet to be well characterized. These non-motor outcomes of STN DBS vary significantly across patients, but decline in working memory and worsening of mood disorders have been observed in multi-center clinical trials [2, 3]. To prevent these non-motor effects and improve the efficacy of STN DBS, there is a need to better understand the neural pathways associated with these non-motor symptoms.
As a first step toward understanding neural engagement responsible for the non-motor effects of STN DBS, we will first investigate and validate the effects of increasing stimulation amplitude on the induction of anhedonia, anxiety, and cognitive decline in a 6-hydroxydopamine-lesioned rat model of PD. Increased stimulation amplitudes should result in greater spread of electrical current. Thus, we expect that increased stimulation amplitude will worsen anhedonia and anxiety-like behaviors by engaging limbic and associative circuitry within the STN. Similarly, we expect that working and spatial memory will worsen as stimulation amplitude is increased. Future work will focus on coupling the effects of STN DBS with in vivo calcium imaging and tractography activation models to create a framework for investigation of the cellular and network effects of STN DBS on the non-motor features of PD.
[1]K. Chaudhuri, D. Healy and A. Schapira, "Non-motor symptoms of Parkinson's disease: diagnosis and management", The Lancet Neurology, vol. 5, no. 3, pp. 235-245, 2006. DOI: 10.1016/s1474-4422(06)70373-8.
[2]J. Rothlind et al., "Neuropsychological changes following deep brain stimulation surgery for Parkinson's disease: comparisons of treatment at pallidal and subthalamic targets versus best medical therapy", Journal of Neurology, Neurosurgery & Psychiatry, vol. 86, no. 6, pp. 622-629, 2014. DOI: 10.1136/jnnp-2014-308119
[3]K. Follett et al., "Pallidal versus Subthalamic Deep-Brain Stimulation for Parkinson's Disease", New England Journal of Medicine, vol. 362, no. 22, pp. 2077-2091, 2010. Available: 10.1056/nejmoa0907083

Disclosures: **S.G. Hillan:** None. **A.J. Asp:** None. **S. Boschen De Souza:** None. **J.L. Lujan:** None.

Poster

132. Parkinson?s Disease Therapeutic Strategies: Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 132.10/G17

Topic: C.03. Parkinson’s Disease

Support: NIH/NINDS R01 NS107336-01

Title: Changes in motor cortex activity evoked by subthalamic nucleus deep brain stimulation in GCaMP6f-expressing Parkinsonian rats

Authors: *S. L. BOSCHEN DE SOUZA[1], S. GORMAN[2], S. PAEK[2], S. WANG[1], L. J. LUJAN[1];
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Abstract: Clinical and preclinical studies suggest that activation of hyperdirect pathway corticofugal projection neurons from the motor cortex (M1) via subthalamic nucleus (STN) deep brain stimulation (DBS) is associated with amelioration of motor deficits in Parkinson's disease. However, little is known about the effects of STN DBS in the patterns of neuronal activity in M1. Our study aims at exploring changes in these patterns of activity via calcium imaging in the context of behavior in a rat model of parkinsonism. To this end, we will induce expression of a calcium indicator in M1 neurons using AAV9-CamKII-GCaMP6f-WPRE.SV40. We will use constrained non-negative matrix factorization (CNMF-E) to detect activity changes in GCaMP6f expressing M1 neurons during open-field behavior in 6-hydroxydopamine lesioned rats using a head-mounted miniature microscope. Finally, we will characterize changes in the patterns of activity of M1 neurons associated with changes in locomotive speed as a function of STN stimulation frequency. This study represents the first steps toward elucidating the cell-specific contributions of the hyperdirect pathway in the effects of STN DBS.

Disclosures: S.L. Boschen De Souza: None. **S. Gorman:** None. **S. Paek:** None. **S. Wang:** None. **L.J. Lujan:** None.

Poster

132. Parkinson?s Disease Therapeutic Strategies: Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 132.11/G18

Topic: C.03. Parkinson's Disease

Support: NIH Grant R25GM075148
NINDS R01 NS 107336

Title: Biophysical model of astrocyte excitation in high frequency stimulation

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Abstract: The goal of neuromodulation therapies, such as deep brain stimulation, is to normalize pathological function within the nervous system. Despite the success of these therapies in treating neurological and psychiatric conditions such as Parkinson's disease and depression, their therapeutic mechanisms are still debated. Astrocytes are key regulators of neuronal excitability and neuroplasticity. Yet, their role in neuromodulation has not been studied as extensively as that of neurons. Given the abundance and homeostatic importance of astrocytes in the nervous system, we seek to model their calcium response to a high frequency electric field to study their interactions with excitable neurons. Membrane bound voltage gated calcium channels (VGCC) are expressed in varying amounts across astrocytic lineages, providing a potential mechanism for calcium spiking in response to electrical stimulation. Here we present a novel stochastic calcium spiking model in astrocytes incorporating Hodgkin-Huxley style VGCC to study electrical excitation of intracellular calcium oscillations at both the individual and network levels. To validate our model we will combine calcium imaging and extracellular electrophysiological recording to explore astrocytic calcium response to 100-130Hz current stimulations in acute hippocampal slices. This represents the first steps toward improving the understanding of the involvement of glial cells in neuromodulation therapies.

Disclosures: K.A. Montejo: None. **L. Balachandar:** None. **A. Moshkforoush:** None. **S.B. De Souza:** None. **A.J. Asp:** None. **S. Wang:** None. **S.G. Hillan:** None. **J.J. Riera:** None. **J. Lujan:** None.

Poster

132. Parkinson?s Disease Therapeutic Strategies: Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 132.12/G19

Topic: C.03. Parkinson's Disease

Support: A.B. - Swedish Research Council Grant 2018-02608
M.P. - Swedish Research Council Consolidator Grant (2017-2022): 2016-00873
M.P. is a New York Stem Cell Foundation Robertson Fellow (NYSCF)
D.B.H. - The Swedish Parkinson Foundation (Parkinsonfonden)

Title: Assessing the impact of a-synuclein pathology on transplanted hESC-derived dopaminergic neutrons in an accelerated a-synuclein rat model of Parkinson's disease

Authors: *D. B. HOBAN[1], S. SHRIGLEY[2], T. CARDOSO[2], B. MATTSSON[2], K. C. LUK[3], A. BJORKLUND[4], M. P. PARMAR[5];
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Abstract: Preclinical assessment of the therapeutic potential of human embryonic stem cell (hESC) derived dopaminergic (DA) neurons has primarily been performed in the 6-hydroxydopamine (6-OHDA) DA depletion rat model of Parkinson's disease (PD). While this is a good model to assess appropriate DA release and graft function, it does not reflect the pathological features or progressive nature of PD. In this study we establish an accelerated model of PD by injecting a mix of preformed human a-synuclein (asyn) fibrils and AAV6 human asyn unilaterally into the rat substantia nigra (SN) and assess how the transplanted cells survive, mature, integrate and innervate the host circuitry after transplantation into the striatum once the pathology has developed .
This model gives rise to inflammation, DA cell dysfunction and progressive loss of DA cells from the SN and terminals in the striatum. Importantly, the model shows extensive asyn pathology in both the substantia nigra and striatum making it an interesting and relevant model in which to examine cell transplantation. After allowing the model to develop for 4-8 weeks, we transplanted hESC-derived DA neurons into the striatum and assessed their survival, maturation, integration and innervation at 6- and 14-weeks post-transplant. Post mortem histology revealed that transplanted cells were capable of innervating the dopamine depleted striatum in a similar, and biologically-relevant pattern previously seen in the 6-OHDA model. We also used monosynaptic tracing based on modified rabies virus to assess that the pathology present in this model did not affect the ability of the graft to integrate into the host circuitry, meaning that the grafted cells are able to receive appropriate and sufficient synaptic contact with the host central nervous system. Finally, on closer examination, we found evidence of asyn pathology in the form of phosphorylated asyn inclusions in the grafted TH+ cells, indicating host-to-graft transfer of asyn pathology.
Further studies to examine a longer time-point where we can assess the maturation and function of the transplanted cells, and if this is affected by the pathology transfer, are currently underway. This will give us a better understanding of the performance of these cells in a more clinically relevant, albeit accelerated, novel asyn model of PD, thus adding to the body of knowledge required as this cell replacement therapy progresses to clinical trials.

Disclosures: D.B. Hoban: None. **S. Shrigley:** None. **T. Cardoso:** None. **B. Mattsson:** None. **K.C. Luk:** None. **A. Bjorklund:** None. **M.P. Parmar:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); M.P. is the owner of Parmar Cells AB and co-inventor of the US patent application 15/093,927 owned by Biolamina AB, and EP17181588 owned by Miltenyi Biotec.

Poster

132. Parkinson?s Disease Therapeutic Strategies: Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 132.13/G20

Topic: C.03. Parkinson's Disease

Support: A.B. - Swedish Research Council Grant 2018-02608
M.P. - Swedish Research Council Consolidator Grant (2017-2022): 2016-00873
M.P. is a New York Stem Cell Foundation Robertson Fellow (NYSCF)
D.B.H. - The Swedish Parkinson Foundation (Parkinsonfonden)

Title: Development of a novel, accelerated a-synuclein rat model of Parkinson's disease for longterm assessment therapeutic interventions

Authors: *B. MATTSSON[1], S. SHRIGLEY[1], L. S. BREGER[2], K. C. LUK[3], A. BJORKLUND[4], M. PARMAR[5], D. B. HOBAN[1];
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Abstract: While the involvement of a-synuclein (a-syn) in the pathogenesis of Parkinson's disease (PD) is well established, many a-syn based animal models of PD do not recapitulate the full range of cellular and behavioural changes that are characteristic of the human disease, nor do they do so in a time-efficient manner that would allow long-term assessment of potential therapeutic interventions in the life-span of the rat.
Therefore, we sought to establish and characterise a novel accelerated model of Parkinson's disease by simultaneously injecting human a-syn fibrils into the rat substantia nigra (SN), in combination with AAV-mediated overexpression of human a-syn, at levels that, by themselves, are unable to induce dopamine (DA) neurodegeneration. To this end, we have found that the combination of human a-syn fibrils and overexpression of a-synuclein lead to extensive a-synuclein pathology in the nigrostriatal pathway. This occurs in a short time-span (as early as 4 weeks post-injection) and results in TH cell loss at the level of the SN, as well as loss of TH expression in the striatum. Upon further investigation, we found that the loss of TH at this early timepoint appears to be a combination of actual cell death and cell dysfunction (indicated by the downregulation of TH in DA cells), whereas at later timepoints, the loss of TH indicates almost complete cell loss, a characteristic which illustrates the progressive nature of the model. Additionally, a significant impairment in the motor function of approximately 50% of the animals was demonstrated as early as 4 weeks after injection using multiple motor tests (rotational asymmetry, stepping adjustment and forelimb use) an attribute of the model which would allow early selection of affected animals for assessment of therapeutic intervention. Finally, a prominent inflammatory response involving activation of resident microglia was observed and microglia were found to enclose or engulf cells and processes containing a-syn pathology, further reproducing a pathogenic feature of the human disease.
In summary, the relatively short time span, and indeed the distinct sequence of pathological and degenerative changes, make this novel combined a-syn model an attractive option as an experimental model for the assessment of neuroprotective and disease-modifying strategies.

Disclosures: B. Mattsson: None. **S. Shrigley:** None. **L.S. Breger:** None. **K.C. Luk:** None. **A. Bjorklund:** None. **M. Parmar:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); M.P. is the owner

of Parmar Cells AB and co-inventor of the US patent application 15/093,927 owned by Biolamina AB, and EP17181588 owned by Miltenyi Biotec. **D.B. Hoban:** None.

Poster

132. Parkinson?s Disease Therapeutic Strategies: Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 132.14/G21

Topic: C.03. Parkinson's Disease

Support: Weston Brain Institute

Title: Binding of [^{11}C]-JNJ-42491239 in the marmoset brain: A positron emission tomography study

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Abstract: [^{11}C]-JNJ-42491293 is a positive allosteric modulator (PAM) that was initially developed as a radio-ligand to image metabotropic glutamate 2 ($mGlu_2$) receptors using positron emission tomography (PET) studies. Whereas *in vitro* binding and *in vivo* imaging studies conducted in rodents showed a brain distribution consistent with that of $mGlu_2$ receptors, a PET study in human suggested otherwise, as [^{11}C]-JNJ-42491293 also appeared to interact with a yet-to-be identified target different to the $mGlu_2$ receptor. Here, we sought to determine if [^{11}C]-JNJ-42491293 binds selectively to $mGlu_2$ receptors, or if it displays affinity for another binding site, in the common marmoset (*Callithrix jacchus*).
Three marmosets underwent brain magnetic resonance imaging (MRI) for PET images registration purposes. Dynamic PET scans with [^{11}C]-JNJ-42491293 alone and in combination with the $mGlu_2$ PAM AZD-8529 (0.1, 1 and 10 mg/kg) were obtained.
In the scans in which [^{11}C]-JNJ-42491293 was administered alone, the brain areas with the highest standardised uptake values (SUVs) were the midbrain, cerebellum and thalamus, while the lowest SUVs were found in the pons. The co-injection of AZD-8529 with [^{11}C]-JNJ-42491293 did not modify the SUVs obtained with [^{11}C]-JNJ-42491293 alone, suggesting that there was no competition between the 2 ligands.
The results we obtained in this study performed in the marmoset add to the mounting evidence in the literature suggesting that *in vivo*, JNJ-42491293 may not be selective for $mGlu_2$ receptors in humans and non-human primates.

Disclosures: **P. Huot:** None. **M.S. Kang:** None. **H. Bdair:** None. **D. Bédard:** None. **S.G. Nuara:** None. **J.C. Gourdon:** None. **K. Ross:** None. **R. Hopewell:** None. **A.P. Mathieu:** None. **C.L. Tardif:** None. **J. Soucy:** None. **G. Massarweh:** None. **P. Rosa-Neto:** None. **A. Hamadjida:** None.

Poster

132. Parkinson?s Disease Therapeutic Strategies: Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 132.15/G22

Topic: C.03. Parkinson's Disease

Support: Weston Brain Institute
Parkinson Canada

Title: Effects of the prototypical $mGlu_2$ positive allosteric modulator BINA on dyskinesia and psychosis in the MPTP-lesioned marmoset model of Parkinson's disease

Authors: *I. FROUNI[1], A. HAMADJIDA[1], S. NUARA[2], J. GOURDON[2], P. HUOT[1,3,4]; [1]Neurodegenerative Dis. Group, Montreal Neurolog. Inst., Montreal, QC, Canada; [2]Comparative Med. & Animal Resource Ctr., Montreal, QC, Canada; [3]Div. of Neurol., McGill Univ. Hlth. Ctr., Montreal, QC, Canada; [4]Dept. of Neurol. and Neurosurg., McGill Univ., Montreal, QC, Canada

Abstract: Psychosis and dyskinesia undermine the quality of life of patients with advanced Parkinson's disease (PD). Available therapies are few, and their use may be limited by adverse effects. We have recently demonstrated that activation of metabotropic glutamate 2 ($mGlu_2$) receptors with a prototypical positive allosteric modulator (PAM), LY-487,379, reduces both dyskinesia and psychosis-like behaviours (PLBs) in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned non-human primate. Biphenylindanone A (BINA) is another $mGlu_2$ prototypical PAM, but with different pharmacological and pharmacokinetic properties. We hypothesised that $mGlu_2$ positive allosteric modulation with BINA is potentially efficacious to reduce dyskinesia and PLBs induced by L-3,4-dihydroxyphenylalanine (L-DOPA), in the MPTP-lesioned common marmoset. Parkinsonism was induced in 6 common marmosets, by injections of MPTP. Following repeated administration of L-DOPA to induce stable dyskinesia and PLBs, marmosets were administered acute challenges of BINA (0.1, 1 and 10 mg/kg) or vehicle, in combinaison with L-DOPA after which the severity of dyskinesia, PLBs and parkinsonism was rated. In combination with L-DOPA, BINA (0.1, 1 and 10 mg/kg) significantly and dose-dependently reduced the severity of peak dose dyskinesia, by ≈ 28.9%, ≈ 52.3 and ≈ 57.8% ($P < 0.05$, $P < 0.01$ and $P < 0.01$), compared to L-DOPA/vehicle. The severity of peak dose PLBs was also significantly and dose-dependently reduced by BINA (0.1, 1 and 10 mg/kg), by ≈ 33.3%, ≈ 55.6 and ≈ 61.1% ($P < 0.05$, $P < 0.01$ and $P < 0.01$), when compared to L-DOPA/vehicle. The anti-dyskinetic and anti-psychotic benefits conferred by BINA were

achieved without interfering with the therapeutic effect of L-DOPA on parkinsonian disability. BINA is the second $mGlu_2$ PAM that was effective at alleviating dyskinesia and PLBs in the gold-standard animal model of PD, the parkinsonian primate. The results presented here further add to the increasing data suggesting that $mGlu_2$ positive allosteric modulation may be effective to diminish both L-DOPA-induced dyskinesia and PD psychosis.

Disclosures: I. Frouni: None. **A. Hamadjida:** None. **S. Nuara:** None. **J. Gourdon:** None. **P. Huot:** None.

Poster

132. Parkinson?s Disease Therapeutic Strategies: Cellular and Animal Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 132.16/G23

Topic: C.03. Parkinson's Disease

Support: parkinson canada
Weston brain institute

Title: Effects of combined $mGlu_2$ positive allosteric modulation and orthosteric stimulation on psychosis like behaviours and dyskinesia in the Parkinsonian marmoset

Authors: *A. HAMADJIDA[1], S. G. NUARA[2], J. C. GOURDON[2], P. HUOT[1];
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Abstract: The treatment of advanced Parkinson's disease (PD) is challenging because several patients experience motor complications, *e.g.* dyskinesia, as well as non-motor manifestations, *e.g.* psychosis.
We have previously demonstrated that activation of metabotropic glutamate 2 ($mGlu_2$) receptors via positive allosteric modulation or orthosteric stimulation alleviates psychosis-like behaviours (PLBs) and dyskinesia in experimental parkinsonism. Here, we hypothesised that a synergistic effect might result upon combining an $mGlu_2$ positive allosteric modulator (PAM) with an $mGlu_2$ orthosteric agonist (OA). To test our hypothesis, we conducted experiments in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned common marmoset, in which we administered the $mGlu_2$ PAM LY-487,379 and the $mGlu_2$ OA LY-354,740, alone and concurrently, in combination with L-3,4-dihydroxyphenylalanine (L-DOPA).
Six marmosets were rendered parkinsonian by MPTP injection. PLBs and dyskinesia were induced by repeated administration of L-DOPA. Once they expressed stable PLBs and dyskinesia, marmosets were administered acute challenges of LY-487,379 (1 mg/kg), LY-354,740 (1 mg/kg), LY-487,379/LY-354,740 (each 1 mg/kg) or vehicle, in combinaison with L-DOPA, after which the severity of PLBs, dyskinesia and parkinsonism was rated.

LY-487,379, LY-354,740 and LY-487,379/LY-354,740 each significantly reduced the severity of the global PLBs score, by 51%, 44% and 56% (each $P < 0.001$), when compared to L-DOPA/vehicle. The combination LY-487,379/LY-354,740 was significantly more effective than LY-487,379 ($P < 0.01$), but not LY-354,740 ($P > 0.05$). The severity of the global dyskinesia score was also significantly reduced by each of LY-487,379, LY-354,740 and LY-487,379/LY-354,740, by 42%, 55% and 64% (each $P < 0.001$), when compared to L-DOPA/vehicle. The combination LY-487,379/LY-354,740 was significantly more effective than each drug alone (both $P < 0.05$). The benefits on PLBs and dyskinesia were achieved without compromising the therapeutic effect of L-DOPA on parkinsonism.
Our results confirm $mGlu_2$ activation, via both positive allosteric modulation and orthosteric stimulation, is a promising strategy to reduce dyskinesia and psychosis in PD. Moreover, they suggest that combining an $mGlu_2$ PAM with an $mGlu_2$ OA may lead to a synergistic effect, thereby providing greater anti-dyskinetic and anti-psychotic effects.

Disclosures: **A. Hamadjida:** None. **S.G. Nuara:** None. **J.C. Gourdon:** None. **P. Huot:** None.

Poster

133. ALS and FTD Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 133.01/G24

Topic: C.06. Neuromuscular Diseases

Title: Characterization of pathological consequences of the F115C Matrin 3 mutation in motor neurons derived from patient iPSCs

Authors: *D. X. MEDINA[1], M. N. DOMINICK[3], R. G. SATTLER[2], R. BOWSER[4];
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Abstract: We recently described four mutations in the Matr3 gene encoding the nuclear matrix protein Matrin 3 that are associated with ALS, including the F115C mutation. In human spinal cord tissue, Matrin 3 immunostaining is present in both motor neurons and glia, predominantly nuclear, and is stronger in ALS patients than in control cases and strongest in an ALS patient with a mutation in Matrin 3. However, the molecular roles of matrin 3 in ALS pathogenesis or disease progression are not understood. To elucidate the role of Matrin 3 mutations in ALS we have generated induced pluripotent stem cells (iPSC) from an ALS patient with the F115C Matrin 3 mutation. Control and Matrin 3 F155C iPSCs were differentiated into motor neurons and aged up to 75 DIV. These cells were co-cultured on top of confluent mouse astrocytes cultured from p0 mouse pups. Motor neurons were immunostained with neuronal marker MAP2 and antibodies for ALS associated proteins such as TDP-43 and FUS. Sholl analysis and dendritic length were quantified from filament tracings performed on confocal images using

ImageJ. Additionally, the synaptic activity of differentiated motor neurons was measured 3 times a week using microelectrode array (MEA). The reprogramming process resulted in iPSCs that maintained a normal karyotype, expressed pluripotency markers, and could differentiate into 3 different germ layers. Further our differentiation process produced a culture highly enriched for motor neurons. Analysis of motor neurons derived from patient iPSCs demonstrated modest changes in dendritic branching and complexity compared to control motor neurons. In addition, MEA analysis demonstrated differences in synaptic activity between Matrin 3 mutant motor neurons and control lines. This study provides evidence for the functional consequences of Matrin 3 mutations in motor neurons from patient derived iPSCs. Further, by utilizing motor neurons derived from patient iPSCs we can begin to identify the molecular mechanisms involved in Matrin 3 driven ALS.

Disclosures: D.X. Medina: None. **M.N. Dominick:** None. **R.G. Sattler:** None. **R. Bowser:** None.

Poster

133. ALS and FTD Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 133.02/G25

Topic: C.06. Neuromuscular Diseases

Support: NIH
ALS Association
Muscular Dystrophy Association
Robert Packard Center for ALS Research
ALSFAC
F Prime

Title: Coordinated disassembly and reassembly of the nuclear pore complex in C9orf72 ALS/FTD

Authors: *A. N. COYNE[1], B. L. ZAEPFEL[1], L. R. HAYES[1], B. FITCHMAN[2], Y. ZALTHBERG[2], K. BOWEN[1], H. TROST[3], A. HAREL[2], C. SVENDSEN[3], D. SAREEN[3], J. D. ROTHSTEIN[1];
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Abstract: An intronic GGGGCC hexanucleotide repeat expansion in the C9orf72 gene is the most common cause of Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). Nucleocytoplasmic transport is tightly controlled by the nuclear pore complex and has recently emerged as a prominent pathomechanism underlying multiple neurodegenerative

diseases including C9orf72 ALS/FTD. Using super resolution structured illumination microscopy, we evaluated the distribution of individual nucleoporins in nuclei isolated from control and C9orf72 iPSC derived motor neurons and postmortem human motor cortex to identify a subset of nucleoporins lost from the nuclear pore complex in an age dependent manner. A combination of overexpression and knock down experiments reveals that POM121, an integral scaffolding nucleoporin, coordinates the disassembly and reassembly of the nuclear pore complex in post-mitotic neurons impacting nucleocytoplasmic transport and cellular toxicity. Together, these data suggest that POM121 is an integral nucleoporin in the maintenance of the nuclear pore complex in post-mitotic neurons and loss of POM121 from the nuclear pore complex in C9orf72 ALS/FTD initiates a pathological cascade affecting nuclear pore complex integrity and function.

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Poster

133. ALS and FTD Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 133.03/G26

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant NS061867
Barrow Neurological Foundation

Title: Longitudinal analysis of CSF biomarkers for amyotrophic lateral sclerosis

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Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease with a highly complex etiology resulting in a heterogeneous patient population. Biomarkers that can be used to segregate this population could be useful to identify those that could respond best to specific therapeutic approaches. Many studies have employed the use of disease progression rate based on the change in the ALS functional rating scale revised (ALSFRS-r) to segregate patients into fast progressing (FP) and slow progressing (SP) ALS to identify candidate biomarkers. However, the majority of studies are cross-sectional and therefore a temporal response of many biomarkers has not been explored. In this study, we employed shotgun proteomics on longitudinally collected cerebrospinal fluid (CSF) obtained from 13 ALS patients in order to identify candidate biomarkers distinguishing FP versus SP ALS. Overall, we identified 1148 proteins in the CSF of

all ALS patients in this study. Pathway analysis determined enrichment of pathways related to complement and coagulation cascades, cell adhesion molecules, and axon guidance. Longitudinal analysis revealed a number of candidate markers that trended differently between FP and SP. Proteins in the complement and coagulation cascades were significantly higher in FPs as compared to SPs. Neural cell adhesion molecules were significantly higher in the SPs while endothelial cell adhesion molecules were higher in the FPs. Proteins related to axon guidance (attractive / repulsive cues) were significantly higher in SPs. Taken together, we identified candidate biomarkers that can be used to segregate the patient population based on disease progression rate. These biomarkers also demonstrate distinct processes that are modulated in FP and SP ALS representing potential therapeutic targets.

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Poster

133. ALS and FTD Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 133.04/G27

Topic: C.06. Neuromuscular Diseases

Support: MEXT KAKENHI 17H0508

Title: Dynactin-1 and TRAPPC9 implicate autophagosome-lysosome fusion in the pathogenesis of neurodegenerative diseases

Authors: *H. ADACHI[1], Z. HUANG[2], K. OKADA[1], K. OHNARI[1], T. HASHIMOTO[1], T. TOYOTA[1], Y. IWANAKA[1];
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Abstract: Background and Purposes: Dynactin is a dynein-activator complex required for most of the cellular functions of cytoplasmic dynein. Dynactin is composed of seven to nine polypeptides, including the dynactin-1 (p150Glued) that forms the sidearm of the complex and binds both to microtubules and to dynein; the Arp1 polypeptide that forms an actin-like filament at the base of the complex; and the dynamitin, or p50, the subunit that localizes to the shoulder between the sidearm and the base. Autophagy is essential for neuronal homeostasis, and its dysfunction has been linked to many neurodegenerative disorders. In this report, we show that regulatory relationship between dynactin-1 and fusion of autophagosomes and lysosomes. Methods: In the present study, we used a combination of molecular biological techniques and morphological methods such as western blot, immunofluorescence, RFP-AcGFP-LC3 reporter assay, and immunoelectron microscopy on lentivirus-mediated dynactin-1 knockdown NSC 34

motor neuron cell line, and we determined the autophagosome-lysosomes fusion efficiency. Results: The levels of dynactin-1 and TRAPPC9 protein expression were decreased in the dynactin-1 and TRAPPC9 knockdown cells. Immunoprecipitation showed dynactin-1 interacted with TRAPPC9. The level of the autophagosome marker LC3-II in cell culture was increased and the autophagosome-lysosome fusion was inhibited in dynactin-1 or TRAPPC9 knockdown NSC 34 motor neuron cells. The levels of mutant SOD1 protein expression were increased in the dynactin-1 knockdown cells. Conclusions: Our study identifies the dynactin-1 and TRAPPC9 as a regulator that controls the fusion of autophagosomes and lysosomes. These findings suggest that dynactin-1 and TRAPPC9 play important roles in the autophagy and implicate autophagosome-lysosome fusion in the pathogenesis of neurodegenerative diseases.

Disclosures: H. Adachi: None. **Z. Huang:** None. **K. Okada:** None. **K. Ohnari:** None. **T. Hashimoto:** None. **T. Toyota:** None. **Y. Iwanaka:** None.

Poster

133. ALS and FTD Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 133.05/G28

Topic: C.06. Neuromuscular Diseases

Title: Isogenic iPSC lines as a tool for identify common neurodegenerative pathways across fALS mutations

Authors: *A. H. HELD[1,2], B. J. WAINGER[1,2];
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Abstract: Amyotrophic Lateral Sclerosis (ALS) is a rapidly progressing and lethal neurodegenerative disease characterized by the degeneration and death of motor neurons. The majority of ALS cases are sporadic (sALS), but 10% of cases can be attributed to familial inheritance (fALS). Mutations in over 25 genes have been linked to the fALS, and these genes can be grouped into three broad functional groups: protein homeostasis, RNA-binding, and cytoskeletal structure. Two critical unanswered questions in the ALS field are 1) whether mutations in these three broad categories lead to neurodegeneration through a common pathogenic mechanism and 2) whether a potential common pathogenic mechanism in fALS is shared in sALS. To begin addressing these questions, we have edited one gene from each functional group into iPSCs derived from a single healthy individual. These $SOD1^{G85R}$, $TDP43^{G298S}$, and $PFN1^{G118V}$ lines represent the protein homeostasis, RNA-binding, and cytoskeletal structure functional groups, respectively, and are directly comparable because they share the same genetic background. We will now determine whether the $SOD1^{G85R}$, $TDP43^{G298S}$, and $PFN1^{G118V}$ mutations cause phenotypes resembling ALS pathology in iPSC-derived motor neurons, including hyperexcitability, neurite retraction, and motor neuron death. We will then

determine whether these mutations disrupt similar cellular processes using RNA-sequencing after the onset of neurodegeneration. We anticipate that these three fALS lines will cause motor neuron degeneration and the intersection of differentially expressed gene sets will represent a common neurodegenerative pathway. Finally, we plan to determine whether the intersection differentially regulated gene sets is represented in sporadic ALS by testing sALS patient spinal cord tissue for RNA-sequencing hits using RNA-scope.

Disclosures: A.H. Held: None. **B.J. Wainger:** None.

Poster

133. ALS and FTD Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 133.06/G29

Topic: C.06. Neuromuscular Diseases

Support: Packard Center
MDA
ALSA
Barrow Neurological Foundation

Title: Human *in vitro* culture systems of C9orf72-ALS/FTD patient-derived iPSC cortical neurons and microglial cells to study mechanisms of synaptopathy

Authors: *I. LORENZINI[1], J. LEVY[5], C. BURCIU[2], D. BHATIA[2], B. RABICHOW[2], M. ROBERTS[3], R. G. SATTLER[4];
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Abstract: The discovery of a hexanucleotide repeat expansion in the *C9ORF72* gene (C9) as the cause of chromosome 9-linked amyotrophic lateral sclerosis (ALS) and frontotemporal degeneration (FTD) provides an opportunity to investigate common pathobiological mechanisms underlying motor and cognitive dysfunction seen in both diseases. Cognitive dysfunction observed during normal ageing parallels the selective loss of synapses, decreased spine density and altered neuronal morphology. Furthermore, published research in Alzheimer's disease and FTD, suggests that synaptic pruning mediated by microglial cells can be re-activated during neurodegeneration, leading to synapse loss and dysfunction. Interestingly, C9orf72 knockout mice display altered immune responses in microglia, with age-related neuroinflammation exhibiting similarities seen in C9 patient tissue. These findings suggest that inappropriate neural-immune interactions may contribute to synaptic impairment and ultimately cognitive decline in C9-ALS/FTD. *We hypothesize that synaptic defects in C9 dementia are mediated by microglial*

cells and the neural-immune complement pathway. To test our hypothesis, we generated patient-derived human stem cell differentiated cortical neurons (hiPSC-CNs) and microglial cells (hiPSC-MGs) from three C9 patient population clinically diagnosed as: C9-ALS, C9-ALS/FTD and C9-FTD. We evaluated neuronal structure and activity by using three dimensional (3D) imaging tools and a multi-electrode array system, respectively. HiPSC-CNs exhibited changes in dendritic branching, dendritic length, spine density, alterations in the expression pattern of synaptic proteins as well as in neuronal excitability. Furthermore, we examined hiPSC-MGs mono-cultures for gene expression changes, specific brain markers and brain function such as phagocytosis. Our results show that synaptic deficits are present in all C9 patient groups with varying degree of severity, which likely contributes to cognitive impairment and neuronal cell death found in C9orf72 patients. Our goal is to establish an *in vitro* co-culture system of hiPSC-derived cortical neuron-microglial cells to determine the role of microglial cells in neuronal synaptic dysfunction in C9 associated diseases. This human *in vitro* co-culture model not only allows for the studies of C9 disease pathogenesis, but any other neurodegenerative disorder characterized by synapse loss and synaptic dysfunction, including other subtypes of FTD, Alzheimer's disease and Down's Syndrome.

Disclosures: I. Lorenzini: None. **J. Levy:** None. **C. Burciu:** None. **D. Bhatia:** None. **B. Rabichow:** None. **M. Roberts:** None. **R.G. Sattler:** None.

Poster

133. ALS and FTD Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 133.07/G30

Topic: C.06. Neuromuscular Diseases

Title: C9ORF72 repeat expansions cause axonal transport defects in iPSC-derived motor neurons

Authors: L. FUMAGALLI[1], F. YOUNG[2], S. BOEYNAEMS[3], M. DE DECKER[1], R. FAZAL[1], W. GUO[1], A. SWIJSEN[1], W. ROBBERECHT[1], P. KOCH[4], P. VANDEN BERGHE[5], C. VERFAILLIE[6], *L. M. VAN DEN BOSCH[1], S. BULLOCK[2], P. VAN DAMME[1];
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Abstract: The hexanucleotide repeat expansion (HRE) GGGGCC in C9orf72 is the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia

(FTD). Repeat-associated non AUG (RAN) translation of the GGGGCC expansion results in five different dipeptide repeat proteins (DPRs: poly GA, poly GP, poly GR, poly PR and poly PA), which have been proposed to play a crucial role in C9orf72 HRE-induced cytotoxicity. Defective axonal transport is an early perturbed event occurring across several models of familial ALS, indicating that it may be a key initiating contributor to the selective vulnerability of motor neurons. At present, very little is known about the effect of the C9orf72 HRE on axonal transport and the mechanism underlying it. Here we used induced pluripotent stem cell (iPSC)-derived motor neurons (MNs) differentiated from multiple C9ORF72 ALS/FTD patients and controls in order to investigate the effect of the C9orf72 HRE on axonal transport and the contribution of DPRs on this phenotype. No difference in the ability to differentiate into mature, functional and Islet1/ChAT positive motor neurons was observed between control and C9ORF72-positive cultures. C9ORF72 iPSC-derived MNs showed RAN translation pathology and increased in p62/SQSTM1 levels compared to control. Moreover, we found that the C9ORF72 expansion resulted in a significant decrease in the number of motile mitochondria along the processes and this phenotype seemed to be more pronounced over time. We also found that treatment with synthetic arginine-rich DPRs (PR20 and GR20), but not GP20, decrease the number of motile mitochondria in iPSC-derived control motor neurons. Together, our data suggest that C9orf72 HRE cause axonal transport impairment in human-derived motor neurons and show that arginine-rich DPRs may play a role in the mechanism underlying the axonal transport deficit observed in the C9ORF72 iPSC-derived MNs.

Disclosures: **L. Fumagalli:** None. **F. Young:** None. **S. Boeynaems:** None. **M. De Decker:** None. **R. Fazal:** None. **W. Guo:** None. **A. Swijsen:** None. **W. Robberecht:** None. **P. Koch:** None. **P. Vanden Berghe:** None. **C. Verfaillie:** None. **L.M. Van Den Bosch:** None. **S. Bullock:** None. **P. Van Damme:** None.

Poster

133. ALS and FTD Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 133.08/G31

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant R01 NS108191

Title: The AR N/C interaction in SBMA- molecular mechanisms and therapeutic potential

Authors: *A. LISBERG[1], R. CADILLA[2], P. EIDAM[2], D. E. MERRY[1];
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Abstract: Spinal and bulbar muscular atrophy (SBMA) is a progressive neurodegenerative disease that causes degeneration of lower motor neurons and atrophy of skeletal muscle. SBMA is caused by a trinucleotide CAG repeat expansion in the androgen receptor (AR) gene that is translated into an expanded polyglutamine (polyQ) tract in AR and causes misfolding and aggregation of AR in the nucleus. However, the molecular mechanisms by which polyQ-expanded AR leads to toxicity are largely unknown. It was previously found that an interdomain interaction between the amino- and carboxyl-termini of AR, termed the N/C interaction, is required for polyQ-expanded AR toxicity in both cell and mouse models of SBMA. The mechanism for this role of the N/C interaction in SBMA, however, is unknown. This study investigates three aspects of AR trafficking and metabolism that may be altered by blocking the N/C interaction to uncover the mechanism by which blocking the N/C interaction is protective in SBMA, including AR nuclear export, dimerization, and protein interactions. Using a heterokaryon assay, we have found that blocking the N/C interaction with a point mutation (F23A) results in a small but consistent increase in nuclear export of AR. Ongoing studies will determine if this increase is functionally significant, but the small magnitude of this increase suggests that the state of AR in the nucleus plays a more significant role in SBMA pathogenesis. To investigate the role of dimerization in SBMA, a novel assay to resolve both dimeric and monomeric forms of AR using Western blotting techniques was developed to investigate changes in AR dimerization upon polyQ expansion or inhibition of the N/C interaction. Additionally, cell lines with mutations in the dimerization domain of AR were made to investigate the effects of blocking dimerization on AR aggregation. Ongoing studies using these methods will elucidate the role of dimerization in SBMA pathogenesis. Finally, changes in the AR interactome that occur in cells expressing N/C-inhibited AR are being investigated using quantitative proteomics. Previous co-immunoprecipitation studies showed that polyQ-expanded and N/C-inhibited AR interact differentially with USP7, suggesting that blocking the N/C interaction might impact the AR interactome. Additionally, we have screened several selective androgen receptor modulators (SARMs) that block the N/C interaction for their effectiveness in SBMA cell models. Preliminary screens have identified several SARMs that are effective in cell models of SBMA and may be used for future therapeutic testing in SBMA.

Disclosures: A. Lisberg: None. **R. Cadilla:** None. **P. Eidam:** None. **D.E. Merry:** None.

Poster

133. ALS and FTD Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 133.09/G32

Topic: C.06. Neuromuscular Diseases

Title: C9orf72 ALS patient iPSC-derived cortical neurons and astrocytes display disease-specific phenotypes

Authors: *V. J. GARCIA[1], H. HEMMATI[1], R. HO[1], G. M. THOMSEN[2], C. N. SVENDSEN[1]; [1]Board of Govenors Regenerative Med. Inst., Cedars-Sinai Med. Ctr., Los Angeles, CA; [2]Avexis, Inc, San Diego, CA

Abstract: Amyotrophic lateral sclerosis (ALS) is characterized by the loss of neurons in the cortex, brain stem and spinal cord, and by the dysfunction of astrocytes and microglia. Specific cortical neuron subtypes are implicated in the disease, including deep layer V pyramidal neurons that project to the spinal cord, as well as layer II/III interneurons that regulate the excitatory activity of projection neurons. In rodent models of ALS, astrocyte-mediated toxicity has been described and linked to loss of motor neurons in the spinal cord. Of all ALS diagnoses, only ~10% are attributed to a genetic mutation with 40% of those being linked to a mutation on chromosome 9 open reading frame 72 (C9orf72) - a mutation also found in 9% of sporadic cases. We have generated cortical neurons and astrocytes from induced pluripotent stem cells (iPSCs) derived from 8 C9orf72 patients and 8 controls. Using immunocytochemistry to delineate the iPSC-derived neural populations, we demonstrate that our co-culture system recapitulates the heterogeneity of cortical tissue for faithful modeling of ALS. In addition, these cell cultures produce hallmark proteins for ALS, such as TDP-43 and ataxin-2. Microelectrode array and calcium imaging assays demonstrate C9orf72 patient-derived astrocytes provide reduced support to co-cultured ALS and control cortical neurons. Using patch-clamp, we describe intrinsic, physiological properties of diseased astrocytes and cortical neurons and define cell-type specific differences in ionic currents. Finally, single cell RNA sequencing assessed differences in gene expression in individual cell types between ALS and control cortical cultures.

Disclosures: V.J. Garcia: None. **H. Hemmati:** None. **R. Ho:** None. **G.M. Thomsen:** None. **C.N. Svendsen:** None.

Poster

133. ALS and FTD Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 133.10/G33

Topic: C.06. Neuromuscular Diseases

Support: Lewis Family fund to support regenerative medicine research
Advancing a Healthier Wisconsin
Melitta S. and Joan M. Pick Charitable Trust Innovation Fund

Title: Using a novel chaperone protein, SRCP1, to reduce toxic proteins in ALS model systems

Authors: S. SANTARRIAGA[1], ***E. R. SEMINARY**[1], E. WELBY[1], M. SCAGLIONE[2], A. D. EBERT[1];

[1]Cell Biology, Neurobiology, and Anat., Med. Col. of Wisconsin, Milwaukee, WI; [2]Mol. Genet. and Microbiology, Ctr. for Neurodegeneration and Neurotherapeutics, Duke Univ., Durham, NC

Abstract: Protein misfolding and aggregation is a common feature of a variety of neurodegenerative diseases, including ALS. The protein quality control system uses molecular chaperones to prevent protein misfolding and aggregation by either attempting to refold the misfolded proteins or by shuttling misfolded proteins to the proteasome or lysosome for degradation. However, the protein quality control network can be overwhelmed by the accumulation of misfolded proteins leading to cellular stress, disruption of downstream cellular functions, and eventual neuronal loss. Therefore, reducing protein aggregation in ALS tissue may be therapeutically valuable. We have recently identified a novel chaperone protein in *Dictyostelium discoidium*, termed serine-rich chaperone protein 1 (SRCP1), that uses its C-terminal domain to effectively prevent polyglutamine aggregation in induced pluripotent stem cell (iPSC) and zebrafish models of Huntington's disease. Considering the evidence that inclusions of TDP-43, FUS, and C9orf72-generated dipeptide repeats also exhibit amyloid-like properties, here, we are testing whether expression of SRCP1 can reduce insoluble protein levels and protect motor neurons in ALS. We have found that SRCP1 can reduce insoluble SOD1 protein levels in HEK293T cells overexpressing either the A4V or G93R SOD1. Importantly, SRCP1 expression did not impact soluble SOD1 protein levels. We next infected mutant SOD1 expressing ALS iPSC-derived motor neurons with a lentivirus expressing SRCP1 or RFP and are currently examining soluble and insoluble SOD1 protein levels and motor neuron survival. Finally, we are monitoring symptom onset and lifespan in pre-symptomatic SOD G93A ALS mice receiving an intracerebroventricular injection of adeno-associated virus expressing SRCP1 or RFP and will examine motor neuron survival and insoluble protein levels in the brain and spinal cord. We have previously found that ALS iPSC-derived motor neurons exhibit low and potentially insufficient activation of the chaperone network, which may contribute to the buildup of insoluble protein species. As such, SRCP1 may represent a novel therapeutic agent to reduce insoluble protein burden and enhance motor neuron survival in ALS.

Disclosures: S. Santarriaga: None. **E.R. Seminary:** None. **E. Welby:** None. **M. Scaglione:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent pending for the use of SRCP1 and derivatives as therapies. **A.D. Ebert:** None.

Poster

133. ALS and FTD Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 133.11/G34

Topic: C.06. Neuromuscular Diseases

Support: Target ALS Springboard Fellowship

Title: Poly(ADP-ribose) modulate the phase separation of C9orf72 dipeptide repeat proteins and enhances their toxicity

Authors: *K. ZHANG[1], B. G. KANG[2], T.-I. KAM[2], L. GUO[3], T. E. LLOYD[4], T. M. DAWSON[5], V. L. DAWSON[6], J. D. ROTHSTEIN[7];
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Abstract: Frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) are age-related, increasingly prevalent, and fatal neurodegenerative diseases with no cure. A GGGGCC hexanucleotide repeat expansion (HRE) in *C9orf72* is the most common genetic cause of familial FTD and ALS, with the underlying molecular mechanism ill-defined. Emerging evidence has suggested that dipeptide repeat proteins (DPRs), abnormal translational products of HRE-containing transcripts, play a critical role in the pathogenesis of *C9orf72*-mediated FTD/ALS (c9FTD/ALS). They undergo liquid-liquid phase separation (LLPS) and disrupts the LLPS of their interacting proteins that play key roles in cellular processes, such as nucleocytoplasmic transport, DNA damage response, membraneless organelle dynamics and functions, etc. Thus, targeting the LLPS of DPRs may mitigate their cytotoxicity. However, it is still unclear how the LLPS of DPRs is regulated. Using *Drosophila*, cell lines, and iPS neurons derived from patients as models, we found that poly(ADP-ribose) polymerase 1 (PARP1), an enzyme that can be activated by DNA damage, plays an important role in the LLPS of DPRs and their cytotoxicity and is amenable to pharmacotherapeutic intervention.
In a genetic screen, we identified that PARP (fly homolog of PARP1) knockdown suppresses neurodegeneration in a *Drosophila* model of c9FTD/ALS. PARP1 catalyzes the reaction to produce poly(ADP-ribose) (PAR). Consistent with our data, overexpression of poly(ADP)-ribose glycohydrolase (PARG) that eliminates PAR also suppresses neurodegeneration in our c9FTD/ALS flies. Moreover, we observed DNA damage and upregulated PAR levels and found that a PARP inhibitor, Olaparib, can suppress neurodegeneration in c9FTD/ALS patient-derived iPS neurons. To gain mechanistic insight in how PAR contributes to neurodegeneration, we found that PAR impairs the LLPS of DPRs and mediates DPR-caused defects in membraneless organelles, including nucleoli and stress granules, which are suppressed by PARP1 knockout. Hence, our data suggest that DPR-mediated DNA damage activates PARP1, leading to excessive PAR that in turn modulate the LLPS of DPRs and enhances their cytotoxicity.

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Poster

133. ALS and FTD Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 133.12/G35

Topic: C.06. Neuromuscular Diseases

Support: ALS Association
ALS Therapy Alliance
NIH/NINDS

Title: Modulators of promyelocytic leukemianuclear body phenotype in models of amyotrophic lateral sclerosis

Authors: *S. CAO[1], H. KO[1], S. PARELKAR[2], H. ZHOU[1], P. THOMPSON[2], L. HAYWARD[1]; [1]Neurol., [2]Biochem. and Mol. Pharmacol., Univ. of Massachusetts Med. Sch., Worcester, MA

Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by preferential motor neuron death in the brain and spinal cord. FUS (Fused in Sarcoma) is a nucleic acid binding protein localized predominantly in the nucleus, and more than 50 mutations in the FUS gene cause ~4% of inherited ALS. In HEK293 cells expressing human ALS-linked FUS mutants (R495X, R521G, or P525L), we observed a phenotype of enlarged promyelocytic leukemia nuclear bodies (PML-NBs) compared to control cells expressing endogenous FUS or a human WT FUS cDNA. Following exposure to arsenic trioxide (ATO) for 24 h, the turnover of PML-NBs was inhibited in mutant cells. Furthermore, primary fibroblasts from ALS patients harboring mutant FUS exhibited enlarged PML-NBs and impairment of PML-NB turnover following ATO exposure. Using an imaging-based phenotypic assay and the LOPAC library of small molecules, we identified that L-buthionine-sulfoximine (BSO) can exacerbate the abnormal PML-NB phenotype upon co-treatment with ATO. The effects of BSO have been further confirmed in primary fibroblasts from ALS patents harboring FUS mutants. An independent assay showed that the glutathione redox potential was significantly higher in primary ALS fibroblasts compared with controls. Overall, our results suggest that a gain of nuclear toxicity by mutant FUS may contribute to the pathogenesis of ALS by perturbing nuclear functions or redox homeostasis.

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Poster

133. ALS and FTD Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 133.13/G36

Topic: C.06. Neuromuscular Diseases

Support: FWO, Doctoral Grant

Title: Targeted *Drosophila* screen reinforces nucleocytoplasmic transport to DPR pathology in C9orf72-associated ALS/FTLD

Authors: M. DE DECKER[1], J. VANNESTE[1], S. BOEYNAEMS[2], E. BOGAERT[3], J. A. STEYAERT[3], T. VERCRUYSSE[4], D. DAELEMANS[4], L. M. VAN DEN BOSCH[1], *P. VAN DAMME[5];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder affecting the upper and lower motor neurons in the motor cortex, brainstem and spinal cord. This leads to a wide variety of symptoms ranging from limb paralysis to losing the ability to speak, eat and ultimately breathe. In 2011 intronic hexanucleotide (GGGGCC) repeat expansions were discovered in chromosome 9 open reading frame 72 (*C9orf72*) as an important genetic cause of ALS. Until now, three mechanisms for *C9orf72* toxicity have been hypothesized: haploinsufficiency, RNA toxicity or dipeptide repeat proteins (DPRs) toxicity. These DPRs are generated by repeat associated non-ATG mediated (RAN) translation and yields five DPRs: GA, GR, PR, PA and GP. We and others have shown that especially arginine-rich DPRs (GR and PR) are toxic. To further investigate this toxicity, we generated *Drosophila* models expressing DPR specifically in the eye using a GMR driver. Expression of the PR dipeptide yielded a moderate rough-eye phenotype, which we used in a targeted RNAi screen focused on nuclear transport. The aim of this study was to investigate whether toxicity induced by GR DPRs could be influenced by the same modifiers as the ones discovered in the PR screen. Therefore, we generated a *Drosophila* model specifically expressing GR in the eye and repeated the initial screen. We could nicely recapitulate the same hits as in the PR screen. The most potent modifiers from both screens were members of the nuclear import and export pathway, respectively transportin-1 and exportin-1. In addition, modifiers were identified in the nuclear pore complex, and the Ran-GTP cycle. The discovery of these modifiers further supports the hypothesis that DPR pathology of the arginine-rich proteins in *C9orf72* ALS disrupts nucleocytoplasmic

transport, and could initiate the ALS disease cascade hallmarked by protein mislocalization and aggregation.

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Poster

133. ALS and FTD Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 133.14/G37

Topic: C.06. Neuromuscular Diseases

Support: The Brain Foundation Australia, Neuro-Trauma-2018

Title: Inhibition of micro RNA 23a in stressed muscle induce key genes that promote regeneration of neuromuscular synapses

Authors: *R. ISLAM, V. FOLETTA, P. DELLA GATTA, A. P. RUSSELL;
Deakin Univ., Melbourne, Australia

Abstract: In skeletal muscle, the endoplasmic reticulum (ER) plays a pivotal role in protein folding and homeostasis. However, an overload of misfolded or unfolded proteins in the ER lumen cause stress, which results in the activation of a signalling network called the unfolded protein response (UPR). UPR activation within muscle occurs in several neuromuscular disorders including motor neurone disease. MicroRNAs are small RNA molecules that function as post-transcriptional regulators of gene expression. In patients with motor neurone disease (MND) and mouse models of MND, we have observed that the progressive loss of motor neurons and muscle wasting is associated with an increase in miR-23a-3p (MicroRNA 23a-3p). We hypothesized that inhibition of miR-23a-3p will improve the potential for neuromuscular junction (NMJ) regeneration in stressed muscle with activated UPR pathway. In order to address this hypothesis, we used differentiated C2C12 myotubes as a cellular model for muscle. Myotubes were treated with chemical inducers of UPR, Tunicamycin (1µg/ml)) and Thapsigargin (1µM) for 24 hrs. MiR-23a-3p was inhibited using an LNA based inhibitor in stressed and non-stressed myotubes. Among the three UPR activated pathways inhibition of mir-23a-3p only changed the ATF6 (activating transcription factor 6) pathway by increasing the ATF6 protein level significantly. Inhibition of MiR-23a-3p did not affect apoptosis in stressed cells. Treatment with the inhibitor also significantly attenuated the depressed level of protein synthesis in stressed muscles. Inhibition of miR-23a-3p significantly increased the level of the expression of MuSK (muscle-specific kinase) mRNA in Tunicamycin treated samples. The results indicate that after UPR activation in muscle, inhibition of miR-23a-3p results in more

adaptive responses. Increases in the Musk mRNA, shows that the inhibitor treatment has the potential to promote neuromuscular junction formation during regeneration. These data place the well-established UPR signalling system at the muscle end of the NMJ, into a novel context. The study identifies miR-23a-3p as a new target for the control of synaptic regeneration in stressed muscle. Further experimentation is required to establish the role of miR-23a-3p at the neuromuscular junction for meaningful therapeutic use.

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Poster

133. ALS and FTD Mechanisms

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Program #/Poster #: 133.15/G38

Topic: C.06. Neuromuscular Diseases

Support: NSERC Grant 210042
NSERC CGS-M

Title: Investigating a role for the sigma-1 receptor in the protein quality control (PQC) network

Authors: *K. L. FLEMING[1,2], R. BERGERON[1];
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Abstract: Background The Sigma-1 Receptor (S1R) is a ubiquitously expressed endoplasmic reticulum (ER) transmembrane protein that is concentrated in lipid rafts at the nuclear envelope and mitochondrial-associated membranes (MAM). Activation of the S1R has been shown to modulate a diverse array of cellular processes, and of note, has shown to be neuroprotective in several models of neurodegenerative disease. Dysfunction of the S1R, caused by single point mutations, has also been shown to cause motor neuron diseases such as distal hereditary motor neuropathy (dHMN) and ALS16. There is little known about the physiological role of the S1R, and even less about the molecular mechanism behind its neuroprotective properties. A hallmark of neurodegenerative disease is the accumulation of protein aggregates. The protein quality control (PQC) network is responsible for detection and clearance of protein aggregates before they generate pathological inclusions. Upon accumulation of misfolded proteins, RNA granules form to halt mRNA translation and reduce the protein burden in the cell until the stress (misfolded proteins) can be remedied. Misfolded proteins are identified by chaperones and clearance is mediated through autophagic and ER-associated degradation pathways (ERAD). Previous studies have shown S1R involvement in ER stress pathways, proteasome function, and more recently modulation of autophagic flux.
Hypothesis Taken together, we propose that the S1R is a facilitative component in the protein

quality control network.
Objectives To determine its role in this network we used S1R overexpression and ligand activation (SKF 10, 047) in HEK293t and knock-out primary mouse embryonic fibroblasts (KO pMEFs) to assess potential modulation of RNA granule assembly and disassembly, autophagic flux, and ERAD-proteasome efficiency. This work also aimed to generate a baseline S1R protein interactome using the BioID technique.
Results Preliminary data suggest that RNA granules are present in the absence of stress and that RNA granule disassembly is perturbed in S1R KO pMEFs. Additionally, over-expression and activation of the S1R induce autophagosome accumulation, and over-expression of the S1R has no effect on degradation of a known ERAD substrate. Finally, the baseline S1R protein interactome shows gene ontology enrichment in protein folding, cellular stress response, and mRNA processing pathways.
Relevance Determining a novel role for the S1R in the PQC network may bring to light the mechanism by which S1R exerts its neuroprotective effects, and enable us to make more informed decisions when using the S1R as a pharmacological target to treat neurodegenerative diseases.

Disclosures: K.L. Fleming: None. **R. Bergeron:** None.

Poster

133. ALS and FTD Mechanisms

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Program #/Poster #: 133.16/G39

Topic: C.06. Neuromuscular Diseases

Support: Alzheimer's Association New Investigator Research Grant: NIRG-12-24156
National Institute of Aging: 1K01AG042500
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NIG-NIGMS Centers of Biolmedical Research Excellence (COBRE): 5P20GM103653
NSF: 1728804
Delaware Economic Development Office Grant from the State of Delaware

Title: Cellular toxicity model of TDP-43 triple mutation

Authors: *M. DOPLER, M. GITCHO;
Delaware State Univ., Dover, DE

Abstract: Transactive Response DNA Binding Protein of 43 kD (TDP-43) is the major pathological protein in Amyotrophic Lateral Sclerosis (ALS). Aggregated TDP-43 pathology

accumulates primarily in the cytoplasm of neurons forming insoluble aggregates. Pathological TDP-43 becomes hyperphosphorylated, ubiquitinated, and proteolytically cleaved where aggregation reaches cytotoxic levels. Multiple familial mutations of the protein have been identified since the discovery of pathological TDP-43 in those with ALS. Though there are models representing some of these mutations, we have developed a cell culture mode that has three TDP-43 (A315T, M337V, and S379P) mutations. This construct when overexpressed in HEK293T cells produces an increase in ubiquitinated proteins and shows an increase in both the 35kDa and 25 kDa C-terminal fragments reminiscent of the human cases. Using fluorescent microscopy and biochemical fractionation, we have observed an increased localization of TDP-43 in the cytoplasm compared to our control. We have developed a cellular model that shows some of the hallmarks comparable to what is found in ALS patients. This cellular model may lead to a better understanding of the pathogenesis of ALS and/or frontotemporal dementia.

Disclosures: M. Dopler: None. **M. Gitcho:** None.

Poster

133. ALS and FTD Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 133.17/G40

Topic: C.06. Neuromuscular Diseases

Title: High content assays for the quantification of axonal degeneration and molecular phenotypes associated with amyotrophic lateral sclerosis in motor neurons derived from human iPSCs

Authors: *S. JAIN[1], M. BSIBSI[1], M. JANUS[1], J. ESSENLINK[1], J. DEGROOT[1], D. F. FISCHER[2], R. KRAUSS[3];
[1]Charles River, Leiden, Netherlands; [2]Discovery, Charles River, Saffron Walden, United Kingdom; [3]Disarm Therapeut., Cambridge, MA

Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that progressively and irreversibly affects motor movement due to the death of motor neurons in the brain and spinal cord. About 90% of ALS cases have no known etiology while the remaining 10% have a genetic cause. Mutations in over 20 genes have been associated with familial ALS with mutations in 4 genes accounting for the majority of familial cases - SOD1, FUS, TDP-43 and C9orf72. Charles River has established cultures of human induced pluripotent stem cells from control and ALS patients, with each patient cell line harboring a mutation in one of the 4 genes which account for the majority of familial ALS. We have also optimized and implemented a robust motor neuron differentiation protocol that is amenable to high throughput screening. Using this protocol, the differentiated neurons express mature motor neuron markers, including Islet1 and SMI32 by day 30 of differentiation and form Ankyrin G positive axonal structures by

day 45 of differentiation. This physiologically relevant cell system allows high throughput screening of small molecule libraries or functional genomics-type approaches (RNAi/ CRISPR-Cas9), using high content imaging or biomarker readouts. Some of the disease relevant readouts that have been developed for screening:
C9orf72 RNA foci formation
C9orf72 di-peptide aggregation and toxicity (RAN translation products)
Quantification of axonal degeneration using a dual reporter system for quantification of a specific subset of axonal structures
Stress granule formation
Nucleo-cytoplasmic transport
TDP-43 mis-localization
We will present data demonstrating the robustness of our assays in iPSCs derived motor neurons and how, in combination with multi-parametric high content imaging and multi-variate data analysis, can help the discovery of novel targets and drugs for therapeutic intervention for this disease with high unmet medical need.

Disclosures: **S. Jain:** None. **M. BsiBsi:** None. **M. Janus:** None. **J. Essenlink:** None. **J. DeGroot:** None. **D.F. Fischer:** None. **R. Krauss:** None.

Poster

133. ALS and FTD Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 133.18/G41

Topic: C.06. Neuromuscular Diseases

Title: Molecular talk between Alsin/ALS2 and NADPH oxidase complex

Authors: *A. C. AZIM, D. O. BONSU, C. NORPHLET;
Chicago State Univ., Chicago, IL

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease that affects both upper and lower motor neurons. There have been several genes, such as SOD1, TDP-43,FUS, C9ORF72, KIF5A, UBQLN2, that have been linked to familial and sporadic form of ALS. Alsin/ALS2 is a guanine nucleotide exchange factor (GEF) for small GTPases. Alsin deficiency has been linked to a number of juvenile recessive motor neuron diseases (MNDs). There are many recent reports that suggest that Alsin interacts with small GTPases Rac and Rab1 *in vitro*. Rac1 and Rac2 have also been shown to interact with the NADPH oxidase complex in phagocytic cells which is involved in the generation of reactive oxygen species (ROS) in Rac dependent manner. In this study we have assessed the interaction of short form of Alsin (Sf ALSin) and long form of Alsin (Lf ALSin) and the whole of the NADPH Oxidase (Nox) complex by pull down assay, immuno-localization, and immuno-precipitation studies. Our

preliminary results suggest that NADPH does not co-precipitate with the Sf ALSin, but co-localizes with NADPH oxidase. Interestingly our data suggest that the NADPH oxidase complex did co-precipitated and co-localized with the Lf Alsin, suggesting an indirect interaction. Our *in vitro* studies also suggest a possible molecular link between SOD1 and Alsin mediated interaction with NADPH oxidase complex.

Disclosures: A.C. Azim: None. **D.O. Bonsu:** None. **C. Norphlet:** None.

Poster

133. ALS and FTD Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 133.19/G42

Topic: C.06. Neuromuscular Diseases

Support: Laurence and Sandi Gluck Foundation

Title: Experimental evidence that disease pathogenesis in sporadic ALS is unique and distinct from that seen in familial ALS

Authors: A. K. ROSELLE[1], S. J. E. SHIMSHAK[1], J. K. WONG[1], ***S. A. SADIQ**[2];
[1]Tisch MS Res. Ctr. of New York, New York, NY; [2]Tisch MS Res. Ctr., New York, NY

Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by motor neuron death. The majority of ALS cases are sporadic ALS (sALS) patients with no known genetic mutations. Only 5-10% of ALS patients have familial ALS (fALS) associated with an identified genetic mutation. Approximately 15-20% of fALS patients carry a mutation in the superoxide dismutase 1 (SOD1) gene. Although sALS and fALS patients present similar clinical symptoms, it is unknown whether the mechanisms underlying disease pathogenesis are the same. We aimed to address this question by assessing the effects of intrathecal delivery of cerebrospinal fluid (CSF) obtained from sALS and SOD1 patients into mice. 8-10 week old female mice underwent laminectomies at cervical levels 4 and 5, and 3µl of either sALS or SOD1 CSF was injected under the dura mater into the subarachnoid space. Control mice received either saline, CSF from healthy individuals, or CSF from other neurological diseases (OND). Forelimb motor deficits were assessed at 1 day post injection, then mice were perfused for histological analyses of the spinal cord. All motor testing and histological analyses were performed blinded. On motor testing, sALS CSF-injected mice exhibited significantly impaired forelimb function compared to controls and SOD1 CSF-injected mice, which were unimpaired. Pathologically, motor neuron death was observed in spinal cords of sALS CSF-injected mice, as determined by a significantly lower number of ChAT-positive motor neurons and increased activated-caspase3 staining. This was not observed in SOD1 CSF-injected mice. Nonphosphorylated neurofilament-H (SMI-32) expression was also significantly

elevated in the grey matter surrounding motor neurons in sALS CSF-injected mice. Evidence of reactive astrogliosis and microglial activation was observed in spinal cords of sALS CSF-injected mice, as revealed by stronger expression of GFAP and amoeboid microglial morphology, respectively. Glutamate transporter-1 expression appeared higher in the ventral horns of sALS CSF-injected mice, suggesting that glutamate excitotoxicity may be involved in inducing functional deficits and pathology. Overall, these findings suggest that disease mechanisms in sALS are distinct from those occurring in fALS.

Disclosures: **S.A. Sadiq:** None. **J.K. Wong:** None. **A.K. Roselle:** None. **S.J.E. Shimshak:** None.

Poster

133. ALS and FTD Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 133.20/G43

Topic: C.06. Neuromuscular Diseases

Support: Indian Council of Medical Research, New Delhi, India
Indian Council of Medical Research

Title: Oligodendrocytes are these cells lone hero against toxic insults of cerebrospinal fluid from amyotrophic lateral sclerosis patients

Authors: R. KURMOIL, S. BHAGAT, S. RAJKUMAR, A. VARGHESE, A. NALINI, T. SATHYAPRABHA, K. VIJAYALAKSHMI, ***T. R. RAJU**;
Natl. Inst. Mentl Hlth. Neurosci, Bangalore, India

Abstract: Cerebrospinal fluid from Amyotrophic Lateral Sclerosis patients (ALS-CSF) induces marked gliosis and degeneration of motor neurons. Our studies reveal ALS-CSF induced activation of both, microglia and astroglia skewing towards detrimental forms with the release of pro-inflammatory cytokines and other neurotoxic substances leading to obvious neuroinflammatory responses. Amongst the glial cells, role of oligodendrocytes in ALS has been overlooked since demyelination is not a prominent feature of ALS. However, recent studies point towards other role of oligodendrocytes including trophic and metabolic support to the neighbouring neurons. With reduced trophic support from activated astrocytes and microglia as well as altered glucose metabolism in the degenerating motor neurons it is intriguing to examine the role of oligodendrocytes in sporadic ALS. To investigate this, human oligodendrocyte cell line, MO3.13 was exposed to CSF from sporadic ALS patients (ALS-CSF) at 10%v/v for 48hrs and expression of CNPase as well as Olig2 was studied using immunocytochemistry followed by confocal microscopy. The intense nuclear labeling of Olig2 in normal controls was found to be diminished in cells exposed to ALS-CSF. The expression of CNPase was also significantly

reduced. Further, to investigate whether oligodendrocytes are protective to the degenerating motor neurons, MO3.13 cells were co-cultured with NSC-34 motor neuron cell line and, also in the presence of conditioned medium of the MO3.13 cells exposed to ALS CSF. We will be discussing our findings of live cell imaging experiments of NSC-34-MO3.13 co-cultures, levels of lactate support and expression of trophic factors like IGF-1 and GDNF by the oligodendrocytes. The findings of our study will reveal whether oligodendrocytes are indeed the "lone hero" to the degenerating motor neurons when the astrocytes and microglia turn topsy-turvy.

Disclosures: T.R. Raju: None. **R. Kurmoil:** None. **S. Bhagat:** None. **S. Rajkumar:** None. **A. Varghese:** None. **A. Nalini:** None. **T. Sathyaprabha:** None. **K. Vijayalakshmi:** None.

Poster

133. ALS and FTD Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 133.21/G44

Topic: C.06. Neuromuscular Diseases

Support: BMBF Grant 01EK1611C

Title: FUS localization is changed from the post-synapse to the pre-synapse during motorneuron development and accumulates in ALS-synapses

Authors: *M. DEMESTRE[1], D. DESHPANDE[2], T. M. BOECKERS[3], J. MICHAELIS[4]; [1]Anat. and Cell Biol., [2]Institue for Biophysics, [3]Inst. for Anat. and Cell Biol., [4]Inst. for Biophysics, Ulm Univ., Ulm, Germany

Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the degeneration of upper and lower motoneurons which leads to progressive muscle weakening and in most cases death due to respiratory failure. In 5% of familial ALS (fALS) cases mutations in FUS (Fused in Sarcoma) have been identified as a genetic cause of the disease. FUS is a RNA binding protein involved in gene expression such as mRNA splicing, translation and mRNA transport for local translation in dendrites. Mainly it is located in nuclei but also presents a dendritic localization. Most of the FUS mutations related with ALS are clustered in the nuclear localization signal, which is involved in the proper transport of the protein. As a result, in brain and in spinal cord of affected patients, FUS aggregates are detected in the cytoplasm. We studied FUS localization in motoneuron synapses, to distinguish specifically between the pre- and postsynaptic compartments super-resolution microscopy was used. We demonstrate a maturation based variation of FUS localization in rodent synapses where a predominantly postsynaptic FUS was observed in early stages of synaptic development, while in mature synapses FUS was localized at pre-synapses. We used human induced pluripotent stem cells (hiPSC) derived

motoneurons to model ALS neuropathology. HiPSCs from a control subject and fALS-FUS patient harboring a juvenile onset mutation were generated. At DIV42 of cultivation in control and patient-motoneurons immature synapses were formed with pre/post synaptic marker co-localisation but at ultrastructural level no clear thickness of the post synaptic membrane was seen. In control motoneurons, FUS was postsynaptic and in motoneurons derived from ALS patients, FUS and other synaptic markers aberrantly accumulated in synapses. Having demonstrated changes in the FUS localization during synaptogenesis, we propose a role of synaptic FUS in both dendritic and axonal cellular compartments, and we suggest a gain-of-toxic-function as a result of the synaptic aggregation of mutant FUS in ALS.

Disclosures: M. Demestre: None. **D. Deshpande:** None. **T.M. Boeckers:** None. **J. Michaelis:** None.

Poster

133. ALS and FTD Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 133.22/H1

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant 5R01NS105756-02

Title: Bait RNA oligonucleotides to antagonize pathological protein aggregation in ALS/FTD

Authors: *J. R. MANN[1], L. GUO[5], A. GLEIXNER[2], J. C. MAUNA[6], K. E. COPLEY[3], E. GOMES[7], B. PORTZ[8], J. SHORTER[9], C. J. DONNELLY[4];
[1]Neurobio., [3]Neurosci., [4]Dept. of Neurobio., [2]Univ. of Pittsburgh, Pittsburgh, PA; [5]Thomas Jefferson Univ., Philadelphia, PA; [6]Neurobio., Univ. of Pittsburgh Dept. of Neurobio., Pittsburgh, PA; [7]Biochem. & Biophysics, [8]Univ. of Pennsylvania, Philadelphia, PA; [9]Biochem. and Biophysics, Univ. of Pennsylvania Dept. of Biochem. and Biophysics, Philadelphia, PA

Abstract: Aberrant aggregation of RNA-binding proteins, such as TDP-43 and FUS, is a common neuropathological hallmark of neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Current cellular models of these neurodegenerative proteinopathies often rely on the overexpression of disease-linked mutant proteins to induce pathological protein aggregation. However, mutations in the *TARDBP* and *FUS* genes each only account for ~5% of familial ALS (fALS) cases. The vast majority of patients do not harbor mutations in these genes, yet still experience aggregation of these proteins. Similarly, rodent models of ALS produced from the overexpression of these mutant proteins have been historically unreliable and often fail to produce pathologically-relevant inclusions. Here we present a novel optogenetic-based technique to induce pathological aggregation of TDP-43 and FUS with a previously unachievable level of spatial and temporal control

(optoTDP43/optoFUS). Using this approach, we discovered that the RNA-binding status of TDP-43 and FUS dictate their propensity to undergo liquid-liquid phase separation (LLPS) and form pathological inclusions. We also show that these aberrant intracellular phase transitions are toxic to human neuronal cultures and that bait RNA oligonucleotides (bONs) mimicking known TDP-43 or FUS binding sequences can prevent light-induced phase transitions and rescue resulting neurotoxicity. In order to work towards a more therapeutically-relevant paradigm, we also investigate the ability of bONs to disaggregate existing TDP-43 and FUS inclusions and examine resulting effects on neuronal survival. This optogenetic strategy can be further applied to a number of different disorders and will aid in the identification of negative regulators of pathological protein aggregation, as well as in-depth investigations into the effects of these pathological aggregates on various cellular pathways and downstream pathological processes.

Disclosures: J.R. Mann: None. **A. Gleixner:** None. **J.C. Mauna:** None. **K.E. Copley:** None. **E. Gomes:** None. **L. Guo:** None. **B. Portz:** None. **J. Shorter:** None. **C.J. Donnelly:** None.

Poster

133. ALS and FTD Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 133.23/H2

Topic: C.06. Neuromuscular Diseases

Support: ALS Canada- Brain Canada Discovery Grant

Title: Better understand the amyotrophic lateral sclerosis with a 3D spinal cord model generated by tissue-engineering

Authors: *A. LOUIT[1], M.-J. BEAUDET[2], F. BERTHOD[1];
[1]Univ. Laval, Quebec, QC, Canada; [2]Lab. d'Organogenese Experimentale LOEX - Univ. Laval, Quebec, QC, Canada

Abstract: Background: Amyotrophic lateral sclerosis (ALS) is an incurable neurodegenerative disease, that affects 2 to 5 of every 100 000 adults, causing motor neuron (MN) degeneration and leading to patient death due to respiratory failure. A mutation in the Superoxide Dismutase 1 (SOD1) gene inducing protein misfolding and accumulation of aggregates in MN has been identified as a cause of ALS in 5% of patients. Recently, studies have shown that the combination of MN, astrocytes, microglia and myoblasts could constitute a metabolic unit and that non-neuronal cells could contribute to the development of ALS. **Objectives:** Our main goal is to develop and characterize by tissue engineering an in vitro murine 3D model of spinal cord reproducing the ALS phenotype. Then, the objective will be to determine the role of each cell type in the development of the ALS phenotype in vitro, using various combinations of healthy or

diseased cells. **Methods:** MN have been extracted from transgenic SOD1G93A spinal cord mouse embryos aged of 14 days of development and astrocytes, microglia, myoblasts and Schwann cells from adult mice, overexpressing the mutant human SOD1 protein, or wild type SOD1WT mice, overexpressing the normal SOD1 human protein. These cells have been characterized with immunofluorescences, purified by gradient density separation, and co-cultured on 3D collagen/chitosane sponges **Results:** In the 3D model, when SOD1G93A MN were cultured with mutant astrocytes and microglia, there was a 25% reduction in the length of TUJ-1 positive neurites, compared to the control seeded with SOD1WT MN, astrocytes and microglia. In addition, a similar decrease in the length of neurites was observed with non-transgenic MN grown in presence of SOD1G93A glial cells, compared to SOD1WT glial cells. Finally, when cultured with SOD1G93A or SOD1WT Schwann cells, axonal grown was enhanced. **Conclusion:** MN are able to organize themselves into nerve fibers in presence of healthy or diseased glial cells, but axonal migration was found 25% shorter in presence of glial cells overexpressing the mutated SOD1, recapitulating in part an ALS phenotype. The main advantages of the 3D model are to allow the combination of MN, astrocytes, microglia, Schwann cells and myoblasts in the same tissue, to make any healthy and diseased cell combination, to explore the process of axonal migration in 3D and to perform long-term culture (over 2 months). Such in vitro ALS modelling should lead to a better understanding of the disease mechanisms, and could serve as a platform for drug screening. Moreover, this 3D model would be adaptable to other type of mutations involved in ALS.

Disclosures: A. Louit: None. **M. Beaudet:** None. **F. Berthod:** None.

Poster

134. Neuroprotective Mechanisms: Preclinical Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 134.01/H3

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: DIFA-NAT18-I
TEMS-NAT-18-I
PAPPIIT-UNAM (IN214117

Title: Decavanadate-metformin (MetfDeca) improves recognition memory and decreases neurodegeneration in the hippocampus of rats with metabolic syndrome

Authors: *A. D. DIAZ[1], A. GRANADOS[2], E. SANCHEZ-LARA[3], E. GONZALEZ-VERGARA[3], G. FLORES[5], B. VENEGAS MENESES[4], J. GUEVARA[6], S. TREVIÑO[2]; [1]Pharm., Facultad De Ciencias Quimicas, BUAP, Puebla, Mexico, Mexico; [2]Pharm., [3]ICUAP, [4]BUAP, Puebla, Mexico; [5]Univ. Autonoma de Puebla / Inst. de Fisiologia, Puebla, Mexico; [6]Biochemestry; Med., UNAM, Ciudad de Mexico, Mexico

Abstract: Reports indicate that the metabolic syndrome (MS) generates oxidative stress and inflammatoryresponse in the brain. Events that together cause morphological changes and neurodegeneration in the hippocampus. Also, MS causinga failure in cognitive function, such as recognition memory and triggering dementia, although the mechanism isstill unclear. Currently, new drugs are being sought to delay the development of these degenerative diseases. Drugs based on vanadium are possible treatments for MS and its complications at the brain level. In this sense, our working group synthesized MetforminiumDecavanadate (MetfDeca), a compound with hypoglycemic and hypolipidemic properties, which was evaluatedin an SM model induced by a hypercaloric diet (DHC) in male Wistar rats. Our objective was to evaluate its effect on recognition memory and neuronal degeneration. Four groups were formed (n = 12 / group): 1) Normocaloric diet (DNC); 2) DNC + MetfDeca; 3) DHC and 4) DHC + MetfDeca, and the diets were administeredfor 150 days. At 90 day of diets administration, treatment with MetfDeca was started. After the treatments, the animals were subjectedto the object recognition test. The neuronal morphology, the inflammatory response, and the neurodegeneration in the Hp of each group were evaluated. Our results showed that the MetfDeca decreases the deterioration of recognition memory. The brains of these animals showed an increase in dendritic arborization and the density of dendritic spines in Hp. Also, reducing reactive astrogliosis and death of Hp neurons. The present work demonstrates that the treatment with MetfDecadecreased the neurodegeneration in the hippocampus of rats with SM and improved the recognition memory. In this sense, MetfDeca can be considered as a new drug to reduce brain aging and control senile dementias.

Disclosures: A.D. Diaz: None. **A. Granados:** None. **E. Sanchez-Lara:** None. **E. Gonzalez-Vergara:** None. **G. Flores:** None. **B. Venegas Meneses:** None. **J. Guevara:** None. **S. Treviño:** None.

Poster

134. Neuroprotective Mechanisms: Preclinical Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 134.02/H4

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Experimental oximes as organophosphate pre-exposure prophylaxis

Authors: *D. E. LORKE[1,2], S. M. NURULAIN[3], M. Y. HASAN[4], K. KUCA[5], G. A. PETROIANU[1];
[1]Cell. Biol. and Pharmacol., Florida Intl. Univ., Miami, FL; [2]Anat. and Cell. Biol., Khalifa Univ., Abu Dhabi, United Arab Emirates; [3]COMSATS Inst. of Information Technology, Islam, Islamabad, Pakistan; [4]Dept. of Pharmacol. & Therapeut., UAE Univ., Al Ain, United Arab Emirates; [5]Dept. of Chem., Univ. of Hradec Kralove, Hradec Kralove, Czech Republic

Abstract: Aims: Organophosphates (OPs), useful agents as pesticides, also represent a serious health hazard due to acetylcholine-esterase (AChE) inhibition. Standard therapy with atropine and established oxime-type enzyme reactivators is unsatisfactory. Better therapeutic results are obtained when reversible AChE inhibitors are administered before OP exposure. We have previously tested the prophylactic efficacy of 5 known AChE inhibitors (physostigmine, pyridostigmine, ranitidine, tacrine, K-27), when given as pretreatment before OP exposure. Best prophylactic efficacy was observed for the experimental oxime K-27. The present study was undertaken to determine the efficacy of two established (pralidoxime, obidoxime) and five experimental K-oximes (K-48, K-53, K-74, K-75, K-203) to protect from the toxic effects of the OP paraoxon. **Methods:** AChE inhibitory activity of the compounds used for pretreatment was quantified by determining their IC_{50}, using Worek's modification of Elman's technique. Therapeutic efficacy was determined by comparing the relative risk of death (RR) by Cox survival analysis in rats that were administered the oximes at equitoxic dosage (25% of LD_{01}) 30 min before the OP paraoxon with the RR of animals pretreated with the FDA-approved pretreatment compound pyridostigmine and with those that had been exposed to paraoxon only without pretreatment (RR=1). **Results:** Pyridostigmine was the strongest in vitro AChE-inhibitor (IC_{50}=0.33 µM), followed by K-74 (IC_{50}=66 µM), K-53 (IC_{50}=83 µM), K-75 (IC_{50}=101 µM) and obidoxime (IC_{50}=193 µM). Pralidoxime (IC_{50}=412 µM) and K-48 (IC_{50}=643 µM) only weakly inhibited RBC AChE activity. All eight tested substances statistically significantly reduced paraoxon-induced mortality. Best protection was conferred by the experimental oxime K-48, reducing the relative risk of death (RR) to 0.10, which was statistically significantly superior to pyridostigmine (RR=0.31). The other oximes reduced the RR to 0.13 (obidoxime), 0.20 (K-203), 0.21 (K-74), 0.24 (K-75) and 0.26 (pralidoxime); which was significantly lower than the no-pretreatment group (RR = 1). **Conclusions:** Best outcome is achieved if K-48 is administered prophylactically before paraoxon exposure. Our data also support the hypothesis that protective efficacy is unrelated to AChE inhibition and indicate that the tested experimental oximes may be considered promising alternatives to the established pretreatment compound pyridostigmine. The authors have **no conflict of interest** to report

Disclosures: D.E. Lorke: None. **G.A. Petroianu:** None. **S.M. Nurulain:** None. **M.Y. Hasan:** None. **K. Kuca:** None.

Poster

134. Neuroprotective Mechanisms: Preclinical Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 134.03/H5

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: La Salle University - Provided Majority of Research Support
Be Tru Organics, Inc. - Donated CBD and Covered Some Expenses

GPHB Neuroscience, LLC - Covered Some Expenses

Title: Marked neuroprotection by the marijuana derivative cannabidiol against piriform cortical brain damage resulting from prolonged seizures and status epilepticus in rats

Authors: D. Y. BALLOUGH, T. J. MICKUS, E. M. PARRISH, I. MARTINEZ-IGLESIAS, A. J. HILLER, S. P. GAINES, ***G. P. H. BALLOUGH**;
Biol., La Salle Univ., Philadelphia, PA

Abstract: The present investigation assessed the neuroprotective efficacy of cannabidiol (CBD) in a kainic acid (KA) seizure model of temporal lobe epilepsy. Sixty-six male Sprague-Dawley rats were used. Each animal was housed individually in a clear polycarbonate chamber (LWH = 45x24x20cm), containing species-appropriate bedding, and covered with a wire lid that allowed free access to food and water. This research was conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* with oversight and protocol approval by La Salle University's IACUC. After a 6-day acclimation period, rats were randomly assigned to one of 8 treatment groups. These groups were named as follows: (1) "Untreated," (2) "Saline-Saline," (3) "Saline-Vehicle," (4) "Saline-CBD," (5) "KA-Sham," (6) "KA-Saline," (7) "KA-Vehicle," and (8) "KA-CBD." On the day of injections, rats weighed an average of 213.3g. Seizures were induced by intraperitoneal (i.p.) injection of 14mg/kg KA dissolved in sterile saline. Controls received equal volume/weight injections of sterile saline. At 40min post-seizure onset, KA rats received i.p. injections of either saline, vehicle or CBD (50mg/kg); an additional KA group received a sham needle insertion but no injection. Non-KA rats received similar injections 80min following initial injections (accounting for onset duration in KA groups). Four hours following seizure onset, each KA rat received a supplemental injection of sterile saline (8ml) for hydration. Non-KA, control rats also received supplemental saline injections, at an equivalent delay. Twenty-four hours post-seizure onset, rats were administered 130mg/kg (i.p.) pentobarbital and sacrificed, upon evidence of labored breathing, via transcardial perfusion with 0.9% physiological saline followed by 4% paraformaldehyde. Brains were removed, processed, and sectioned at 5μm. Serial sections (bregma -3.3mm) were stained using hematoxylin and eosin (H&E) and Fluoro-jade B (FJB). On H&E stained slides, morphometric image analysis was used to measure necrotic core cross-sectional areas in the piriform cortex and contiguous brain structures (e.g., amygdala). Fluorescent microscopy and image analysis was used to count FJB-positive degenerating hippocampal neurons. CBD was exceptionally neuroprotective in the piriform cortex (i.e., reduced necrosis by 71.6% in the KA-CBD group compared to KA-Vehicle). In contrast, the KA-CBD group showed a significant elevation in hippocampal FJB positive neurons in hippocampal field CA3. Overall, CBD was markedly neuroprotective against KA-induced seizure-related brain damage.

Disclosures: D.Y. Ballough: None. **T.J. Mickus:** None. **E.M. Parrish:** None. **I. Martinez-Iglesias:** None. **A.J. Hiller:** None. **S.P. Gaines:** None. **G.P.H. Ballough:** None.

Poster

134. Neuroprotective Mechanisms: Preclinical Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 134.04/H6

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: Sanofi

Title: Oligodendrocyte precursor maturation is associated with accumulation of endogenous intermediate sterols

Authors: *G. SHENG[1], D. WANG[2], Q. PAN[3], K. RADZWILL[1], J. FARLEY[1], C. GARRON[1], A. BYRNE[3], L. CHAI[3], B. ZHANG[3], T. A. SAMAD[1], C. PEDRAZA[1];
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Abstract: Novel therapies for autoimmune, neurological diseases such as Multiple Sclerosis are aimed to enhancing repair of damaged CNS myelin, thus improving and preserving neuronal function. Pharmacological enhancement of myelination through maturation of endogenous oligodendrocyte progenitor cells (OPC) has been demonstrated in response to several stimuli, including inducers of accumulation of cholesterol biosynthesis intermediates. However, the dynamics of intermediate sterol accumulation during in vitro spontaneous maturation and during remyelination after injury remains undefined. We assessed the temporal dynamics of sterol intermediate accumulation during the process of OPC differentiation and myelination in vitro and in an animal model of de/remyelination. A signature of 8 sterol intermediates showing changes during OPC maturation (DIV, 0-7) was detected with two core sterols, zymostenol and zymosterol showing high accumulation. This indicates a role for the sterol isomerase Emopamil Binding Protein (EBP), which converts 8,9 into 7,8-unsaturated sterols, an essential step in cholesterol biosynthesis. Treatment of cultured OPCs with EBP inhibitors dramatically increased maturation as a consequence of elevated sterol accumulation. Inhibition of EBP activity also resulted in increased expression of cholesterol biosynthesis enzymes potentially as a compensatory mechanism to altered key intermediate sterol levels. In vivo, we observed a robust accumulation of zymosterol in mouse brains as early as 7hr after peripheral injections of tamoxifen (Estrogen receptor modulator) and Clemastine (M1/M3 antagonists, H1 inhibitor), both known partial inhibitors of EBP activity, and inducers of OPC maturation. Moreover, EBP inhibition significantly enhanced myelin repair after demyelination caused by focal injections of lysolecithin in the mouse spinal cord. Our data support EBP as an emerging and viable target for remyelination therapies in MS.

Disclosures: **G. Sheng:** None. **D. Wang:** None. **Q. Pan:** None. **K. Radzwill:** None. **J. Farley:** None. **C. Garron:** None. **A. Byrne:** None. **L. Chai:** None. **B. Zhang:** None. **T.A. Samad:** None. **C. Pedraza:** None.

Poster

134. Neuroprotective Mechanisms: Preclinical Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 134.05/H7

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: JSPS KAKENHI Grant 18K07376

Title: Neuroprotective roles of apolipoprotein e containing lipoproteins in neurons and glia of retina

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Abstract: Apolipoprotein E-containing lipoproteins (E-LPs) released from glia are one of the major lipoproteins and have important roles of lipid metabolism and transport in the central nervous system. We have previously reported that glia-derived E-LPs stimulate axon growth of retinal ganglion cells (RGCs) and show a protective effect against glutamate-induced RGC degeneration via a low density lipoprotein receptor-related protein 1 (LRP1). It has been shown that alpha 2-macroglobulin (a2M), which is one of the LRP1 ligands, is increased in aqueous humor of glaucoma patients. Here we investigated protective mechanisms of E-LPs against neurodegeneration in neurons and glia of retina. Primary cultured RGCs and glia from retina were used for in vitro studies, and *N*-methyl-D-aspartate was injected intravitreally to induce glaucomatous optic neuropathy for in vivo studies. We found that intravitreal injection of E-LPs protected RGCs from degeneration and also decreased the upregulated a2M level in aqueous humor of the glaucoma model. The protective effect by E-LPs in primary cultured RGCs was interfered by a2M. On the other hand, mRNA and proteins of a2M in primary cultured retinal glia were reduced by adding E-LPs via an LRP1. In addition to our previous findings of direct protection of RGCs by E-LPs, this study indicates a possible therapeutic strategy of E-LPs by attenuating the a2M derived from glia in the retinal degeneration, such as glaucoma.

Disclosures: **H. Hayashi:** None. **M. Mori:** None. **M. Yamada:** None. **B. Yuan:** None. **N. Takagi:** None.

Poster

134. Neuroprotective Mechanisms: Preclinical Models

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Program #/Poster #: 134.06/H8

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: The National Natural Science Foundation of China 81271396

Title: Expression of GPNMB after peipheral nerve injury and the effect of recombinant human GPNMB on Schwann cells

Authors: Y. ZHENG, C. HUANG, F. LIU, X. YANG, ***Z. ZHANG**;
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Abstract: Glycoprotein non-metastatic melanoma protein B (GPNMB) has been recently reported to have neuroprotective effects on neurodegenerative diseases such as amyotrophic lateral sclerosis and cerebral ischemia reperfusion injury in the central nervous system. However, the expression and function of GPNMB in the peripheral nervous system still remain unknown. In this study, long noncoding RNA (lncRNA) microarray analysis was performed to profile the lncRNAs and mRNAs in distal sciatic nerve at 0, 1, 3, 7, 14, 21, 28 d following the resection in rat. Short Time-series Expression Miner (STEM) analysis was performed to obtain lncRNA and mRNA expression profiles. It was showed that there were 12 gene expression profiles with significant tendency. The profile 41 had the similar tendency as the proliferation of schwann cells (SCs) after injury,which,consisting of 105 lncRNAs and 389 mRNAs, was increased at 1d after injury, peaked at 7d, and then decrased. Gene ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analysis were performed to find they were involved in cell proliferation, including cell cycle. GPNMB was selected from the most significantly varied mRNAs for validation by quantitative real-time PCR and Western blotting. To valuate the function of GPNMB on SCs, recombinant human GPNMB (rhGPNMB) was added when culturing denervated SCs from distal stumps of resected sciatic nerve. The proliferation, expression and secretion of neurotrophic factors and neural adhesion molecules of schwann cells were also detected. Our data demonstrated that GPNMB expression was quickly increased and peaked at 7d in distal sciatic nerve after injury. rhGPNMB increased the proliferation of SCs and expression and secretion of neurotrophic factors and neural adhesion molecules *in vitro*. Thus, GPNMB could be a novel strategy for peripheral nerve regeneration and repair.

Disclosures: Y. Zheng: None. **C. Huang:** None. **F. Liu:** None. **X. Yang:** None. **Z. Zhang:** None.

Poster

134. Neuroprotective Mechanisms: Preclinical Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 134.07/H9

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Protective effect of Brazilian green propolis against oxidative stress-induced cell death in mouse hippocampal HT22 cells

Authors: *M. TAKASHIMA[1,2], K. ICHIHARA[2], Y. HIRATA[1];
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Abstract: Propolis is a sticky dark-colored substance produced by honey bees and comprises resin, balsam, wax, essential and aromatic oils, pollen, and several other substances; it is used in food and beverages to improve health and prevent diseases. The contained components of propolis are characterized by the botanical origin collected by honey bees. We studied the neuroprotective effects of extracts of Brazilian green propolis in the mouse hippocampal cell line HT22. The botanical origin of Brazilian green propolis is *Baccharis dracunclifolia*. It is also known that these chemical components and their amounts contained in extracts of propolis differ depending on the extracting solvent. Ethanol extracts of Brazilian green propolis (EEP) had a more potent preventive effect on oxidative stress-induced cell death, oxytosis/ferroptosis, in HT 22 cells than water extracts of Brazilian green propolis. Among the primary constituents of ethanol extracts of Brazilian green propolis, only artepillin C, kaempferide, and kaempferol demonstrated neuroprotective effects against oxytosis/ferroptosis. On the other hand, EEP and these main components did not have protective effects on anticancer drug induced-apoptosis. The flavonoid derivatives kaempferide and kaempferol are antioxidants with radical-scavenging abilities that additionally induce antioxidant response element-mediated transcriptional activity, suggesting that upregulation of endogenous antioxidant defenses protects against oxidative stress. In contrast, artepillin C attenuated reactive oxygen species production; however, it did not induce antioxidant response element activation. These findings indicate that the ethanol extracts of Brazilian green propolis help to prevent oxidative stress-related neuronal cell death that is involved in the pathogenesis of neurodegenerative diseases such as Alzheimer's and Parkinson's disease. The main beneficial ingredients of propolis are artepillin C and kaempferide, which scavenge reactive oxygen species directly or indirectly.

Disclosures: **M. Takashima:** None. **K. Ichihara:** None. **Y. Hirata:** None.

Poster

134. Neuroprotective Mechanisms: Preclinical Models

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Program #/Poster #: 134.08/H10

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: IN216817

Title: Effect of prolactin on the CREB transcription factor expression in primary cultures of rat hippocampal neurons under excitotoxicity

Authors: *V. RODRIGUEZ CHAVEZ[1], N. A. RIVERO-SEGURA[2], G. MOLINA-SALINAS[1], M. A. CERBÓN-CERVANTES[1];
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Abstract: It has been reported that the prolactin (PRL), is capable to induce neuroprotection against excitotoxicity in the hyppocampus, both in *in vivo and in vitro* models. However, information about the regulation exerted by PRL on transcription factors involved in neuroprotection has not been completely described. Therefore, the present project focuses on describing the effect of PRL on the CREB transcription factor (cAMP response element) involved in neuroprotection via BDNF and BCL-2 expression. Primary cultures of hippocampal neurons obtained from rat embryos from 17.5 days were used. The cultures were divided in four groups: Control (CTRL, saline), PRL (10 ng/ml), glutamate (Glu, 100 μM) and PRL/Glu (10 ng/ml/100 μM). The nuclear translocation of the transcriptional factor CREB, the expression of total CREB and its phosporylated form (p-CREB) were evaluated, as well as the BCL-2 and BDNF protein content. Our results demonstrate that p-CREB/CREB ratio, evaluated by Western Blot, does not show statistically significant changes between treatments. Similarly, we observed, by fluorescent immunocytochemistry, that treatment with PRL (10 ng/ml for 72 h) does not induce translocation to the nucleus of the transcription factor CREB. Concomitantly, we evaluated the protein expression of BDNF and Bcl-2, both direct targets of CREB. While the protein expression of BDNF showed that the treatment with PRL does not modify the content of this protein, the treatment with Glu, decreases significantly it content, with respect to the control. Importantly, when we performed a simultaneous treatment of PRL and Glutamate the BNDF content increases significantly. Besides, the expression of Bcl-2 in neurons treated with PRL increases significantly with respect to the control, both alone or with glutamate excitotoxicity. Taken together, our results suggest that PRL treatment does not induce activation of the transcriptional factor CREB at this point, however, PRL treatment induce an increase in BNDF and Bcl-2 proteins indicating that these proteins may participate in neuroprotection and are activated by PRL. Further experiments are required to discard whether or not PRL may induce CREB activation in shorter treatment times, participating in PRL-induced neuroprotection.

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Poster

134. Neuroprotective Mechanisms: Preclinical Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 134.09/H11

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: RG14811

Title: Vitamin E promotes neurite outgrowth via Bcl-xL upregulation

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Abstract: B-cell lymphoma-extra large (Bcl-xL) is a pro-survival protein localized to mitochondria. Bcl-xL supports brain health by enhancing neuronal energy metabolism, synapse formation, and neurite outgrowth. In this study, we investigated strategies to manipulate Bcl-xL in primary hippocampal neurons using the nutrient alpha-tocotrienol. Alpha-tocotrienol, a member of the vitamin E family, has been reported to protect the brain against neurotoxic stimuli used to mimics various disease states. Here, we show that Bcl-xL is a major target of alpha-tocotrienol-mediated protection, supporting mitochondrial function, restoring intracellular energy, and promoting hippocampal outgrowth. Primary hippocampal neurons were grown for 3 weeks in media with or without alpha-tocotrienol. Pre-conditioned media was changed once per week. Hippocampal neurons in both groups grew normally, without displaying abnormal morphology. However, neurons treated with alpha-tocotrienol had increased branching and total length of neurites. We also found that alpha-tocotrienol treatment increased the abundance of Bcl-xL which has been shown to improve mitochondrial function. Although the underlying mechanisms are still under investigation, we found that Bcl-xL increases maturation of brain-derived neurotrophic factor (BDNF), a protein required for neuronal outgrowth. We speculate that post-translational modification of BDNF may require mitochondrial involvement. Our findings suggest that alpha-tocotrienol may be a clinically relevant strategy to promote neuronal growth during normal development or possibly to enhance recovery after neuronal injury.

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Poster

134. Neuroprotective Mechanisms: Preclinical Models

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Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: T32EB006359
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Title: Acidic nanoparticles formulated from biodegradable poly lactic-co-glyolic acid can restore lysosomal function in Parkinson's disease models

Authors: *A. MARTIN[1], J. ZENG[1], O. SHIRIHAI[2], X. HAN[1], M. GRINSTAFF[1];
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Abstract: Lysosomal dysfunction has been implicated in the progression of both familial and idiopathic Parkinson’s Disease (PD). However, the identification of the lysosome as a viable target for therapeutic intervention has been stifled due to a limited number of techniques specifically directed at elevating lysosomal function. Nanoparticles formulated from biodegradable poly lactic-co-glycolic acid (PLGA) effectively traffic to lysosomes though endocytic mechanisms. In diseased lysosomes with elevated pH (>5.0), PLGA nanoparticles degrade, acidify the lysosomal environment, and restore lysosomal function. Using neurotoxins such as 1-methyl-4-phenylpyridinium (MPP+) to recreate PD, treatment with nanoparticles demonstrate lysosomal pH modulating capabilities. MPP+ injury results in decreased lysosomal acidity, inhibited autophagic flux, and significant cell death. PLGA nanoparticles restore these effects in a compositional dependent manner, where nanoparticles formulated from PLGA polymers with a higher glycolic acid to lactic acid ratio demonstrate improved degradation rate, lysosomal pH modulation, and autophagic flux restoration. The nanoparticles target lysosomes using native endocytic mechanisms and yield modular restoration of lysosomal function dependent on the compositional of the polymeric nanoparticles. These formulations demonstrate the ability to controllably modulate lysosomal function in PD models.

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Poster

134. Neuroprotective Mechanisms: Preclinical Models

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Program #/Poster #: 134.11/H13

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NICHD Intramural Research Program

Title: Carboxypeptidase E - Neurotrophic factor- α 1: Obliteration of the enzymatic activity uncovers novel receptor-mediated neuroprotection and cognitive functions in hippocampal CA3 neurons in mice

Authors: *L. XIAO[1], V. K. SHARMA[2], L. TOULABI[3], X. YANG[4], A. PELTEKIAN[3], I. ARNAOUTOVA[3], D. ABEBE[3], Y. LOH[5];
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Abstract: Stress causes various pathophysiology in the brain including neuronal degeneration, compromised neuronal network and cognitive dysfunction, and leads to diseases such as Alzheimer Disease and Major Depressive Disorder. Neurotrophic/growth factors such as BDNF, NGF and NT3 have been linked to these pathological conditions. Carboxypeptidase E (CPE), a proneuropeptide/prohormone processing enzyme, also named neurotrophic factor-α1(NFα1) is highly expressed in the stress-vulnerable hippocampal CA3 neurons, and was shown to have neuroprotective activity from *in vitro* studies. Here we investigated if CPE-NFα1 functions *in vivo*, independent of its enzymatic activity, and the mechanism underlying its action. We generated knock-in mice expressing a non-enzymatic form of CPE, CPE-E342Q, but not wild-type CPE. The CPE-E342Q mice showed significantly decreased neuropeptide content and exhibited obesity, diabetes and infertility due to lack of prohormone processing activity, similar to CPE-KO mice. However, they showed no hippocampal CA3 degeneration, exhibited neurogenesis in the dentate gyrus, and displayed normal spatial learning and memory, similar to CPE wild-type mice, after weaning stress; unlike CPE-KO mice which showed hippocampal CA3 neuronal degeneration and cognitive deficits. Binding studies showed that radiolabeled CPE bound hippocampal cell membrane specifically, in a saturable manner. Binding of CPE and CPE-E342Q to hippocampal neurons activated Erk signaling and pre-treatment with either of these proteins protected neurons against H_2O_2- or glutamate-induced neurotoxcity by increasing BCL2 expression. *In vitro* and *in vivo* inhibitor studies demonstrated that this neuroprotective effect was independent of tyrosine kinase receptor signaling. Taken together, the data provide evidence that CPE-NFα1 is a unique neurotrophic factor which acts through a non-tyrosine

kinase receptor to activate Erk-BCL2 signaling to protect hippocampal CA3 neurons against stress-induced neurodegeneration and maintaining normal cognitive functions in mice.

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Poster

134. Neuroprotective Mechanisms: Preclinical Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 134.12/H14

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NICHD intramural research program

Title: NF-α1/CPE neuroprotects against oxidative stress by activating ERK/CREB/BCL2 signaling via HTR1E receptor

Authors: *V. K. SHARMA[1], Y. XUYU[1], D. S. SANCHEZ[2], Y. LOH[1];
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Abstract: Neurotrophic factor-α1/Carboxypeptidase E (NF-α1/CPE) is known to protect hippocampal and and cortical neurons against oxidative stress caused by H_2O_2. To protect the cells against oxidative stress, NF-α1/CPE acts extracellularly through a cell surface receptor to execute this function. Previously we have shown that human HTR1E serotonin receptor serves as a binding partner (or receptor) for NF-α1/CPE using high throughput screening of human orphan GPCRs followed by PRESTO-Tango reporter assay system and in co-immunoprecipitation studies. Furthermore, we now show in immunocytochemical studies, the colocalization of HTR1E with NF-α1/CPE in human hippocampal CA3 and dentate gyrus neurons. We have reported that NF-α1/CPE increased ERK phosphorylation via HTR1E receptor when added to culture media. Based on these findings we sought to determine the other downstream effectors of NF-α1/CPE induced ERK signaling via HTR1E. We studied the effect of recombinant NF-α1/CPE on pCREB at different time points and found a significant increase in the level of phosphorylated CREB after 20 minutes of NF-α1/CPE treatment in HEK293 cells stably expressing HTR1E receptor. CREB is known to regulate various functions including neuroprotection via increasing pro-apoptotic protein BCL2. We then checked the effect of NF-α1/CPE on BCL2 levels during H_2O_2 induced oxidative stress. We found that there was a marked decrease of BCL2 in HTR1E stably transfected HEK293 cells treated with H_2O_2 whereas cells pretreated with NF-α1/CPE for 24h followed by H_2O_2 treatment showed no decrease in BCL2. These results show that NF-α1/CPE binds HTR1E receptor which then activates

ERK/CREB/BCL2 signaling pathway to mediate protection of these cells against oxidative stress.

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Poster

134. Neuroprotective Mechanisms: Preclinical Models

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Program #/Poster #: 134.13/H15

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH NS047264

Title: Exercise is neuroprotective following partial motoneuron depletion via androgen action at the target muscle

Authors: *C. CHEW, D. R. SENGELAUB;
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Abstract: We have previously demonstrated that partial depletion of motoneurons innervating the quadriceps muscles induces dendritic atrophy in remaining motoneurons. Furthermore, systemic treatment with supplemental androgens is neuroprotective, and dendritic atrophy following partial motoneuron depletion is attenuated. Blockade of the androgen receptor at the target muscle prevents the neuroprotective effects on motoneuron dendrites in rats treated with supplemental androgens. We have recently shown that exercise is also neuroprotective on motoneuron dendrites following partial motoneuron depletion, and circulating levels of androgens have previously been shown to increase following exercise. Together, these results suggest that exercise may be neuroprotective via androgen action at the muscle. In the present study, we examine whether blockade of androgen receptors at the target musculature would prevent the neuroprotective effects of exercise on dendrites following partial motoneuron depletion. Motoneurons innervating the vastus medialis muscle in adult male rats were selectively killed by intramuscular injection of cholera toxin-conjugated saporin. Simultaneously, some saporin-injected rats were given implants of the androgen receptor antagonist hydroxyflutamide, either directly at the quadriceps musculature or interscapularly as a systemic control. Following saporin injections, some animals were allowed free access to running wheels attached to their home cages. Four weeks later, motoneurons innervating the ipsilateral vastus lateralis muscle were labeled with cholera toxin-conjugated horseradish peroxidase, and dendritic arbors were reconstructed in three dimensions. Compared with untreated males, partial motoneuron depletion resulted in decreased dendritic length in remaining quadriceps motoneurons. Early data suggests that following partial motoneuron depletion, exercised males with androgen receptor blockade at the quadriceps show dendritic lengths that

are significantly shorter than those of exercised males with no treatment, while dendritic lengths in exercised males with interscapular implants do not differ from those of exercised animals without implants. These findings suggest that exercise may be protective against dendritic atrophy via androgens binding at the target musculature.

Disclosures: C. Chew: None. **D.R. Sengelaub:** None.

Poster

134. Neuroprotective Mechanisms: Preclinical Models

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Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

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Title: Catfish (Arius bilineatus, Val.) skin protein preparation improves histopathological and ultrastructural alterations in the sciatic nerve myelinated fibers in STZ-diabetic rats

Authors: *W. M. RENNO[1], H. L. SADEK[1], J. K. KUMAR[1], P. GEORGE[1], J. M. AL-HASSN[2]; [1]Anat., Kuwait Univ. Fac. of Med., Kuwait, Kuwait; [2]Biol. Sci., Kuwait Univ. - Fac. of Sci., Kuwait, Kuwait

Abstract: Our preliminary investigations and clinical trials showed that soluble protein Fraction B (SPF-B) from catfish skin preparations (Arius bilineatus, Val.) exhibits potent anti-inflammatory and healing properties for the healing of resistant diabetic foot ulcers, chronic back pain, and some other neurological disorders. Recently we showed that SPF-B treatment lessens neurobehavioral deficits, enhances axonal regeneration and ameliorates histomorphological alterations following sciatic nerve injury. Further, SPF-B protects spinal neurons and enhances subcellular recovery by decreasing the astrocytic activity and GAP-43, thus improves nerve regeneration and functional recovery. The present study proposal explored the effects of the SPF-B on the sciatic nerves of STZ-induced diabetic neuropathy in the rat model. Morphological analysis of the sciatic nerve of diabetic animals showed a significant decrease in the area of axons, the axon diameters and the average axonal diameters compared to the control group. However, SPF-B treatment of STZ-diabetic rats significantly increased the area of axons, the axon diameter and the average axonal diameters. The axon diameter increase was even higher in SPF-FB-treated STZ-diabetic animals than the control group. Further, the myelin sheath area and myelinated fiber cross-sectional area, which were decreased in diabetic rats compared with control rats, were alleviated by in the SPF-B treated group. Likewise, the average optical density of myelin sheath area and myelinated fiber cross-sectional area were decreased in diabetic rats compared with control rats and these changes were also alleviated by SPF-B administration.

Ultra-structurally, sciatic nerve of diabetic rats showed shrunken axons, myelin destructions with onion-bulb form protrusion on the myelin sheath and the axonal myelin showed vacuolization and lamellar separation. Degenerative changes were also observed in mitochondria and Schwann cells. In contrast, the SPF-B-treated animals showed remarkable recovery of the myelin and axons ultrastructural features and the myelin breakdown decreased markedly. Further, vacuolization and lamellar separation of the axonal myelin were less evident. The fine structure of Schwann cells was seemingly normal. In conclusion, the SPF-B may provide a therapeutic potential for future treatment of diabetic peripheral neuropathy.

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Poster

134. Neuroprotective Mechanisms: Preclinical Models

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Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: SAF2017-84283-R
CB06/05/0024
2014SGR-525

Title: Epigallocatechin-3-gallate protects from the effect of 3-nitropropionic acid, Huntington's disease mice model, modulating the synapsis

Authors: *M. ETTCHETO[1,4,5,6], A. CANO[2,7], O. BUSQUETS[1,8,5,6], C. AULADELL[3,8,5], M. GARCÍA[2,9], J. FOLDH[6,8], A. CAMINS[1,8,5];
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Abstract: The Epigallocatechin-3-gallate (EGCG) is the main polyphenol of the green tea. During the last years, this compound has been widely investigated due to its known multiple health benefits such as cognitive improvement and antioxidant effects, among others. Thus, EGCG has been considered a promising drug for several brain disorders, however, its molecular

target remains unclear. Huntington´s disease is a brain progressive pathology mainly characterized by uncontrolled movements and cognitive decline. Therefore, the aim of this study was to evaluate the effect of EGCG administration in C57Bl6 (wild-type; WT) mice previously treated with 3-Nitropropionic (3-NP) acid, a Huntington´s disease mice model.
3-NP was intraperitoneally (i.p) administered for 5 consecutive days at a dose of 70 mg/kg/day to 1,5-month-old male mice. EGCG was also administered i.p. at a dose of 50 mg/kg/day one hour prior to 3-NP injection. Before being sacrificed, several behavioral tests involved in motor assessment were carried out. Protein levels and mRNA expression of molecules related to memory, apoptosis, unfolded protein response (UPR), among others were analyzed in hippocampus. Moreover, targets involved in inflammatory and synapsis processes were also studied through immunofluorescence experiments.
Our results demonstrated that this compound induced alterations in the motor neuron system which were clearly reverted after EGCG treatment. At molecular level, protein levels involved in synapsis process such as neuroligin, neurexin and debrin showed a significant increase in WT + 3-NP mice treated with EGCG compared to WT+ 3-NP mice. Moreover, the neuroinflammation was decreased after EGCG treatment in comparison to 3-NP treated mice.
In conclusion, our study demonstrates EGCG could confer neuroprotection against the 3-NP effect restoring the synapsis loss, suggesting EGCG as a potential drug for the treatment of Huntington´s disease.

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Poster

134. Neuroprotective Mechanisms: Preclinical Models

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Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NRF Grant 2018R1A6A1A03025221

Title: Vagus nerve stimulation modulates inflammatory responses in the poly-I:C-induced animal model of chronic fatigue syndrome

Authors: ***K. LEE**[1,2], H.-J. AHN[1], B.-G. JO[1], C.-G. SON[1,2], U. NAMGUNG[1,2];
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Abstract: Polyinosinic-polycytidylic acid (poly-I:C), virus-mimicking synthetic double-stranded RNA, is a ligand of toll-like receptor-3 (TLR3) and is able to initiate rapid innate immune responses. Chronic fatigue syndrome (CFS) is characterized by prolonged, extreme disabling

fatigue with unknown specific causes. Also, CFS is induced by impairment of neuro-endocrine-immune interactions including activation or dysfunction of glial cell in central nervous system. It was previously reported that poly-I:C-induced rat model of CFS showed a sustained decrease in spontaneous running wheel activity that is accompanied by enhanced glial activation and expression of interferon-alpha (IFN-α), 5-HTT and IL-1β in the brain. Vagus nerve stimulation (VNS) has been explored in multiple experimental animal models and clinical trial of epileptic seizure, and drug-resistant depression. In the present study, we investigated whether VNS has a useful effects on the poly-I:C-induced animal model of CFS. Four groups of rats (control, VNS only, poly-I:C and poly-I:C with VNS) were challenged with saline or poly-I:C (3mg/kg) and stimulation was given at the right vagus nerve and the prefrontal cortex analyzed by western blotting and immunohistochemistry. Compared to saline control, animal group administered with poly-I:C induced activation of microglia and astroglia in prefrontal cortex, then their activation level was reduced by VNS. Also, we found that the expression level of phospho-Akt in poly-I:C-treated group with VNS was increased compared with poly-I:C group, suggesting the neuro-survival effects by VNS. In addition, VNS attenuated poly-I:C-induced increases in IL-1β production in prefrontal cortex. These findings suggest that VNS has anti-inflammatory effects on neurons in the prefrontal cortex in poly-I:C-induced animal model of CFS.

Disclosures: **K. Lee:** None. **H. Ahn:** None. **B. Jo:** None. **C. Son:** None. **U. Namgung:** None.

Poster

134. Neuroprotective Mechanisms: Preclinical Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 134.17/H19

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: FAPESP
CNPq
CAPES

Title: Impact of environmental enrichment in cognition, behavioral parameters and BDNF levels of rats subjected to the lithium pilocarpine model of epilepsy

Authors: *L. F. ARAÚJO, Sr, J. E. DA SILVA, M. J. FERNANDES;
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Abstract: Introduction: Epilepsy affects more than 50 million people worldwide. Temporal Lobe Epilepsy (TLE) is the most common form of epilepsy in adults, and hippocampal sclerosis is one of the commonest pathologies associated with Mesial TLE. Approximately 30% of patients receiving antiepileptic drugs have inadequate seizure control. Patients with TLE often show cognitive deficits, including changes in executive and working memories, attention,

decision-making, language, planning and judgment. Furthermore, psychiatric comorbidities as anxiety, psychosis and depression are also associated with TLE, dramatically reducing the quality of life of these people. The pilocarpine model may reproduce the main pathophysiological characteristics of the TLE and is very useful to study mechanisms involved with epileptogenesis and changes associated with seizures. It allows us to study alternative therapies to reduce the impact of seizures. Environmental Enrichment (EE) protocols have shown beneficial effects on neuronal plasticity by changing neuronal morphology, neurogenesis and adaptive properties. **Objectives:** In the present study we evaluated the effects of an EE protocol on behavioral changes (latency and frequency of seizures, cognitive process and anxiety) in rats submitted to lesional epilepsy model induced by lithium pilocarpine (LIP) and changes in BDNF levels in the hippocampus. **Methods:** Male Wistar rats PND 21 were exposed to an EE protocol or to a standard environment for 5 weeks. After this period, the animals of both groups (EE and standard) were randomized and injected with lithium pilocarpine or lithium saline solution, and video monitored for 44 days to evaluate the latency and the frequency of seizures. After this period, the animals were subjected to the following behavioral tests: Elevated Plus Maze, Open Field, Rearing and Novel Object Recognition. The ELISA method was used to evaluate the BDNF expression in the hippocampus. **Results:** The EE decreased hyperactivity, preserved short-term memory and increased the latency to first seizure in rats from LIP group compared to rats from LIP group raised in standard conditions. However, no differences in the anxiety behavior or seizure frequency were observed. The BDNF level was higher in animals exposed to an enriched environment subjected to the LIP model when compared to rats raised in standard conditions. **Conclusions:** Our data show that EE can improve memory and increase BDNF expression in the hippocampus of rats. These data indicate that BDNF may be a mechanism involved with memory improvement observed in LIP model. EE can be a safe and effective strategy to improve neuroplasticity following seizures.

Disclosures: **L.F. Araújo:** A. Employment/Salary (full or part-time):; Sao Paulo Federal University. **J.E. da Silva:** None. **M.J. Fernandes:** None.

Poster

134. Neuroprotective Mechanisms: Preclinical Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 134.18/H20

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH NINDS NS-019108
NCATS UL1 TR000430
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The George Schultz Innovation Fund from the Polsky Center for Entrepreneurship and Innovation at the University of Chicago

Title: CNS biodistribution of nasally administered IFNγ stimulated exosomes

Authors: *A. D. PUSIC, K. M. PUSIC, R. P. KRAIG;
Dept. of Neurol., Univ. of Chicago, Chicago, IL

Abstract: Exosomes from IFNγ-stimulated dendritic cells (IFNγ DC-Exos) contain miRNAs which promote myelination, reduce oxidative stress (OS), and preferentially enter oligodendrocytes in brain slice cultures. Nasally administered IFNγ DC-Exos improve remyelination following lysolecithin-induced demyelination (as a model of multiple sclerosis) and inhibit spreading depression (SD). SD is the likely cause of migraine with aura, and a well-established model of migraine that triggers increased OS and transient demyelination. While we can infer that exosomes entered the CNS through measurement of these functional effects, we have not yet directly tracked exosomes through imaging studies. Determining the route of entry and distribution over time of intranasally administered exosomes will be an important step toward therapeutic development. Exosomes were labelled with Xenolight DiR, a lipophilic near infrared dye that stably stains cytoplasmic membranes with negligible dye transfer between cells. Xenolight DiR-Exos were nasally administered and animals imaged at multiple time points in an IVIS Spectrum 2000 imaging system. We detected fluorescence in the rostral portion of the brain, but had concerns about whether fluorescent signal from the exosomes could penetrate through a rat's skull and brain. To circumvent this, we performed *ex vivo* imaging of excised brain (therapeutic target), liver and spleen (clearance/toxicity), and lung (for error in nasal delivery). Within 30 minutes of nasal administration, exosomes could be detected throughout the brain, and faint signal could even be detected in the cervical spinal cord. We observed little to no signal in control animals nasally administered PBS or unlabeled exosomes. We next examined whether IFNγ DC-Exos enter oligodendrocytes *in vivo* as they do *in vitro*. Exosomes were transfected with mCherry mRNA prior to nasal administration. To maximize mCherry expression, animals were nasally administered two doses of transfected exosomes twelve hours apart. Brains and spinal cords were harvested six hours after the final dose for immunohistological verification of mCherry expression. mCherry positive cells were found in coronal sections taken along the rostrocaudal axis as far caudal as the brainstem. These cells morphologically resembled oligodendrocytes, and indeed a subset co-stained for pre-oligodendrocyte/oligodendrocyte markers. Our results provide evidence that IFNγ DC-Exos enter the brain, distribute throughout the parenchyma, and functionally deliver their contents. This data further supports use of IFNγ DC-Exos as a novel therapeutic for neurodegenerative disorders.

Disclosures: **A.D. Pusic:** None. **K.M. Pusic:** None. **R.P. Kraig:** None.

Poster

134. Neuroprotective Mechanisms: Preclinical Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 134.19/H21

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Exercise-induced hemoglobin expression in rat CNS

Authors: A. E. WEAVER[1], S. FREEMAN[2], K. ALKHAYER[2], N. K. SINGHAL[3], J. MCDONOUGH[1], *E. J. FREEMAN[2];
[1]Biomed. Sci., [3]Dept. of Biol. Sci. and Sch. of Biomed. Sci., [2]Kent State Univ., Kent, OH

Abstract: Growing evidence suggests that exercise can improve outcomes in multiple neurodegenerative disorders through stimulation of neurotrophic factors, anti-inflammatory effects and angiogenesis. In addition, we suggest neuronal expression of Hbb may also play an important role in the beneficial effects of exercise on brain function. The presence of hemoglobin mRNA and protein within human and rat neurons in the central nervous system (CNS) has been reported but regulation of its expression and its function in cortical neurons is still unclear. We have previously reported that the beta subunit of hemoglobin (Hbb) is expressed in cortical neurons and enriched in pyramidal neurons of deeper cortical layers. Further, we have observed that Hbb levels are significantly reduced in nuclear fractions isolated from postmortem multiple sclerosis (MS) cortex compared to controls and reduced Hbb is associated with dysregulation of mitochondria and impaired energetics in cortical neurons. Therefore, we suggest a link between Hbb expression and mitochondrial function in neurons. In this study, we examined the impact of exercise on Hbb expression in rat brain. Using 5-10 week old Sprague Dawley rats (n=5/group) in a 5 week voluntary wheel running protocol we observed an increase in neuronal Hbb expression in running animals compared to sedentary using both western blot and confocal microscopy. This was correlated with an increase in the trimethylation of histone H3 on lysine 4 (H3K4me3), an epigenetic mark we have previously reported to regulate genes involved in mitochondrial respiration. Exercise was also associated with decreases in several inflammatory markers, including NOS2, TNFa inducible protein 2 and ceramides when compared to sedentary controls. Thus, exercise may provide a mechanism for improved CNS protection in the face of inflammatory neurodegenerative disease states through increased Hbb expression and reduced inflammation.

Disclosures: **A.E. Weaver:** None. **S. Freeman:** None. **K. Alkhayer:** None. **N.K. Singhal:** None. **J. McDonough:** None. **E.J. Freeman:** None.

Poster

134. Neuroprotective Mechanisms: Preclinical Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 134.20/H22

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: Internal WVU Collaboration Grant

Title: Constitutively active proteasome expression in *C. elegans* improves lifespan and resistance to oxidative stress

Authors: *R. T. ANDERSON, D. M. SMITH;
Biochem., West Virginia Univ. Sch. of Med., Morgantown, WV

Abstract: Virtually all neurodegenerative diseases are characterized by the accumulation of proteins that are thought to play a significant role in disease pathogenesis. One of the cell's primary systems for the removal and degradation of misfolded or damaged proteins is the Ubiquitin Proteasome System (UPS). However, numerous studies have shown significant impairment of the UPS in essentially all of these neurodegenerative diseases. We have shown previously that oligomeric forms of the proteins involved these diseases directly interact with the proteasome and drastically hinder its function. Furthermore, we have shown that these oligomers do not inhibit a constitutively active proteasome. With this in mind, we contacted Nemametrix to build the first ever animal model expressing a constitutively active proteasome in *Caenorhabditis elegans* (*C. elegans*) using CRISPR technology. We have shown that the nematodes expressing this mutant proteasome have an increased lifespan and show resistance to oxidative stress. Also, we have crossed this constitutively active nematode with one expressing tau, which is a protein involved in Alzheimer's disease, and we are in the process of collecting data from these animals. We hypothesize that crossing this mutant nematode with neurodegenerative models of nematodes will help ameliorate some of the neurodegenerative phenotypes in these models.

Disclosures: R.T. Anderson: None. **D.M. Smith:** None.

Poster

134. Neuroprotective Mechanisms: Preclinical Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 134.21/H23

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: Illinois Society for the Prevention of Blindness (AKG)
American Society for Pharmacology and Experimental Therapeutics Summer Undergraduate Research Fellowship(KAO)
Richard A. Peritt M.D. Charitable Foundation(SK)
Dr. John P. and Therese E. Mulcahy Endowed Professorship in Ophthalmology (SK)
Experimentica Ltd.

Title: Cell-type specific cytoprotection by xanthohumol

Authors: *H. N. HARIANI[1], A. K. GHOSH[1,2,3,4], S. ANKIREDDY[2,3], K. A. ORLOFF[2], J. J. HAKKARAINEN[5], S. KAJA[1,2,3,4];
[1]Grad. Program in Neurosci., [2]Dept. of Mol. Pharmacol. and Therapeut., [3]Dept. of Ophthalmology, Loyola Univ. Chicago, Maywood, IL; [4]Res. Service, Edward Hines Jr. VA Hosp., Hines, IL; [5]Res. and Develop. division, Experimentica Ltd., Kuopio, Finland

Abstract: Xanthohumol (Xn; 2',4',4-trihydroxy-6'-methoxy-3'-prenylchalcone) is a natural polyphenol chalcone present in *Humulus lupulus* (hops). Xn has gained attention for its broad pharmacologic activity, which includes direct reactive oxygen species (ROS) scavenging and activation of the antioxidant response element due to modulation of the transcription factor Nuclear factor (erythroid-derived 2)-like 2 (*Nrf2*). Given the pathological role of elevated cellular levels of oxidative stress in age-related ophthalmic conditions, we here quantified Xn-mediated cytoprotection against oxidative stress in three ocular cell types, corneal epithelial cells, optic nerve head astrocytes, and photoreceptor cells. Human corneal epithelial cells (HCE-T; Riken; Japan), primary rat optic nerve head astrocytes (ONHAs) and mouse 661W photoreceptor cells (generously provided by Dr. Muayyad Al-Ubaidi) were maintained as previously described. Cells were seeded in 96-well plates and exposed to increasing concentrations (1 nM-100 μM) of Xn to determine possible cytotoxicity. In a different set of experiments, cells were pre-treated with Xn (0.1-5 μM) and subsequently exposed to chemically-induced oxidative stress using *tert*-butyl hydroperoxide (tBHP) for 6 hr. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) uptake and lactate dehydrogenase (LDH) release assays for cell viability and proliferation were performed to determine the cytoprotective activity of Xn. Intracellular signaling pathways were quantified by immunoblotting. Xn did not exert cytotoxicity at concentrations below 50 μM in any of these three cell types. In HCE-T cells, Xn resulted in a statistically significant dose-dependent protection against oxidative stress. At 5 μM Xn, the IC_{50} for tBHP in the MTT assay shifted from 15.2±0.6 μM to 33.3±3.4 μM (P<0.001, n = 6). Similarly, Xn protected both resting and activated ONHA against tBHP-induced oxidative stress by shifting the IC_{50} by -22.1±3.9 μM (n=4-5, P < 0.001) and -14.6±3.9 μM (n=4-5, P<0.001), respectively. Intriguingly, Xn did not show any cytoprotection in 661W cells (P>0.05; n=4). Data from the LDH assay confirmed the results of the MTT assay. Analysis of phase II antioxidant enzymes revealed statistically significant differences in the temporal expression profiles and between cell types. In conclusion, Xn exerted potent cytoprotection in corneal epithelial cells and optic nerve head astrocytes, while no antioxidant activity was observed in

photoreceptor cells. Current studies are investigating the cytoprotective and glioprotective potential of Xn in *in vivo* models for dry-eye disease and optic neuropathy.

Disclosures: H.N. Hariani: None. **A.K. Ghosh:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Experimentica Ltd, K&P Scientific LLC, eyeNOS Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); eyeNOS Inc.. F. Consulting Fees (e.g., advisory boards); K&P Scientific LLC. **S. Ankireddy:** None. **K.A. Orloff:** None. **J.J. Hakkarainen:** A. Employment/Salary (full or part-time):; Experimentica Ltd.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Experimentica Ltd. **S. Kaja:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Experimentica Ltd, K&P Scientific LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Experimentica Ltd, K&P Scientific LLC.

Poster

134. Neuroprotective Mechanisms: Preclinical Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 134.22/H24

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: CEU/Banco de Santander MPC30/18

Title: Cellular characterization of the potential food-addiction biomarker clusterin

Authors: C. RODRIGUEZ-RIVERA, ***C. GONZALEZ-MARTIN**, M. D. PÉREZ-CARRIÓN, C. ANSEEUW, L. F. ALGUACIL, M. J. POLANCO;
Univ. CEU San Pablo, Madrid, Spain

Abstract: Overnourishment, specially with high fat diets, has been related to changes in feeding habits and motivation towards reinforcers. On the other hand, undernourishment during perinatal periods has also been described to increase vulnerability to develop addictions. We have recently communicated that clusterin, a protein related to feeding control, is differently expressed in the nucleus accumbens of mice fed on high fat diets showing increased motivation for sucrose, as well as in the nucleus accumbens of undernourished rats. Moreover, clusterin levels are increased in the plasma of patients with obesity that exhibit poor control over eating (Rodríguez-Rivera et al., World J Surg 2019, 43: 744-759). All these findings strongly suggest an involvement of clusterin in food addiction. To better understand the cellular mechanisms that could underlie all these observations, we have mimicked over- and under-nourishment conditions in SH-SY5Y cells *in vitro* by modifying glucose and protein concentrations in cell media during

different periods of time. The effects of these incubations on clusterin gene and protein expression (qPCR, Western Blotting and ELISA), cell morphology, proliferation and metabolism (MTT) and mitochondrial functionality (Mitotracker) were analysed. We observed that neuronal changes produced by different nutritional status are associated to a different expression and location of clusterin within the cell to preserve cell survival in an adaptive and non-permanent way, suggesting a crosstalk between clusterin and nutrition-related neuronal changes neuroblastoma cells. Bearing in mind that the dopaminergic phenotype of SH-SY5Y cells is similar to that of neurons in the ventral tegmental area, it is possible that nutritional changes like those mimicked here could be relevant to food addiction and involve changes of clusterin function potentially neuroprotective.

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Poster

134. Neuroprotective Mechanisms: Preclinical Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 134.23/H25

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NRT grant IGE1747486

Title: Effect of caffeine administration timing, sex, and prenatal condition on cognitive outcomes in premature infants

Authors: *R. M. MCLEOD[1], T. S. ROSENKRANTZ[2], R. FITCH[1];
[1]Psychology - Behavioral Neurosci., Univ. of Conneticut, Storrs, CT; [2]Dept. of Pediatrics/Neonatology, Univ. of Connecticut Sch. of Med., Farmington, CT

Abstract: Caffeine and other methylxanthines are commonly used drugs in the care and treatment of premature infants. Caffeine and theophylline are used to treat apnea of prematurity, a common problem in premature infants that results from weakness in muscles used for respiration, as well as immaturity of neural respiratory mechanisms. Recent evidence suggests that caffeine may also act as a neuroprotectant against hypoxic-ischemic brain injury. Hypoxia-ischemia (HI), or the loss of blood supply and/or oxygenation, is very common in the preterm brain following episodes caused by cardiopulmonary immaturity and failure. These episodes can include interventricular hemorrhage, ischemic insult, reperfusion tissue damage, and/or intermittent tissue hypoxia. All of these events lead to neuronal death. If caffeine can protect against these injuries, then evaluation of optimal timing and dosage is needed to insure efficacy. Retrospective human studies have shown that early caffeine administration (within 72 hrs. of birth) -- but not later -- leads to better cognitive outcomes at 18 months and 24 months. This

finding could greatly impact how infants are treated at birth, and may mean that caffeine should be given as soon as possible, similarly to requirements for cooling protocols for term infants with HI. Importantly, none of these studies take into account other factors that could influence susceptibility to HI, and associated cognitive and motor outcomes. These other factors include prenatal events that may make preterm infants more susceptible to injury, such as preeclampsia, chorioamnionitis, administration of magnesium sulfate, and fetal intolerance to labor. Moreover, it is well documented that female infants tend to do better and have less severe impairments due to ischemia-hypoxia than males under the same condition, meaning that sex may further affect the efficacy of caffeine treatment. For the current study, medical record data was taken for infants born 23-30 weeks gestational age between the years of 1991 and 2017 who were treated with caffeine or theophylline. Infants who received treatment within the first 48 hours of life were compared to infants who received methylxanthines after 48 hours, using subsequent cognitive outcomes obtained in follow-up assessments as an index of efficacy. Infants were further split into groups by sex and prenatal conditions for comparison. We predicted that after controlling for co-morbid conditions, infants who received earlier treatment with caffeine would have better cognitive outcomes. We also predicted that within the early and late caffeine-treated groups, females would show better cognitive outcomes than males.

Disclosures: R.M. McLeod: None. **T.S. Rosenkrantz:** None. **R. Fitch:** None.

Poster

134. Neuroprotective Mechanisms: Preclinical Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 134.24/H26

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Retinal ganglion cell axon regeneration is regulated by presynaptic circuits

Authors: *P. R. WILLIAMS;
Ophthalmology and Visual Sci., Washington Univ., Saint Louis, MO

Abstract: Axon damage following CNS injury or neurodegenerative disease leads to a non-recoverable loss of communication among long-distance neural circuits. It was long thought that the inhibitory environment of the post-lesion CNS was a main contributing factor to this failed regenerative response. However, injury to adult neurons also leads to a number of homeostatic changes that prevent regeneration. Roughly a decade ago, it was discovered that intrinsic manipulations could allow injured retinal ganlgion cells (RGCs) to regenerate after optic nerve injury. We have recently discovered, that not only are RGCs negatively impacted by their own axotomy, but they also communicate their injury to their local environment in the retina with a detrimental outcome. An important result of this local injury signal is the hyperactivation of amacrine cells, the sole presynaptic inhibitory inputs onto RGCs. We found that globally

blocking amacrine cell activity relieved RGCs of this suppressing input and allowed them to respond to growth factors by mounting a regenerative response. Our preliminary results suggest that this effect could be limited to a subset of RGCs, and therefore may be resultant to the activity of only a handful of amacrine cell types. As such, we are currently attempting to dissect which amacrine cells become hyperactivated by RGC axotomy, and how they are signaled to do so by injured RGCs. Ultimately, we wish to examine if there is any circuit level logic that may be expanded out to other systems, thus allowing for circuit manipulation as a strategy for axon regeneration after trauma throughout the CNS.

Disclosures: P.R. Williams: None.

Poster

134. Neuroprotective Mechanisms: Preclinical Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 134.25/H27

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: Bright Focus Foundation/G2018168
NIH EY10343
NIH R56 DE023806
LASURI - UIC

Title: Extracellular vesicles as novel targeted therapy for retinal damage and repair in MS

Authors: S. TRAN[1], L. G. ACHA[1], M. NAYYAR[1], L. A. TORRES[1], S. KALININ[1], C.-C. HUANG[1], D. FEINSTEIN[1], S. RAVINDRAN[1], S. ROTH[2], B. MATHEW[2];
[2]Anesthesiol., [1]Univ. of Illinois at Chicago, IL, Chicago, IL

Abstract: Optic Neuritis, characterized by optic nerve fiber and retinal ganglion cell degeneration, is responsible for impaired, and often irreversible, vision loss in about 20% of MS patients. Both MS and its animal model experimental autoimmune encephalomyelitis (EAE), display characteristic neuronal loss including RGC death and apoptosis in retina. Current treatment therapies focus only on minimizing MS disease symptoms and preventing disease relapse. However, none of these offer a cure for RGC and axonal loss. It is therefore imperative that the treatment focus be shifted to developing neuroprotection, stimulating axonal growth, and regeneration of RGCs and axons.
Stem cell-based RGC replacement is an encouraging approach. Recently it has been shown that stem cells release extracellular vesicles (EVs), nanoparticles that facilitate cell-to-cell communication. MSCs-EVs decrease neuronal cell death after hypoxia/ischemia *in vitro* and *in vivo*, stimulate axonal growth, and attenuate inflammation and oxidative stress. They can be administered cross-species, do not proliferate, and their stability, biocompatibility, biological

barrier permeability, and low toxicity make them attractive therapeutic delivery vehicles. EVs are taken up by cells; unlike stem cells, which rely upon integration into tissues, or diffusion of secreted contents to the cells, EVs deliver their cargo directly. Our previous research using *in vitro* and *in vivo* retinal ischemia models, demonstrated sustained EV uptake into retinal neurons and microglia and amelioration of functional impairment of retina, reduction in RGC loss and apoptosis after prolonged acute retinal damage. Here we aimed to modify EVs to specifically target RGCs. EVs isolated, labelled and modified using carrier peptide to enable RGC targeting. Endocytosis of modified EVs (EEVs) were compared to normal EVs using mixed primary retinal neurons. To compare the functional impact of EEVs and EVs on RGCs in a cell death model, we subjected cells to oxygen-glucose deprivation (OGD) for 24 h, following 24 pre-incubation with EVs, EEVs, CM-EVs, or peptide alone. Cell death quantified using LDH and apoptosis assays. Immuno-staining of retinal cells using neuronal markers performed to compare relative uptake of EEVs by different cell types. Our data demonstrates remarkably enhanced neuroprotection and uptake by retinal neurons when treated with EEVs.

Disclosures: S. Tran: None. **L.G. Acha:** None. **M. Nayyar:** None. **L.A. Torres:** None. **S. Kalinin:** None. **C. Huang:** None. **D. Feinstein:** None. **S. Ravindran:** None. **S. Roth:** None. **B. Mathew:** None.

Poster

134. Neuroprotective Mechanisms: Preclinical Models

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 134.26/H28

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: SM07883, a novel, oral DYRK1A inhibitor, improves cognition and protects against amyloid and tau pathologies in the 3xTg-AD mouse model of Alzheimer's disease

Authors: *K. X. DUONG-POLK[1], B. GUNER[1], S. HABROUN[1], S. ANDERSON[2], C. LAI[3], B. MELCHIOR[3];
[1]SAMUMED, LLC, San Diego, CA; [2]Samumed, San Diego, CA; [3]Samumed, LLC, San Diego, CA

Abstract: Dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A) regulates Amyloid Precursor protein (APP) and tau phosphorylation. DYRK1A is overexpressed in Alzheimer’s disease (AD) brains and correlates with disease pathology. We hypothesized that DYRK1A inhibition may reduce AD pathology. The effectiveness of SM07883, a small-molecule DYRK1A inhibitor currently being tested in a clinical trial, was assessed on amyloid, tau, and neuroinflammation pathology together with cognition in two independent studies using the 3xTg-AD mouse model.
To test the effect of SM07883 on amyloid processing *in vitro*, a serial dilution was tested on SH-

SY5Y cells and APP phosphorylation at Threonine 668 was measured by Western blot. SH-SY5Y cells overexpressing wild type human APP were treated with SM07883 and Aβ40 secretion was measured by MSD. For *in vivo* assessment of amyloid and tau pathology, 10- and 12-month-old female 3xTg (APP/PSEN/Tau P301L) mice were orally administered SM07883 (QD, 5mg/kg) or vehicle for 6 months. Mice were evaluated for cognitive behavior in the Novel Object Recognition (NOR) and Y-maze spontaneous alternation tests. At termination (6 months), left hemispheres were stained for amyloid burden (6E10, thioflavin), astrocytes activation (GFAP), activated microglia (Iba1), and tau pathology (AT8) compared to vehicle. Hippocampal and surrounding cortical areas were analyzed for tau and APP phosphorylation by Western blot while amyloid, tau fragments, and proinflammatory mediators were analyzed by immunoassays. *In vitro* data showed that SM07883 reduced APP phosphorylation and Aβ40 production in cells compared to DMSO controls with EC_{50} of 187 nM and 798 nM, respectively. Transgenic 3xTg mice treated with vehicle had elevated tau, APP phosphorylation, gliosis, and activated microglia in the hippocampal area compared to age-matched wild type littermates ($p<0.05$). All SM07883 results showed significance ($p<0.05$) when compared to vehicle at termination. A reduction of elevated tau and APP phosphorylation as well as amyloid fragments were reduced in lysates. Immunostaining showed amyloid and tau pathology reduction in the hippocampal area while GFAP and Iba-1 showed reduced gliosis, which was associated with a reduction in proinflammatory cytokines. SM07883-treated mice performed better in NOR and SM07883 prevented cognitive deficit in the Y-maze.
In two independent studies in triple-transgenic mice, daily oral administration of SM07883, a DYRK1A inhibitor, showed reduction of pathological AD hallmarks (tau and amyloid), associated neuroinflammation, and protected against cognitive deficits compared to vehicle.

Disclosures: **K.X. Duong-Polk:** A. Employment/Salary (full or part-time):; Samumed, LLC. **B. Guner:** A. Employment/Salary (full or part-time):; Samumed, LLC. **S. Habroun:** A. Employment/Salary (full or part-time):; Samumed, LLC. **S. Anderson:** A. Employment/Salary (full or part-time):; Samumed, LLC. **C. Lai:** A. Employment/Salary (full or part-time):; Samumed, LLC. **B. Melchior:** A. Employment/Salary (full or part-time):; Samumed, LLC.

Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.01/H29

Topic: C.09.Stroke

Support: NSFC Grant 81671229
NSFC Grant 81871032
NIH Grant NS085019

Title: Alpha1-chimaerin limits dendritic spines, synapses and functional recovery after stroke

Authors: *S. LI[1,2], H. LIANG[3], M. T. JOY[4], S. P. BRIDGES[2], J. LUO[5], M. MACHNICKI[2], H. YAO[1], A. JIMENEZ[2], R. GHANIAN[2], A. BRUMM[2], H. ZHANG[6], J. XIAO[6], G. LIANG[6], L. LIN[6], S. CARMICHAEL[2], X. LI[7];
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Abstract: Ischemic stroke induces formation of axonal sprouting in peri-infarct cortex, which is casually associated with behavioral recovery in this disease. We previously reported that α1-chimaerin (α1CHN), a Rho GTPase-activating protein, reduced peri-infarct cortical connections of stroke mice. Nevertheless, it remains unknown if α1CHN affects dendritic spines and synapses in these areas, and behaviors after stroke. We therefore first specifically quantified dendrites and dendritic spines of the lentiviral infected neurons in peri-infarct cortex 3-week after transfection of α1CHN-GFP or control-GFP lentiviral vectors. α1CHN induction resulted in a significant reduction of dendritic spine density as well as spine total numbers in peri-infarct motor cortex. Spine classification analysis indicated that filopodia and stubby spines were statistically decreased in lentiviral α1CHN-treated mice post-stroke. We then measured co-localized synaptic numbers in peri-infarct cortical tissues. There was a statistically significant decrease in synapses in α1CHN-transfected mice compared to control lentivirus after stroke or stroke alone. Knockdown of α1CHN increased synapses in peri-infarct cortex following stroke. To address the functional significance of α1CHN in behavioral recovery after stroke, we examined mice on forelimb motor tasks using the same forelimb motor cortex stroke in which axonal sprouting was previously quantified. Stroke produced an impairment in forelimb motor control as seen in deterioration in performance, with spontaneous recovery towards baseline at 45-day or longer. This recovery was blocked in the α1CHN overexpression group in both pasta handling task and cylinder task. On the contrary, blockade of αCHN expression in the peri-infarct temporally enhances functional recovery after stroke. In gird walking performance, however, no statistical significance at any time point was seen after the treatment with lentiviral α1CHN-GFP or αCHN siRNA. This data identifies functional roles of α1CHN distinct from those of ATRX and GDF10 in behavioral recovery after stroke and suggests alternative therapeutic strategies in the development of neural repair drugs in this disease.

Disclosures: S. Li: None. **H. Liang:** None. **M.T. Joy:** None. **S.P. Bridges:** None. **J. Luo:** None. **M. Machnicki:** None. **H. Yao:** None. **A. Jimenez:** None. **R. Ghanian:** None. **A. Brumm:** None. **H. Zhang:** None. **J. Xiao:** None. **G. Liang:** None. **L. Lin:** None. **S. Carmichael:** None. **X. Li:** None.

Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.02/H30

Topic: C.09.Stroke

Support: NSFC 81571277

Title: The study of VBM-based gray matter remodeling of tele-rehabilitation therapy on promoting motor function recovery in stroke patients

Authors: *C. REN[1], J. CHEN[2], S. ZHANG[2];
[1]Shanghai East Hospital,Tongji Univ., Shanghai, China; [2]Neurol., Shanghai No. 5 Hosp., Shanghai, China

Abstract: OBJECTS: to study the potential neurorehabilitation mechanism of tele-rehabilitation therapy by studing the volume change of whole brain gray matter using fMRI in stroke patients. **METHODS:** 44 patients with motor dysfunction were selected and divided into TR and CR. Fugl-Meyer motor function scale (FMA) was evaluated before and after rehabilitation intervention. Phillip 3.0T MRI was used to perform three-dimensional T1WI scans. Voxel-based morphometry (VBM) was used to measure the T1 structural images. Inter-group comparisons of relative gray matter volume (RGMV) of the whole brain were measured in three groups before and after rehabilitation and intra-group comparisons of brain RGMV before and after rehabilitation in TR and CR groups. **RESULTS:** There were no significant differences in FMA score between TR group and CR group before and after rehabilitation intervention ($P > 0.05$); after 12 weeks of rehabilitation intervention, FMA both in TR group and CR group increased significantly ($P < 0.05$). Before rehabilitation intervention, RGMV in anterior cingulate gyrus and corpus callosum in TR group decreased significantly, while RGMV in anterior cerebellum, left and right middle occipital gyrus and left suboccipital gyrus increased significantly ($P < 0.05$). There was no significant difference in whole brain RGMV between TR group and CR group ($P > 0.05$). After rehabilitation intervention, RGMV in left pons was significantly decreased and RGMV in left upper frontal lobe was significantly increased in TR group ($P < 0.05$). There was no significant difference in whole brain RGMV between TR group and CR group ($P > 0.05$). In TR group, RGMV in left and right occipital middle gyrus decreased significantly at T2, RGMV in right occipital middle gyrus, inferior gyrus, right central anterior gyrus and right temporal superior and inferior gyrus increased significantly than at T1 ($P < 0.001$). Correlation analysis showed that RGMV changes in right precentral gyrus were positively correlated with FMA scores change before and after rehabilitation intervention in TR group ($r = 0.423$). **CONCLUSIONS:** There is a specific spatial distribution pattern of brain structure of gray matter changes in patients after stroke, brain atrophy involves the cerebral

cortex and subcortical structure; after tele-rehabilitation and conventional rehabilitation treatment, the spatial distribution pattern of brain structure of gray matter changes. The neurological mechanism of gray matter structure reorganization induced by tele-rehabilitation therapy is different from that induced by conventional rehabilitation therapy.

Disclosures: **C. Ren:** None. **J. Chen:** None. **S. Zhang:** None.

Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.03/H31

Topic: C.09.Stroke

Support: NIH 5K02NS093014
VA 1I01RX001640
NRF 2018R1A6A3A03013031

Title: Changes in the coupling of slow-waves with spindles tracks motor recovery after stroke

Authors: ***J. K. KIM**[1,2], A. HISHINUMA[1,2], L. GUO[1,2], S.-J. WON[2], K. GANGULY[1,2]; [1]Dept. of Neurol., Univ. of California San Francisco, San Francisco, CA; [2]Neurol. and Rehabil. Service, San Francisco Veterans Affairs Med. Ctr., San Francisco, CA

Abstract: There is growing evidence that sleep can promote motor recovery after stroke, but little is known about the underlying mechanisms. Importantly, the precise temporal coupling of spindles (10-15 Hz) to slow-waves activity (SW; <1 Hz) during non-rapid-eye-movement (NREM) sleep has been proposed to support memory consolidation in healthy cortical areas (e.g., prefrontal cortex and primary motor cortex). Here we tested the hypothesis that such sleep dynamics are important for recovery after stroke. Here, we show that the temporal coupling of spindles to SW tracks motor recovery after stroke and can be a target for modulation of recovery. In rats, the temporal coupling of spindles to SW, measured in the local field potential of the perilesional cortex after a lesion to the primary motor cortex, was increased over rehabilitation days after stroke; such restoration of sleep dynamics was closely related to the recovery of motor performance. In addition, motor deficits involving arm and hand movements were more severe in rats with a large stroke area compared to rats with a small stroke area, and the quality of temporal coupling of spindles to SW predicted the two degrees of stroke. Interestingly, the duration of sleep after rehabilitation training was closely correlated with motor task offline gains. Taken together, our results suggest that sleep, in general, and the restoration of the precise temporal coupling of spindles to SW, in specific, are important for recovery of upper-limb function after stroke.

Disclosures: **J.K. Kim:** None. **A. Hishinuma:** None. **L. Guo:** None. **S. Won:** None. **K. Ganguly:** None.

Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.04/H32

Topic: C.09.Stroke

Title: Sex differences in cerebral autoregulation dynamics following ischemic stroke

Authors: *A. P. BLABER[1], R. RUEDL[1], K. TAVAKOLIAN[2], F. FAZEKAS[3], N. GOSWAMI[4]; [1]Biomed. Physiol. and Kinesiology, Simon Fraser Univ., Burnaby, BC, Canada; [2]Electrical Engin., Univ. of North Dakota, Grand Forks, ND; [3]Dept. of Neurol., [4]Inst. of Physiol., Med. Univ. of Graz, Graz, Austria

Abstract: Introduction: Following rehabilitation, ambulatory stroke patients are known to have increased incidents of falls and injuries, as well as higher risks in reoccurrence of stroke. We hypothesized that damage to cerebral arterioles and capillaries after stroke would result in dysfunctions of cerebral autoregulation. **Methods**: This study was conducted in the Department of Neurology at the Landeskrankenhaus with Medical University Graz, Graz, Austria. Only patients (5 female, 7 male) with transient or mild stroke syndromes (Modified Rankin scale < 3) within six months of measurement, and who were without other neurological disorders such as severe disabilities or intracranial stenosis, were included. Age/sex-matched controls (10 female, 10 male) without any history of stroke and fulfilling the inclusion criteria were also recruited. Following at least 5 minutes seated, at least 5 minutes of standing beat-by-beat cerebral blood flow velocity of the middle cerebral artery (MFV) and mean arterial blood pressure adjusted to brain level (MAP_{brain}) were simultaneously collected. An index of cerebrovascular resistance (CVRi) was calculated as MAP_{brain}/MFV. Wavelet transform coherence analysis and convergent cross mapping causality methods were used to extract indices characterizing the cerebral autoregulation interaction time (*fraction of time active, FTA*), response gain (*gain*), and control directionality (*causality*) between MAP_{brain} and MFV. Two-way ANOVA across sex and condition (stroke/control) were performed on these data. **Results**: Female stroke patients had higher CVRi than male stroke patients and controls (p=0.032). No differences were seen in the autoregulation transfer gain between females and males or between patients and controls; however, FTA was decreased in stroke patients compared to controls (p=0.023) with FTA significantly decreased in females compared to males (p=0.044). Finally, when a simple regression of patient data with elapsed time from stroke was conducted, a significant linear decrease in autoregulation causality (MAP_{brain} --> MFV) was observed. Further analysis revealed a greater rate of reduction in female compared to male patients. **Conclusions**: These data

provided new insight into cerebrovascular autoregulation post-stroke. Along with increased cerebrovascular resistance, we were able to show that while women showed a stronger impairment after ischemic stroke, they had a faster recovery compared to men.

Disclosures: A.P. Blaber: None. **R. Ruedl:** None. **K. Tavakolian:** None. **N. Goswami:** None. **F. Fazekas:** None.

Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.05/H33

Topic: C.09.Stroke

Title: Mouse headfixed reaching for water after stroke provides a high throughput system for assessing motor circuit level plasticity

Authors: *M. BALBI, D. XIAO, J. BOYD, T. MURPHY;
Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Motor deficits are the most common and visible symptom after stroke leading to a diminished quality of life. Unfortunately, many paradigms for stroke assessment in mice do not capture long term deficits or cannot be scaled for high-throughput analysis. Here we present a behavioral assessment of stroke that incorporates headfixed in vivo imaging of mesoscopic cortical activity during motor task performance in GCAMP6 mice. Mice were cued by sensory stimuli and learned to reach for water droplets, extending work by Galinanes et al. 2018[(1)]. Following successful training on the reaching task and awake photothrombotic stroke induction in the target area between sensory and motor cortex, stereotactic coordinates (1.5; 0.5) mm from bregma, there was a significant reduction in behavioral performance scores with impaired kinematics that were pronounced for at least 3 days and recovered to varying degrees over the period following stroke. Mapping of the sensory cortex before and during stroke recovery via whisker stimulation revealed cortical reorganization. Laser speckle imaging and neurological deficit scoring were carried out in parallel to the task in order to validate changes in cerebral blood flow and assess motor function across a wide range of motor programs, respectively.
(1) Galinanes et al. 2018, Cell Report.

Disclosures: M. Balbi: None. **D. Xiao:** None. **J. Boyd:** None. **T. Murphy:** None.

Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.06/H34

Topic: C.09.Stroke

Support: NIH 1R01NS104117 (NGB and LB)

Title: Mesencephalic astrocyte-derived neurotrophic factor and triggering receptor expressed on myeloid cells-2 are modulated by docosahexaenoic acid after ischemic stroke

Authors: *N. G. BAZAN[1], S. HONG[2], R. S. FERITAS[1], H. MENGHANI[1], S. MARCELL[1], P. MUKHERJEE[1], L. KHOUTOROVA[1], L. BELAYEV[1];
[1]Louisiana State Univ. Hlth. Sci. Ctr., New Orleans, LA; [2]Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX

Abstract: Mesencephalic astrocyte-derived neurotrophic factor (MANF) has been identified as a secretion protein belonging to a novel, evolutionally conserved family of neurotrophic factors that promote tissue repair after various injuries to neurons *in vivo* or *in vit*ro. The microglia cell surface receptor (triggering receptor expressed on myeloid cells-2; TREM2) is expressed on microglia cells and significantly regulated the production of pro- and anti-inflammatory mediators after ischemic stroke. We aimed to examine the profile of MANF and TREM2 expression induced by middle cerebral artery occlusion (MCAo) and investigated if docosahexaenoic acid (DHA) treatment affects MANF and TREM2 expression and provides additional neuroprotection. Male Sprague-Dawley rats were subjected to 2 h of MCAo. DHA or vehicle was administered IV at 3 h after the onset of MCAo. Behavior was evaluated on days 1, 3, 7, and 14; MANF and TREM2 expression were measured by immunohistochemistry and western blotting. MANF was found to be extremely upregulated in neurons and astrocytes on days 1, 7, and 14 and TREM2 is expressed on macrophages infiltrating the tissues from the circulation and astrocytes in the ischemic penumbra and dentate gyrus (DG) on days 7 and 14. DHA improved behavioral deficit and attenuated infarct size on days 7 and 14, increased MANF and decreased TREM2 expression in ischemic core, penumbra, and DG. In conclusion, MANF and TREM2 protein abundance are robustly increased after focal cerebral ischemia. We are demonstrate, that DHA treatment potentiated MANF, decreased TREM2 expression, improved behavior and provided additional neuroprotection. We are further exploring the mechanisms involved by assessing docosanoids and elovanoids as potential mediators.

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Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.07/H35

Topic: C.09.Stroke

Support: Health and Medical Research Fund, the Food and Health Bureau, The Government of the Hong Kong Special Administrative Region (03142256)

Title: Exacerbated outcomes in type I diabetic mice after experimental stroke are associated with CHOP and Erk while neurological deficits can be attenuated by lutein treatment

Authors: *A. C. LO, D. T. C. NG, K. TAM, A. K. W. LAI;
Dept. of Ophthalmology, The Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: PURPOSE: Type 1 diabetic patients are more prone to mortality after stroke and display more severe post-ischemic outcome. Their median survival is only half compared with the general population. Here, we aimed to elucidate potential mechanisms contributing to the exacerbation and test the effects of lutein, a safe anti-inflammatory and antioxidative agent.
METHOD: Ins2Akita/+ mice, a type 1 diabetic mouse model, and their wildtype littermates (12-15 weeks old) were challenged with experimental stroke by middle cerebral artery occlusion (MCAO): short ischemia (0.5 h) or long ischemia (2h) followed by 23.5h (0.5hI/23.5hR) or 2h or 22h of reperfusion, respectively (2I/2hR or 2hI/22hR). Survival rate and neurological deficits were assessed. Brain slices were stained with 2, 3, 5-triphenyltetrazolium chloride for estimation of infarct, hemispheric swelling and hemorrhagic area. Western blot analysis was used to compare levels of ZO-1, VEGF and pErk for assessment of blood vessel integrity and inflammation. ER-stress and autophagy responses were assayed using real-time PCR. Lutein (0.2 mg/kg) was given 1h before and 1h after reperfusion in the 2I/2hR animal group while the 0.5hI/23.5hR group received a single dose of lutein (2 mg/kg) 10min before reperfusion.
RESULTS: Earlier death and higher mortality rate were observed in Ins2Akita/+ mice. More hemorrhagic transformation observed since 2h after reperfusion was further exaggerated at 22h of reperfusion, with decreased ZO-1 and increased MMP-9 immunoreactivity. Western blot analyses demonstrated down-regulation of ZO-1 but up-regulation of VEGF, p-Erk1/2 and p-p38. mRNA expression of ER stress-related CHOP and autophagy-related LC3-b was elevated during early stages of reperfusion. Ins2Akita/+ mice (0.5hI/23.5hR) displayed infarcts similar to those induced by 2h long ischemia but milder neurological deficits, which could be successfully reduced by lutein administration.
CONCLUSION: Ins2Akita/+ mice displayed high mortality after MCAO, similar to type 1 diabetic patients upon stroke. Augmented cerebral hemorrhage and decreased ZO-1 expression indicated lower blood vessel integrity. Provoked inflammatory response and ER-stress are

important in the exacerbation of ischemic hyperglycemic brain. These results after 2h long ischemia suggested that hyperglycemia plays a significant role in exacerbation of stroke at an early stage by compromising blood vessel integrity and exerting inflammatory response. Meanwhile, results of 0.5h short ischemia demonstrated Lutein's neuroprotective effect against experimental stroke, making lutein a potential treatment for diabetic stroke patients.

Disclosures: **A.C. Lo:** None. **D.T.C. Ng:** None. **K. Tam:** None. **A.K.W. Lai:** None.

Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.08/H36

Topic: C.09.Stroke

Title: Cross-cultural differences and parietal lesioning: Computational modelling work

Authors: *E. MAVRITSAKI, P. RENTZELAS;
Birmingham City Univ., Birmingham, United Kingdom

Abstract: Parietal lesions studies after stroke are strongly influenced by research concentrated among participants of mainly a European American cultural background. This in extend is influencing the rehabilitation approaches identified that are based on the outcomes of this research. However, it has been found that one area that individualist European and American cultures differ from collectivist East Asian culture is the picture perception (Nisbett & Masuda, 2003). Collectivists in comparison to Europeans are more likely to attend to perceptual field as a whole rather than the salient item. Furthermore, continuing in this line of work similar differences are found in attentional processes (Ueda et al., 2018). To investigate the affect that this cross-cultural difference has in stroke patients we employed the spiking Search over Space and Time model (sSoTS) that has previously simulated cross-cultural differences (Mavritsaki et al., 2015) and has been used in Neuropsychology (Mavritsaki & Humphreys, 2016) to simulate lesioning in collectivists group. We followed the same line of research and applied changes in the model supported from our previous work (Mavritsaki & Humphreys, 2016; Mavritsaki et al., 2015). The outcomes from the research highlighted that the lesioning affect is more pronounced in collectivist than individualist group while reduced performance is also observed on ipsilesional side of collectivist group. These result provide the first step to understand cross-cultural differences in neuropsychology and can support further work on personalising rehabilitation or support after stroke.

REFERENCES

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Disclosures: E. Mavritsaki: None. **P. Rentzelas:** None.

Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.09/H37

Topic: C.09.Stroke

Support: Fullerton Foundation Grant

Title: Ischemic stroke patients with pre-stroke depression and thrombolysis therapy

Authors: *T. I. NATHANIEL[1], L. WORMACK[2];
[1]Univ. of So Carolina Sch. of Med. Greenville, Greenville, SC; [2]Univ. of South Carolina, Greenville, SC

Abstract: Objectives. To investigate clinical risk factors that may be associated with functional ambulatory recovery following thrombolysis therapy in ischemic stroke patients with pre-stroke depression. **Methods**.Multivariate analyses were performed to identify predictors of functional ambulatory outcomes, and clinical risk factors served as predictor variables. We used multicollinearity analysis, variance inflation factors, and goodness-of-fit to determine the fitness of the predictive model.**Results**. Carotid artery stenosis (OR= 11.577, 95% CI, 1.281 - 104.636, P=0.029) and peripheral vascular disease (OR= 18.040, 95% CI, 2.956-110.086, P=0.002) may be associated with improved ambulatory outcome, while antihypertensive medications (OR= 7.810, 95% CI, 1.401 -43.529, P=0.019),previous TIA (OR= 0.444, 95% CI, 0.517 -0.971, P=0.012), and congestive heart failure (OR= 0.217, 95% CI, 0.318 -0.402, P=0.030) were more likely to be associated with a non improvement in functional ambulation following thrombolysis therapy.**Conclusion.** Even after adjustment for covariates more clinical risk factors were associated with a non-improvement when compared with an improvement in pre-stroke depressed stroke population that received rtPA.

Disclosures: T.I. Nathaniel: None. **L. Wormack:** None.

Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.10/H38

Topic: C.09.Stroke

Support: AHA Predoctoral Fellowship 19PRE34380333
NIH K25HD096116

Title: Increasing shoulder abduction load negatively affects accuracy in judging elbow flexion torque

Authors: *N. M. CAI[1], J. P. DEWALD[1,2], N. GURARI[1];
[1]Physical Therapy and Human Movement Sci., [2]Biomed. Engin., Northwestern Univ., Chicago, IL

Abstract: Purpose
Previous research studied how accurately individuals can judge their self-generated rotational forces. i.e., torques, at a single joint. However, previous research has yet to investigate how accurately individuals can judge a torque at a single joint during multi-joint movements, as often occurs during activities of daily living. Therefore, this study is, to the best of our knowledge, the first to determine how accurately individuals can judge a torque at a single joint while simultaneously generating a torque at a second joint. We investigate how accurately individuals can judge torques at their elbow while simultaneously lifting at their shoulder.
Methods
Six participants without neurological impairments (mean±standard deviation: 59±7 years of age) took part in this study. Participants sat in a Biodex chair with a seatbelt and arm straps fastened to restrict trunk and shoulder movement. Their testing forearm was affixed to an instrumented mechatronic device, which measured the participant's isometric movements. Participants first generated 0, 20 or 40% of their maximum voluntary shoulder abduction torque (MVST), and then produced a reference torque of 10Nm about their elbow in flexion while maintaining the shoulder abduction load. Next, the participant rested for 6 seconds. Proceeding, the participant followed visual feedback to match the shoulder abduction load in the same arm. Finally, the participant matched the reference elbow torque without receiving feedback. This elbow torque-matching task was repeated eight times at each shoulder abduction level for each arm. The mean absolute error in matching elbow torques (i.e., absolute error) was estimated across the eight trials for each of these testing conditions.
Results
Participants matched their elbow torque less accurately as the shoulder abduction load increased ($p<0.001$). The absolute error with 40% MVST (5.05±2.39Nm) was higher than with no shoulder

abduction (2.07±1.12Nm, p<0.001) and 20% MVST (2.58±1.19Nm, p=0.001). Absolute error did not differ for the no shoulder abduction and 20% MVST conditions. Additionally, absolute error did not differ for the dominant and non-dominant arm at each of the three shoulder abduction loads.

Conclusions

In individuals without neurological impairments, increasing the shoulder abduction load negatively impacts how accurately they can judge torques generated at their elbow. This finding may have implications for our future work in which we will investigate how individuals with chronic hemiparetic stroke, who have abnormally coupled elbow torques when activating their shoulder, perceive their elbow torques while lifting.

Disclosures: **N.M. Cai:** None. **J.P. Dewald:** None. **N. Gurari:** None.

Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.11/H39

Topic: C.09.Stroke

Support: Royal Society of New Zealand Project Grant
Ministry of Business, Innovation, and Employment, New Zealand

Title: Stroke induced impairment in spatial working memory on the trial unique nonmatched to location task

Authors: ***J. M. HOULTON**, C. CLARKE, D. K. BARWICK, A. N. CLARKSON;
Anat., Univ. of Otago, Dunedin, New Zealand

Abstract: Introduction: Stroke-induced cognitive impairments are of significant concern, however mechanisms that underpin these impairments remain poorly understood and researched. Recently, we have established a model of stroke that results in delayed impairment in spatial memory. To further characterise cognitive impairments in our model and to align our assessments with what is used clinically, we have employed the use of touchscreen-based behavioural testing systems.

Methods: Young C57BL/6 mice were trained in operant touchscreen chambers to complete the trial-unique nonmatched-to-location (TUNL) task. Based on baseline performance animals were given either stroke (n=12) or sham (n=12) surgery using a photochemical model, bilaterally to the prefrontal cortex (PFC). Upon recovery, post-stroke spatial working memory was assessed through varying the degree of separation and delay within the TUNL trials. Seven weeks after surgery animals received prelimbic injections of the retrograde tracer cholera toxin B (CTb) to access PFC-thalamic connectivity. Tissue was then processed histologically and

immunohistochemically to assess infarct volume and connectional maps respectively.
Results: Two-way ANOVA and multiple comparisons revealed stroke animals took significantly longer ($p=0.017$) and performed worse ($p=0.0003$) during reacquisition of the TUNL task, relative to shams. Moreover, all animals performed worse when delay duration ($p=0.013$) or separation level ($p<0.0001$) was increased or decreased, respectively, which was exacerbated in the stroke animals (delay, $p<0.0001$; separation, $p=0.0054$). Preliminary cell counts of CTb-positive neurons show a loss of connections between the PFC and thalamus after stroke.
Discussion: To the best of our knowledge, the current study is the first to show stroke-induced spatial working memory impairments in mice completing the TUNL task. Stroke patients with impairments in this form of memory find even the smallest tasks extremely difficult. Our findings contribute to a better understanding of the neural mechanisms underlying such impairments. Future experiments will investigate therapeutic interventions in the hope of promoting functional improvement in cognition.

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Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.12/H40

Topic: C.09.Stroke

Support: Resslor Family Foundation

Title: Elucidating the intercellular interactome in white matter stroke

Authors: *M. TIAN, Y. KOMURO, J. D. HINMAN, T. S. CARMICHAEL;
Dept. of Neurology, The David Geffen Sch. of Med. at UCLA, Los Angeles, CA

Abstract: White matter strokes (WMS) constitute 25 percent of clinical stroke. It is also implicated in neurodegenerative diseases, including dementia. However, due to the distinct anatomical characteristics and specific cellular component in white matter, the molecular mechanism underlying WMS remain largely unknown. The micro-environment at the stroke site consists of astrocytes, endothelial cells, oligodendrocyte progenitor cells (OPCs), pericytes, and axonal bundles. These cells form a niche and communicate through ligand-receptor interactions, which is important for recovery. Our lab has previously developed a reproducible model to induce WMS in mouse brain by injecting a vasoconstrictor (L-NIO). Bulk RNAseq results showed that WMS has very different transcriptomic changes compare with cortical stroke, though these data lack the cell-type resolution. Given that there is no cure for WMS, it is important to elucidate the molecular signaling changes that are cell-specific after WMS. In

detail, it is important to understand how the cells that are affected in WMS communicate to each other: the ligand and receptor interactions that related to WMS recovery in different cell types. This might be termed the “neurovascular interactome” in WMS. Due to the hypermyelinated structure of white matter, it is difficult to obtain single cells for every cell type by simple dissociation approaches. Therefore, we use viral labeling and transgenic mouse lines, combined with translating ribosome affinity purification (TRAP), to immunoprecipitate mRNA from specific cell types. By doing do, we were able to label and harvest mRNA from astrocytes, endothelial cells, OPCs and pericytes. The enrichment of cell-specific genes was validated in RNAseq data and real-time PCR. Several interesting ligand-receptor couple genes that indicate the change of cell-cell communication after WMS were identified. Our current study may promote the understanding of mechanism underlying WMS recovery. Hopefully, new targets for the treatment of WMS can be discovered.

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Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.13/H41

Topic: C.09.Stroke

Support: NRF Korea Grant NRF-2016M357A1904391

Title: Catechin alleviates decrease of hippocalcin in middle cerebral artery occlusion animal model

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Abstract: Calcium is one of the major ions that involved in physiological functions, including metabolism, gene expression, cell proliferation, and apoptosis. Hippocalcin is a calcium buffering protein that has a high-affinity for calcium binding. It regulates the intracellular calcium concentration and prevents neuronal cell death from oxidative stress. Catechin has an excellent antioxidant property and exerts a neuroprotective effect. We studied the modulation of hippocalcin expression by catechin administration in focal cerebral ischemic injury and glutamate-induced neuronal cell damages. Male Sprague-Dawley rats were injected with vehicle or catechin (50 mg/kg) immediately before middle cerebral artery occlusion (MCAO). Cerebral cortical tissues were isolated 24 h after MCAO. Catechin alleviated MCAO-induced neuronal movement deficit and infarction. MCAO induced a decrease of hippocalcin expression in

cerebral cortex. However, catechin administration prevented MCAO-induced this decrease. In cultured hippocampal cells, glutamate excitotoxicity dramatically increased the intracellular calcium concentration, whereas catechin attenuated this increase. We observed a reduction of hippocalcin expression in glutamate-exposed cells. Catechin prevented glutamate-induced this decrease. Moreover, we showed that caspase-3 expression in hippocalcin siRNA transfection condition is higher than that of un-transfection condition. Catechin administration prevented an increase of caspase-3. In conclusion, our findings suggest that the catechin exerts a neuroprotective effect through prevention of intracellular calcium overload and alleviation of down-regulated hippocalcin expression in MCAO-induced injury and glutamate-induced damage.

Disclosures: P. Koh: None. **D. Park:** None. **J. Kang:** None. **M. Kim:** None.

Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.14/H42

Topic: C.09.Stroke

Support: MOST 104-2314-B-003-MY3
TCRD 108-56

Title: Inhibition of proteasome over-activation alleviates neuroinflammation and neurological deficit in intracerebral hemorrhage rats

Authors: *H.-K. LIEW[1], W.-F. HU[1], B.-C. P. LIN[3], P.-K. WANG[2], P.-Y. A. TSAI[4], C.-Y. PANG[1], T.-Y. CHEN[2];
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Abstract: Background: Neuroinflammation is a hallmark in intracerebral hemorrhage (ICH) induce secondary brain injury, leading to neuronal cell death. Uncontrolled inflammation response is highly related to endoplasmic reticulum (ER) dysfunction, namely endoplasmic reticulum (ER) stress. ER stress triggered-apoptosis and protein accumulation/aggregation play an important role in various neurological disorders. The consequences of ER stress and proteostasis disruption phenomenon have not been fully understood during the course of ICH development.
Methods: ICH was induced by infusion of collagenase VII-S at the right striatum of the rat. Animals were sacrificed at 0, 3, 6, 24, and 72 hours post-ICH. Rats were determined for body weight changes, hematoma volume, and neurological deficits. Brain tissues were harvested either for ELISA (oxidative stress, proteasomal activity, ubiquitination level, pro-inflammatory

cytokines expression), immunoblot analysis (ER stress molecular signaling), RT-qPCR (ER stress molecular signaling), protein aggregation, or for histological examination to assess the cells apoptosis and it co-localization. A non-selective proteasome inhibitor, MG132 was administered into the right striatum three hours prior to ICH induction.
Results: We found that ICH-induced excessive oxidative stress could lead to proteostasis disturbance as early as at three hours post-ICH, with evidence of elevation of ubiquitinated protein accumulation, proteasome activity, and protein aggregation. This proteostasis disturbance that occurred majorly around the perihematomal region was investigated under ER stress context: the ER stress-associated GRP78 was rapidly degraded in the injured right striatum at 3 hours after ICH and gradually restored to basal level at 24 hours post-ICH. The pro-apoptotic CHOP was elevated in neurons, microglia, and vascular endothelial cells in the injured brain, and colocalized to TUNEL+ apoptotic cells in the perihematomal region. These subsequence responses were accompanied by pro-inflammatory cytokines via NFkB activation. Pre-treatment with proteasome inhibitor - MG132 significantly ameliorated the ICH-induced ER stress/proteostasis disturbance, pro-inflammatory cytokines, and neurological deficits.
Conclusions: ICH-induced rapid ER stress/proteostasis disturbance and proteasome over-activation leading to exaggerated the neuroinflammation might be a critical event in acute ICH pathology.

Disclosures: H. Liew: None. **W. Hu:** None. **B.P. Lin:** None. **P. Wang:** None. **P.A. Tsai:** None. **C. Pang:** None. **T. Chen:** None.

Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.15/H43

Topic: C.09.Stroke

Support: Duke Anesthesiology DIG grant

Title: Long term assessment of cardiac abnormalities in mouse subarachnoid hemorrhage

Authors: Z. CAO, Z. YANG, X. LI, P. WANG, D. WARNER, W. YANG, ***H. SHENG**; Duke Univ. Med. Ctr., Durham, NC

Abstract: Introduction: Cardiac arrhythmias occur frequently in subarachnoid hemorrhage (SAH) patients including atrial and ventricular arrhythmias, alterations in QRS configuration, Q-T interval prolongation, T-wave abnormalities, and S-T segment elevation or depression. In humans, these arrhythmias have been associated with catecholamine release, global cerebral hypoperfusion, increased long-term mortality, and the risk of other cardiac events. Cardiac abnormalities in SAH have also been reported in dog, rabbit and rat. Currently only acute

preclinical studies have been performed. The aim of this study was to investigate cardiac dysfunction after long-term SAH survival in mice and establish a model applicable to therapeutic development. **Methods:** 16 male C57/Bl6 mice (8-10 weeks old) were anesthetized with isoflurane, intubated and ventilated. Rectal temperature was maintained at 37°C. Mice were randomly divided into sham and SAH groups and received either no blood or 120 μl prechiasmatic blood injection. Body weight was measured. Rotarod performance and EKG signals were examined prior to surgery and 28 days post-surgery. Heart tissue was harvested from an additional 6 mice (4 SAH and 2 naïve mice) at 1 or 3 h post-surgery for western blot analysis of phosphorated elf2α, an endoplasmic reticulum stress marker. A researcher blind to group assignment collected all data that were analyzed in Prism 6 using unpaired T test for Rotatod and EKG, one way ANOVA for p-elf2α and repeated measures ANOVA for body weight.**Results:** All SAH mice recovered from injury and survived for 28 days. Loss of body weight persisted in SAH mice compared to the sham group ($p < 0.01$). No difference was found between groups in pre-injury rotarod performance or the EKG. However, the latency to fall from the rotarod was decreased in SAH mice at 28 days after injury (SAH 193±49 seconds vs. sham 281±95 seconds, $p = 0.036$). We did not detect a difference in the QRS complex (SAH 0.74±0.06 mV and sham 0.63±0.14 mV, $p=0.09$) or T waves (SAH 0.07±0.02 mV and sham 0.14±0.18 mV, $p=0.3$) at 28 days. Heart rate was similar in both groups (SAH 579±44 beats/minute and sham 575±35 beats/minute, $p=0.8$). Compared to the naïve group, SAH mice had an increased p-elf2α (naïve 1.200±0.148, SAH 1 h 2.207±0.026 and SAH 3 h 1.657±0.352, $p=0.047$). **Conclusions:** This SAH model successfully induced a significant body weight loss and reduction of rotarod latency. SAH induced a two-fold increase in heart ER stress protein response was present, although a difference in EKG abnormalities was absent. Measurement of hemodynamic events and echocardiography are planned. Further investigation of the subcellular stress response is warranted.

Disclosures: H. Sheng: None. **Z. Cao:** None. **Z. Yang:** None. **X. Li:** None. **P. Wang:** None. **D. Warner:** None. **W. Yang:** None.

Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.16/H44

Topic: C.09.Stroke

Title: Novel microRNA PC-5P-12969 and ischemic stroke

Authors: *M. VIJAYAN, P. H. REDDY;
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Abstract: The purpose of our study is to characterize the novel miRNA PC-5P-12969 in an *in vitro* ischemic stroke (IS) model. In our recent global microRNA expression study, we found two previously unreported miRNAs in serum samples from patients with ischemic stroke. Further validation of differentially expressed miRNAs, we found miRNA PC-5P-12969 up-regulated in serum IS samples, postmortem IS brains, lymphoblastoid IS cell lines, oxygen-glucose deprived human, mouse neuroblastoma cells, HT22 cells, IS stroke mouse models. In the present study, we sought to characterize miRNA PC-5P-12969 in an oxygen glucose deprivation/reoxygenation-OGD/R model. SHSY-5Y cells were transfected with miRNA PC-5P-12969 agomir and antagomir using Lipofectamine 2000 transfection reagent for 24 h in order to check their transfection efficiency and expression patterns. Agomir showed a good upregulation but antagomir did not show any down-regulation of PC-5P-12969. It is indicated that the PC-5P-12969 is expressing only in the disease condition. Further, cells were treated with agomir, antagomir and underwent for OGD/R to mimic the ischemic condition. Interestingly, miRNA agomir expression was increased significantly compared to normal condition transfected with agomir. Cell viability, apoptotic, ATP, cytochrome C oxidase activity, H_2O_2 production and lipid peroxidation were assessed in the SHSY5Y cell lines from control, OGD/R cells and agomir treated OGD/R cells. Increased cell viability, ATP production, cytochrome oxidase activity and decreased apoptotic cells, free radical production and lipid peroxidation were found in agomir treated OGD/R cells. We found significantly decreased number of mitochondria and increased mitochondrial length in agomir treated OGD/R cells relative to control and OGD/R cells suggesting that agomir reduces excessive mitochondrial fragmentation and increased mitochondrial length in ischemic condition. Our *in silico* analysis revealed that PC-5P-12969 is associated with the regulation of the stroke-related gene(s). To the best of our knowledge, this is the first study identified/validated/ characterized potential novel candidate PC-5P-12969 in ischemic stroke. Based on these observations, we conclude that miRNA PC-5P-12969 is a potential biomarker for ischemic stroke.

Disclosures: M. Vijayan: None. **P.H. Reddy:** None.

Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.17/H45

Topic: C.09.Stroke

Support: NIH Grant

Title: Prostaglandin E2 EP3 receptor contributes to acute intracerebral hemorrhage-induced brain Injury

Authors: *H. REN[1], T. WU[1], X. YANG[2], J. WANG[3];
[1]Johns Hopkins Sch. of Med., Baltimore, MD; [2]Dept. of Anesthesiol. and Critical Care Med., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [3]Anesthesiology/Critical Care Med., Johns Hopkins Univ., Baltimore, MD

Abstract: Intracerebral hemorrhage (ICH) is a neurologically destructive type of stroke for which no valid treatment is available. Activation of prostaglandin E2 receptors (EP1-EP4) by prostaglandin E2 (PGE2) produces different biologic effects in the setting of experimental stroke or ICH, but the pathogenesis is still not fully understood. PGE2 EP3 receptor (EP3R) is the most abundantly expressed EP receptor subtype in the brain. In the current study, we found that blocking or knocking out EP3R reduced ICH-induced brain injury volume, brain edema, and secondary neuronal cell death in mice. Blood-brain barrier (BBB) damage, as assessed by Evans Blue leakage, was markedly decreased in EP3R knockout mice and in mice treated with ONO-AE3-240, a highly selective EP3R antagonist. The loss of BBB tight junction protein occludin was significantly attenuated in the perihematomal region of the EP3R knockout mice. Moreover, gelatin gel zymography results showed that the activity of matrix metalloproteinase (MMP) 9 (MMP), but not that of MMP-2, was increased after ICH. However, gelatinolytic activity decreased in the perihematomal region of the EP3R knockout mice and mice treated with EP3R antagonist ONO-AE3-240 when compared with that of their respective controls. At the same time, the activation of microglia/macrophages and astrocytes decreased in both EP3R knockout and mice treated with ONO-AE3-240. Infiltrating neutrophils and neutrophil-derived reactive oxygen species were also reduced by both EP3R knockout and inhibition. Finally, EP3R knockout and ONO-AE3-240 each ameliorated neurologic deficits after ICH. These results suggest that EP3R inhibition could be a novel therapeutic strategy to improve outcomes after ICH.

Disclosures: H. Ren: None. **T. Wu:** None. **X. Yang:** None. **J. Wang:** None.

Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.18/H46

Topic: C.09.Stroke

Support: Burrows Wellcome Fund 1009855
California National Primate Research Center

Title: Perilesional motor and somatosensory cortex dynamics with recovery of dexterous function after cortical strokes in non-human primates

Authors: *P. KHANNA[1], D. TOTTEN[2], R. J. MORECRAFT[3], K. GANGULY[1];
[1]UCSF, San Francisco, CA; [2]Univ. of California, San Francisco, San Francisco, CA; [3]Univ. South Dakota Schl Med., Vermillion, SD

Abstract: Stroke related disability and early death is set to double worldwide within the next 15 years [1]. Despite therapy, 50% of stroke survivors have impaired hand function [2] which strongly impacts activities of daily living. Hand dysfunction includes weakness, poor finger individuation, and lack of coordination, preventing subjects from achieving the dexterity needed for object manipulation tasks performed in daily life. Somatosensation deficits are a strong predictor of chronically impaired hand function [3,4]. Somatosensory deficits can be observed even when motor-only areas are effected by stroke, highlighting the importance of the dense somatosensory to motor connectivity needed for dexterous actions.

To understand how these connections are re-established between perilesional somatosensory (sPLC) and perilesional motor (mPLC) cortical areas to support dexterous hand control following stroke, we trained male rhesus macaques between 5 and 7 years of age to perform a reach-to-grasp task and a center-out reaching task. To model a stroke, aspiration was used to remove the forelimb region of primary motor cortex unilaterally after cauterization of the primary arterial supply. In the same surgery, chronic microwire electrodes were implanted into dorsal premotor cortex (mPLC) and primary somatosensory cortex (sPLC) to monitor the neural correlates of behavioral recovery. Here, we report on how mPLC-sPLC dynamics change as animals recover dexterity following the lesion.

Finally, one promising method of enhancing mPLC-sPLC communication during recovery is with oscillating epidural stimulation. Previous work in rodents has identified that low-frequency activity in mPLC is correlated with the recovery of motor function after a primary motor cortex lesion [5]. Boosting this low-frequency activity with epidural stimulation delivered through cranial screws in impaired rodents improved motor function. A critical translational step is to modify this approach for application to improvements in dexterous control within the primate brain, so we also tested epidural stimulation as a means to boost motor recovery after stroke in non-human primates and track how stimulation influences underlying mPLC-sPLC dynamics. Together, our results provide a possible path to improve motor function after stroke using targeted modulation of circuit dynamics.

References: 1. Feigin, V.L., et al. (2014). The Lancet 383, 245-255., 2. Go, A.S., et al. (2013). Circulation 127, e6-e245., 3. Hill, V.A., et al. (2014). Top. Stroke Rehabil. 21, 339-346., 4. Pavlova, E.L., et. al. (2018). J. Mot. Behav. 50, 134-143., 5. Ramanathan, D.S., et. al. (2018). Nat. Med. 24, 1257-1267.

Disclosures: P. Khanna: None. **D. Totten:** None. **R.J. Morecraft:** None. **K. Ganguly:** None.

Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.19/I1

Topic: C.09.Stroke

Support: Robertson Fund for Cerebral Palsy Research

Title: Performance on bilateral object hitting task in children with perinatal stroke

Authors: *R. L. HAWE[1], A. M. KUCZYNSKI[2], A. KIRTON[3], S. P. DUKELOW[1];
[1]Clin. Neurosciences, [2]Cumming Sch. of Med., [3]Pediatrics, Cumming Sch. of Med., Univ. of Calgary, Calgary, AB, Canada

Abstract: Introduction: Motor deficits in children with perinatal stroke are often assessed with reaching and/or grasping tasks. However, many real-world activities are bilateral, and require integration of motor and visuospatial skills. The objective of this study was to use a robotic object hitting task to assess bilateral sensorimotor control and visuospatial skills in children with hemiparesis due to perinatal arterial ischemic stroke (AIS) or periventricular venous infarct (PVI).
Methods: Forty-nine children with perinatal stroke (AIS: n=28, 12.4±4.0 years; PVI: n=21, 11.7±3.8 years) and 155 typically developing (TD) children (12.5±4.0 years) participated. Participants performed a bilateral object hitting task using the KINARM Exoskeleton Robot in which virtual paddles displayed at the fingertips are used to hit balls that fall from the top of the screen with increasing speed and frequency over 2.3 minutes. Performance was quantified across 13 parameters including number of balls hit with each hand, movement speed and area, biases between hands, and spatial biases. Normative ranges accounting for age effects were calculated from TD children, using the non-dominant and dominant arms as comparisons for the affected and less affected arms, respectively. Children with perinatal stroke underwent clinical assessments using the Assisting Hand Assessment, Melbourne Assessment, and Behavioral Inattention Test.
Results: Overall, 82% of children with AIS and 57% of children with PVI hit fewer balls with their affected arm compared to TD children. Deficits were also seen in the less affected arm, with 43% of children with AIS and 14% of children with PVI falling outside of normative ranges. Despite hitting fewer balls, <18% children with AIS or PVI were identified as impaired in terms of movement speed or area with either arm. Across the different parameters, impairments were more common and severe in children with AIS compared to PVI. The number of balls hit with the affected hand was found to correlate with clinical measures (Assisting Hand Assessment and Melbourne Assessment) in children with AIS (R^2=0.47 and R^2=0.49 respectively). Hits with the less affected hand were correlated with scores on the Behavioral Inattention Assessment (AIS:

R^2=0.21, PVI: R^2=0.32).
Conclusions: Children with perinatal stroke had significant deficits on the object hitting task, including the less affected arm. The combination of motor impairments and decreased visuospatial attention likely contributes to the impaired performance. Clinical assessments and interventions should address the interplay between motor and visuospatial skills.

Disclosures: R.L. Hawe: None. **S.P. Dukelow:** None. **A.M. Kuczynski:** None. **A. Kirton:** None.

Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.20/I2

Topic: C.09.Stroke

Title: Ischemic conditioning causes an acute pressor response in chronic stroke survivors

Authors: S. Z. ALQAHTANI[1], M. DURAND[2], S. C. RAAB[1], J. NGUYEN[2], B. D. SCHMIT[3,2], *A. S. HYNGSTROM[1];
[1]Physical Therapy, Marquette Univ., Milwaukee, WI; [2]Med. Col. of Wisconsin, Milwaukee, WI; [3]Dept. of Biomed. Engin., Marquette Univ. Dept. of Biomed. Engin., Milwaukee, WI

Abstract: Ischemic conditioning (IC) has been shown to improve motor performance in individuals with stroke, but the neural mechanisms of motor improvement are not well understood. Although multifactorial, one proposed mechanism is that IC engages the autonomic nervous system through stimulation of chemosensitive muscle afferents, resulting in increased sympathetic outflow. It is plausible that if IC engages the autonomic nervous system, there may be an acute pressor response to the IC stimulus. The purpose of this study was to quantify the effects of IC on the pressor response in individuals post-stroke. **Methods:** Nine individuals with chronic stroke (>1 year post-stroke) aged 60 ±10 years participated in this prospective, controlled, cross-over study (males n=5, females n=4). To administer either IC or IC Sham, a rapid inflate/deflate cuff (Hokanson, Inc) was placed over the proximal paretic thigh and inflated and deflated for five cycles of five minutes at 225 or 10 mmHg (IC and IC-Sham conditions, respectively; order counterbalanced). Blood pressure and heart rate (NOVA, Finapres Medical Systems) were measured continuously before, during and after the IC or IC- Sham conditions. **Results:** There was a greater percent increase in systolic blood pressure in the IC vs. IC-Sham condition (IC mean change = 17.20%±1.50, IC-Sham mean change = 5.80%±1.94, respectively, p= 0.029). In addition, there was greater percent increase in the rate pressure product in the IC vs. IC-Sham group (IC mean change = 13.06 %±0.85, IC-Sham mean change = -3.01 %±1.43, respectively, p=0.003). These data suggest that a single session of IC may be sufficient to engage the autonomic nervous system to increase sympathetic outflow in stroke survivors.

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Poster

135. Stroke I

Location: Hall A

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Program #/Poster #: 135.21/I3

Topic: C.09.Stroke

Support: NHRI-EX108-10803NI
MOST 107-2320-B-039-067

Title: Focal ischemia causes heterokaryotic fusion of neuron and hematopoietic cell

Authors: H.-L. WANG[1], ***T. W. LAI**[1,2,3,4];
[1]Grad. Inst. of Clin. Med. Sci., [2]Grad. Inst. of Biomed. Sci., [3]Drug Develop. Ctr., China Med. Univ., Taichung, Taiwan; [4]Translational Med. Res. Ctr., China Med. Univ. Hosp., Taichung, Taiwan

Abstract: Bi-nucleated neurons are rare in post-mortem brain tissues from human subjects without brain diseases, yet they are found in patients that had multiple sclerosis, Alzheimer's disease, CNS atropy, and other brain diseases. Emerging evidence suggests that these bi-nucleated neurons arise from heterokaryotic fusion of infiltrated hematopoietic cells and neurons in the cerebellum. Using a novel animal model, we demonstrate that hematopoietic-neuronal cell fusion contributed to the generation of bi-nucleated neurons in the motor cortex of mice subjected to focal ischemia. Specifically, we generated mice that express emGFP in hematopoietic cells and DsRed in other cell types, and subjected these mice to distal middle cerebral arterial occlusion. Indeed, fusion of emGFP+ hematopoietic cells and DsRed+ neurons were found in the motor cortex of these mice. We suggest that this model may be used to study the role of these bi-nucleated neurons in stroke pathogenesis, and further demonstrate how the same model can be applied to study and quantify heterokaryotic fusion of neuron and hematopoietic cell in other brain diseases. Funding: This work is supported by research funding from the National Health Research Institutes (NHRI-EX108-10803NI).

Disclosures: **H. Wang:** None. **T.W. Lai:** None.

Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.22/I4

Topic: C.09.Stroke

Support: Wellcome Principal Research Fellowship110027/Z/15/Z
NIHR Oxford Biomedical Research Centre support
Wellcome Trust core funding 203139/Z/16/Z

Title: Real time fMRI neurofeedback for stroke rehabilitation

Authors: *M. K. FLEMING, Z.-B. SANDERS, T. SMEJKA, M. MARZOLLA, C. ZICH, S. W. RIEGER, C. SAMPAIO-BAPTISTA, H. JOHANSEN-BERG;
Wellcome Ctr. for Integrative Neuroimaging, Univ. of Oxford, Oxford, United Kingdom

Abstract: Stroke is a leading cause of adult disability and strategies to improve motor recovery are vital. Real-time functional magnetic resonance imaging (fMRI) neurofeedback aims to drive brain activation towards optimal patterns through online feedback. The patient is instructed to try to move their affected hand to promote an increase in lateralisation of motor cortex (M1) activity towards the ipsilesional hemisphere. This double blinded, randomised controlled trial aims to investigate whether 1) patients can increase lateralisation of M1 activity over 3 sessions of neurofeedback training, 2) increases in lateralisation are maintained after the feedback is removed and 3) real neurofeedback leads to improvements in upper limb function. Following written informed consent, chronic stroke survivors (> 6 months post-stroke), with upper limb impairment are randomised to receive 3 sessions of real or sham fMRI neurofeedback over four days, with minimisation of between group differences in initial upper limb function (Action Research Arm Test (ARAT) score) and time since stroke. Lateralisation of brain activity is assessed using fMRI in each session while participants move their affected hand, with and without the feedback display. Change in lateralisation during a visuomotor squeeze task is assessed by comparing baseline and 1 week post-neurofeedback. Upper limb function is assessed at each session using the Jebsen Taylor Test and at baseline and 1 week post-neurofeedback using the ARAT and the upper extremity section of the Fugl-Meyer Assessment (FMA). Participants' perception of control over the neurofeedback visual display is rated each session from 1 (no control) to 5 (full control). Preliminary data (n=12) demonstrate an increase in lateralisation of M1 activity during neurofeedback for the real fMRI neurofeedback group, suggesting that participants can utilize the feedback. Participants report that they are "somewhat in control of the red bar" (representing activity from the ipsilesional M1), with an average control rating of 3.3 out of 5. The movements/strategies that participants regularly report to be effective include wrist flexion/extension, sometimes in combination with opening or closing the

hand, imagining finger movements such as playing the piano and tapping fingers to the thumb. There is also a tendency for a greater increase in lateralisation of M1 activity during the visuomotor squeeze task and greater improvement in upper limb function for the real fMRI neurofeedback group in comparison with sham.

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Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.23/I5

Topic: C.09.Stroke

Title: A new generation of plasminogen activator for safer clearance of brain hematoma following intracerebral hemorrhage in rats

Authors: R. GOULAY[1], T. GABEREL[1], M. NAVEAU[2], D. VIVIEN[1], ***J. PARCQ**[3]; [1]INSERM U1237, Caen, France; [2]Univ. of Caen, Caen, France; [3]Op2lysis, Caen, France

Abstract: Intracerebral hemorrhage (ICH) is a life-threatening disease that leads to a massive extravasation of blood anywhere within the brain parenchyma. Up to now, no treatment has been approved by the authorities for ICH to reduce the heavy burden of this disease.
MisTIE phase 3 clinical trial was a program to evaluate the combination of minimally invasive surgery and clot lysis with recombinant tissue Plasminogen Activator (rtPA, actilyse®) to remove brain hematoma following ICH. Results published in 2019 showed that (1) the minimally invasive surgery protocol was safe and effective to allow thrombolytic agent injection in the hematoma and hematoma evacuation; (2) there was a strong correlation between the volume of blood evacuated and the clinical benefit and (3) that rtPA was not enough effective (only 58% blood evacuation in the surgical group). Moreover, in a model of ICH in pigs tPA treatment has been shown to double the volume of residual edema, thus reducing benefits of hematoma drainage, an effect associated to an overactivation of the NMDA receptor dependent pathway. We hypothesized that rtPA pro-neurotoxicity was an obstacle to the clinical improvement of ICH patients treated in the surgical group with rtPA.
We produced an original de-poisoned thrombolytic derived from the current human tPA, named Optimized tPA (OptPA) and investigated its safety and efficacy for *in situ* fibrinolysis in a rat model of ICH. Magnetic resonance imaging analyses of hematoma and edema volumes, behavioural tasks and histological analyses were performed to measure the effects of treatments. *In vitro,* OptPA was equally fibrinolytic as rtPA without promoting NMDA-dependent neurotoxicity. *In vivo, in situ* fibrinolysis using OptPA reduced hematoma volume, like rtPA, but

it also reduced the evolution of peri-hematomal neuronal death and subsequent edema progression. Overall, this preclinical study demonstrates beneficial effects of OptPA compared to rtPA for the drainage of ICH.
We thus propose OptPA as the future for improved fibrinolysis following ICH in humans.

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Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.24/I6

Topic: C.09.Stroke

Support: CIHR MOP 106662
Heart and Stroke Canada Grant-in-Aid
ORF-RE-04-47
AIHS PGF

Title: Upper limb proprioceptive acuity is not dependent on motor function post-stroke

Authors: *J. A. SEMRAU[1], J.-L. MARNET[2], S. H. SCOTT[3], S. P. DUKELOW[4];
[1]Univ. of Delaware, Newark, DE; [3]Dept Biomed. and Mol. Sci., [2]Queen's Univ., Kingston, ON, Canada; [4]Clin. Neurosciences, Univ. of Calgary, Calgary, AB, Canada

Abstract: Recent work has shown that proprioception is impaired in 50-60% of individuals with stroke. Further, work using instrumented assessments of post-stroke proprioception at the elbow and wrist have shown impairments in proprioceptive thresholds. While these studies have been useful in characterizing the impairments that occur due to stroke, few studies have examined the relationship of post-stroke proprioception to motor function. Recently, we have shown that impairments in proprioception can occur independently of impairments in motor performance throughout stroke recovery, and vice versa. Here we aim to examine if proprioceptive thresholds measured during a unilateral proprioceptive discrimination task are related to motor function after stroke. Twenty-three unilateral stroke subjects and 12 controls performed robotic tasks of proprioceptive and motor function: 1) Single Arm Proprioception (SAP)-A two-alternative forced-choice (2-AFC) task to measure proprioceptive thresholds of the stroke-affected arm, 2) Position Matching (PM)-A bilateral position matching task where the robot moved the subjects' arm, and subjects mirror-matched the location with the opposite arm, 3) Kinesthesia (KIN)-A bilateral movement matching task where the robot moved subjects' arm, and subjects mirror-

matched the speed, direction, and magnitude of movement with the opposite arm. 4) Visually Guided Reaching (VGR)-A 4-target reaching task to measure motor function (e.g., Reaction Time). For stroke subjects in tasks 1-3, the robot moved the stroke-affected arm. For SAP, a median proprioceptive threshold was calculated from the magnitude of movements delivered by the robot to compare against PM and KIN, and motor measures of VGR. Overall, we found that subjects with stroke had significantly higher proprioceptive thresholds than controls (Stroke=3.8±4.1cm; Control=0.6±0.2cm, $p<0.001$), and that performance on the SAP task was well-correlated to performance in the PM ($r=0.7$, $p<0.001$) and KIN ($r=0.68$, $p<0.001$) tasks. Further, when we compared proprioceptive thresholds (SAP) against performance in the motor task (VGR) for both groups, we found that the two tasks were not significantly correlated when corrected for multiple comparisons ($r=0.35$, $p=0.04$). We found that a traditional 2-AFC measure of proprioception correlated well with bilateral measures of proprioception. However, impairment in motor function did not always correspond to measured impairments in proprioceptive thresholds, suggesting the importance of independent measurement of motor and proprioceptive function in stroke survivors.

Disclosures: **J.A. Semrau:** None. **J. Marnet:** None. **S.H. Scott:** Other; Co-Founder and CSO of BKIN Technologies. **S.P. Dukelow:** None.

Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.25/I7

Topic: C.09.Stroke

Support: MOST104-2320-B-010 -015 -MY3 from Ministry of Science and Technology, Taiwan

Title: TrkB activation attenuates white matter injury and motor deficit induced by hypoxia-ischemia in neonatal rats

Authors: S.-P. HSU, C.-C. HUNG, P.-C. HSU, Y.-J. HUANG, Y.-C. LAN, *Y.-H. LEE; Physiol., Natl. Yang-Ming Univ., Taipei, Taiwan

Abstract: Neonatal hypoxic-ischemic (HI) brain injury is the leading cause of cerebral palsy and neurocognitive deficit in children, which preferentially damages the brain white matter and causes demyelination. Unfortunately, there is no effective therapy for neonatal hypoxic brain injury to date. Previous studies reported that activation of tropomyosin-related kinase B (TrkB) by brain-derived neurotrophic factor (BDNF) has marked neuroprotective effect against ischemic brain injury in adult animals. However, high concentration of BDNF would activate a pro-death p75 receptor and give undesirable effects. In this study, we investigated whether LM22A-4

(LM22A), a small-molecule BDNF loop-domain mimetic and TrkB activator that was reported to enhance neuronal survival, would protect against white matter injury in a neonatal transient hypoxia-ischemia (tHI) rat model. Postnatal day 7 (P7) rat pups received LM22A by intraperitoneal injection (i.p.) at 1 h after recovery from tHI. By using immunofluorescence staining, we found that LM22A reduced tHI-induced astrogliosis and microglial activation in the corpus callosum at 24 h after tHI injury. The oligodendrocyte progenitor cells labeled with neural/glial antigen 2 (NG2) were also increased in the corpus callosum after LM22A treatment. Further histological examination of white matter integrity at P34, the preadolescent stage, indicated that the number of node of Ranvier decreased in the corpus callosum by tHI was preserved in LM22A-treated group, as determined by double staining with antibodies against nodal sodium channels Nav1.6 and paranodal protein caspr (contactin-associated protein). Finally, behavior assessments for motor and cognitive functions at P34 indicated that neonatal tHI impaired motor coordination, not recognition memory, and the effect could be ameliorated by LM22A treatment. In conclusion, these results suggest that TrkB activation by LM22A may benefit white matter development and subsequent motor outcome in neonatal rat brain after HI insult by reducing gliosis and facilitating OPC differentiation.

Disclosures: S. Hsu: None. **C. Hung:** None. **P. Hsu:** None. **Y. Huang:** None. **Y. Lan:** None. **Y. Lee:** None.

Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.26/I8

Topic: C.09.Stroke

Title: Effects of unilateral and bilateral tDCS over M1 on the kinematics of sensorimotor function in chronic stroke patients

Authors: *T. MUFFEL[1,2,3,4], P.-C. SHIH[1,4], B. KALLOCH[1,5], V. NIKULIN[1,6], A. VILLRINGER[1,2,3,4], B. SEHM[1,4];
[1]Neurol., Max Planck Inst. For Human Cognitive and Brain Sci., Leipzig, Germany; [2]Mind Brain Body Inst., Humboldt-Universität zu Berlin, Berlin, Germany; [3]Charité - Universitätsmedizin Berlin, Berlin, Germany; [4]Day Clin. for Cognitive Neurol., Univ. Hosp. Leipzig, Leipzig, Germany; [5]Leipzig Univ. of Applied Sci., Leipzig, Germany; [6]Ctr. for Cognition & Decision Making, Higher Sch. of Econ., Moscow, Russian Federation

Abstract: The improvement of acute stroke treatment has increased survival, leaving more people in rehabilitative care. The recovery of function, however, typically stagnates after initial progress. Transcranial direct current stimulation (tDCS) has been suggested to facilitate recovery beyond this plateau. However, inconsistent results of tDCS effects were found in chronic

patients, probably due to varying behavioural assessments with low spatial or temporal resolution, a selection bias introduced through group designs and small sample sizes. Here, we used high-resolution kinematic assessments to inspect a wide range of sensorimotor functions using established protocols for unilateral (utDCS) and bilateral tDCS (btDCS) in a cross-over, double-blind and sham-controlled clinical trial. 24 patients underwent all tDCS conditions (sessions separated by one week) concurrent to kinematic assessments. tDCS-induced effects were measured on 4 tasks (Fig. 1). 40 kinematic parameters were extracted across these tasks and corrected for age and Fugl-Meyer score. The estimates were then compared between sham vs. utDCS and sham vs. btDCS, respectively, using paired-samples t-tests and classified as (1) non-significant changes, (2) significant increases or (3) decreases in performance. Class distributions across parameters were then compared between real and permuted data to account for falsely positive significance tests. utDCS induced significant increases in 7 and decreases in 18 parameters, btDCS increases in 11 and decreases in 15 parameters (Fig. 1). The class distributions differed significantly between real and permuted data, indicating a profound tDCS effect. Our approach provides a new avenue for investigating tDCS effects across different sensorimotor domains after stroke. The results demonstrate the efficacy of tDCS to modulate sensorimotor function after stroke, but also indicate that the induced effects are complex and bidirectional.

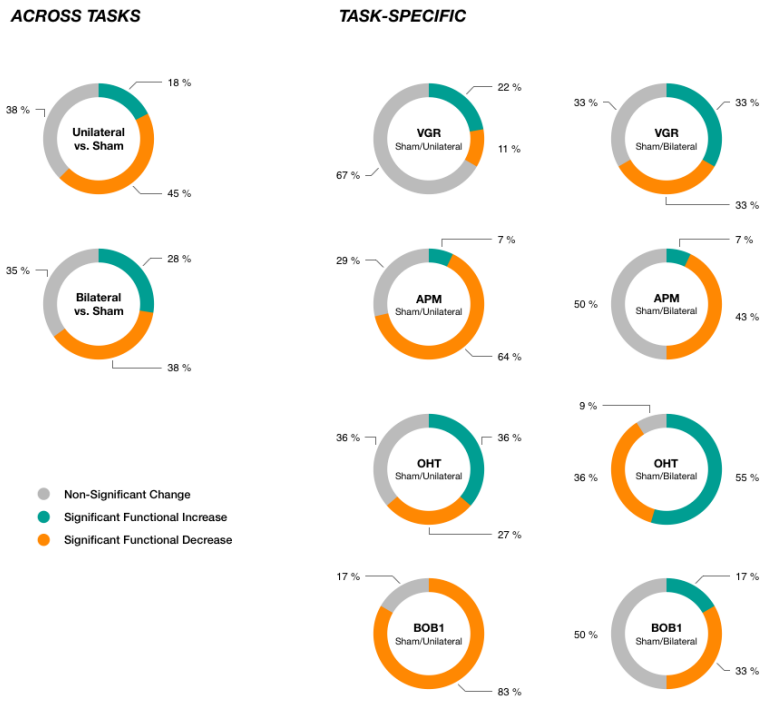

Fig. 1: Circles represent change classes across all parameters and tasks (left) and specific for each task (right). Across tasks, utDCS induced a larger proportion of functional decreases, in contrast to previous findings. Decreases originated mainly from the proprioceptive and bimanual coordination tasks and increases were observed in unimanual reaching.

Abbreviations: *APM* = Arm Position Matching, *BOB* = Ball on Bar (cooperative bimanual coordination), *OHT* = Object Hitting Task (independent bimanual coordination), *VGR* = Visually Guided Reaching.

Disclosures: **T. Muffel:** None. **P. Shih:** None. **B. Kalloch:** None. **V. Nikulin:** None. **A. Villringer:** None. **B. Sehm:** None.

Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.27/I9

Topic: C.09.Stroke

Support: Ontario Research Fund ORF-RE 04-47
GSK Chair in Neuroscience
CIHR Operating Grant MOP 106662

Title: Separating transient ischaemic attack and migraine from healthy individuals using one-class classification

Authors: *L. E. R. SIMMATIS, S. H. SCOTT, A. Y. JIN;
Queen's Univ., Kingston, ON, Canada

Abstract: Transient ischaemic attack (TIA) and migraine are pathophysiologically distinct, yet they can be difficult to identify and differentially diagnose. The present diagnostic gold-standard remains expert neurologist opinion which can be subjective because of reliance on patient self-report of symptoms. Here, we investigate the use of the KINARM to assess 1) separating individuals who had TIAs or migraines from healthy control participants, and 2) identifying whether or not variables derived from robotic assessment have differing importance in TIA and migraine. Individuals who had TIAs or migraines were recruited from the Kingston Health Sciences Centre stroke prevention clinic. Control participants were recruited using local classified advertisements. In total, we recruited 50 people who were diagnosed with TIAs and 31 diagnosed with migraines. We recruited 240 to 642 individuals in the control cohorts (task-dependent). Participants performed a series of robotic tasks testing motor, sensory, and cognitive behaviours. We focus here on 4 tasks: Visually-Guided Reaching (VGR), Reverse Visually-Guided Reaching (RVGR), Object Hit (OH), and Object Hit-and-Avoid (OHA). Z-scores (age, sex, and handedness adjusted) were derived from task parameters that quantify spatial and temporal features of performance. We additionally calculated Task Scores and M-Scores, which describe overall task performance. Data were split into train/test/validation (control) or test/validation (TIA, migraine) sets for classification. We trained a bagged one-class SVM (OCSVM) classifier on control data (hyperparameters optimised by grid-search). The highest test accuracy was 80% (OHA: TIA vs control). The highest validation accuracy was 79% (OHA: migraine vs control). Controls could be identified correctly up to 80% of the time (VGR). The task with the greatest similarity in parameter ranking between TIA and migraine was RVGR, whereas the task with the greatest difference in parameter ranking between TIA and migraine was OHA. Thus, we were able to separate individuals who had TIAs or migraines from healthy

controls with relatively high accuracy in the majority of tasks, although tasks testing cognitive-motor integrative ability allowed better separation in test- and validation-sets.

Disclosures: L.E.R. Simmatis: None. **S.H. Scott:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); GSK Chair in Neuroscience. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BKIN Technologies Ltd.. **A.Y. Jin:** None.

Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.28/I10

Topic: C.09.Stroke

Support: NIH Grant NS094896

Title: Red blood cell-derived microparticles treatment improves post-intracerebral hemorrhage long-term outcomes in rats

Authors: *S. CHO[1], A. K. REHNI[1], H. NAVARRO QUERO[2], C. J. KEATLEY[1], S. GAJAVELLI[3], S. KOCH[1], Y. S. AHN[2], M. A. PEREZ-PINZON[1], W. JY[2], K. R. DAVE[1]; [1]Neurol., [2]Med., [3]Miami Project to Cure Paralysis, Univ. of Miami Miller Sch. of Med., Miami, FL

Abstract: As spontaneous intracerebral hemorrhage (sICH) is the deadliest stroke sub-type with no therapeutic options, the prevention of hematoma expansion is a potential therapeutic target. Our earlier studies showed that treatment with red blood cell-derived microparticles (RMP), a hemostatic agent[1], lowered post-sICH hematoma volume 24 h post-collagenase injection. The goal of this study was to evaluate the potential of RMP therapy in improving primary and secondary long-term outcomes in a rat model of sICH. RMPs were prepared from human RBCs[1]. sICH was induced in young Sprague-Dawley male rats by injecting collagenase into the right striatum. Rats were randomly assigned to vehicle, RMP ($2.55x10^{10}$ particles/kg, b.w. i.v.), or recombinant Factor VIIa (rFVIIa) (positive control, 120 µg/kg, b.w. i.v.) treatment groups. RMP dose and treatment paradigms were determined based on earlier pharmacokinetic, dose response, and multiple paradigm comparison studies. On day 28 post-sICH, rats were euthanized to collect brains for histological assessment. In an earlier study to be presented at the Brain 2019 conference, we reported that both RMP- and rFVIIa-treatments significantly lowered neurological deficit scores than the control group up to 14 days post-sICH. We also reported that in the ladder rung walking test when compared to the baseline, the control group had a significantly greater percentage of contralateral foot faults up to 28 days post-sICH, and the rFVIIa group had significantly greater foot faults up to 21 days post-sICH. However, the RMP

group did not have foot faults significantly different than the baseline at any time point. We next evaluated brain histopathology, focusing on bregma levels +3.0 to -3.0. Animals with more than 2 sections missing at the level of interest (6 animals) and improper injection location (4 animals) were excluded from the analysis. The RMP-treated group was compared with the control and rFVIIa-treated groups using Student's t-test. Based on our results so far, we observed that the damaged brain volume was significantly lower in the RMP group (n=11) than the control ($p<0.05$, n=9) and rFVIIa ($p<0.05$, n=12) groups by 28% and 29%, respectively. We are in the process of evaluating histopathology of the remaining animals belonging to all experimental groups. Our results indicate that RMPs have the potential to not only lower hematoma growth, but also improve long-term outcomes post-sICH.

References: 1) Thrombosis and Haemostasis. 2013;110(4):751-60.

Disclosures: S. Cho: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; The present study was supported by NIH grant NS094896.. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); RxMP Therapeutics provided the testing material for the study.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The University of Miami has partial ownership in RxMP Therapeutics. The University of Miami is a co-inventor of 2 US patents related to red cell microparticles. **A.K. Rehni:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; The present study was supported by NIH grant NS094896. **H. Navarro Quero:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; The present study was supported by NIH grant NS094896.. **C.J. Keatley:** None. **S. Gajavelli:** None. **S. Koch:** None. **Y.S. Ahn:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Dr. Ahn received grant support from RxMP Therapeutics.. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); RxMP Therapeutics provided the testing material for the study.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dr. Ahn and the University of Miami have partial ownership in RxMP Therapeutics. Dr. Ahn is a co-inventor of 2 US patents related to red cell microparticles. **M.A. Perez-Pinzon:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; This study was supported by NIH grant NS094896. **W. Jy:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; This study was supported by NIH grant NS094896. Dr. Jy received grant support from RxMP Therapeutics.. C. Other Research Support (receipt of

drugs, supplies, equipment or other in-kind support); RxMP Therapeutics provided the testing material for the study.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dr. Jy and the University of Miami have partial ownership in RxMP Therapeutics. Dr. Jy is a co-inventor of 2 US patents related to red cell microparticles. **K.R. Dave:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; The present study was supported by NIH grant NS094896.. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); RxMP Therapeutics provided the testing material for the study.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The University of Miami has partial ownership in RxMP Therapeutics. The University of Miami is a co-inventor of 2 US patents related to red cell microparticles..

Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.29/I11

Topic: C.09.Stroke

Support: NIH R01 NS085167
NIH R01 NS094384

Title: Development of a novel system for motor and sensory rehabilitative therapy after neurological injury

Authors: *D. PRUITT, E. MEYERS, Y.-N. DUONG, J. WRIGHT, J. EPPERSON, R. AFFENIT, S. HAYS, M. KILGARD;
Univ. of Texas at Dallas, Richardson, TX

Abstract: Stroke and spinal cord injury are the leading causes of long-term acquired disability. Despite the magnitude of the problem, available rehabilitation programs often provide only limited improvements in function. Rising medical costs and an inability to travel to a rehabilitation clinic without assistance lead to missed rehabilitation sessions and less-than-optimal dosage of movement repetitions. We have developed an Android tablet-based therapy system paired with novel motion-tracking devices designed for both clinic and at-home use with stroke and spinal cord injury patients. Using this system, we demonstrate that at-home compliance with prescribed rehabilitation exercises can be upwards of 97%. Additionally, we have found that quantitative outcome assessments using our system are highly correlated with GRASSP and Jebsen scores, two measures commonly used in the clinic with stroke and spinal

cord injury patients. Ongoing studies will continue to investigate the usage of this system for recovery of both motor and sensory function in patients with neurological injuries.

Disclosures: D. Pruitt: None. **E. Meyers:** None. **Y. Duong:** None. **J. Wright:** None. **J. Epperson:** None. **R. Affenit:** None. **S. Hays:** None. **M. Kilgard:** None.

Poster

135. Stroke I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 135.30/I12

Topic: C.09.Stroke

Support: NIH R01 NS085167
NIH R01 NS094384
DARPA N66001-15-2-4057
DARPA N66001-17-2-4011

Title: An at-home and clinical system for arm and hand rehabilitative therapy after stroke and spinal cord injury

Authors: *E. C. MEYERS[1], D. T. PRUITT[1], R. AFFENIT[1], Y.-N. DUONG[1], J. WRIGHT[2], J. EPPERSON[2], R. L. RENNAKER[1], S. A. HAYS[3], M. P. KILGARD[4];
[1]Texas Biomed. Device Ctr., [2]Bioengineering, The Univ. of Texas at Dallas, Richardson, TX; [3]Bioengineering, [4]Behavioral and Brain Sci., Univ. of Texas at Dallas, Richardson, TX

Abstract: Loss of arm and hand function after neurological injury such as stroke and spinal cord injury affects millions of people worldwide. Physical rehabilitation is the standard of care for helping patients regain function in the affected hand, and most patients undergo some amount of rehabilitation upon discharge from the hospital. However, many patients have difficulties attending appointments due to factors including a lack of travel assistance, the high cost of care, and limited access to therapists. Furthermore, the dosage of movement repetitions provided in the clinic is substantially lower than the effective dosage suggested by recent preclinical studies. To overcome these challenges, we have developed a suite of tools that provide an automated rehabilitation regime that collects highly sensitive and quantitative measurements of arm and hand function for delivery of rehabilitation both in the clinic and at home. These tools interface with a custom software suite comprised of multiple video games and assessment packages. We find that these devices provide a useful assessment of upper limb function over time in participants, substantially increase movement repetitions, and outcome measures are well-correlated with standard clinical assessments such as GRASSP. Ongoing studies are investigating the at-home utility and usability of these devices in patients with stroke and spinal cord injury.

Disclosures: **E.C. Meyers:** None. **D.T. Pruitt:** None. **R. Affenit:** None. **Y. Duong:** None. **J. Wright:** None. **J. Epperson:** None. **R.L. Rennaker:** None. **S.A. Hays:** None. **M.P. Kilgard:** None.

Poster

136. Spinal Cord Injury: Responses and Repair

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 136.01/I13

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant NS104422

Title: Role of blood pressure in pain-induced hemorrhage after spinal cord injury: Does blocking the nociception-induced rise in blood pressure have a protective effect?

Authors: *D. T. JOHNSTON[1], M. M. STRAIN[2], K. E. HUDSON[1], M. K. HENWOOD[1], G. N. FAUSS[1], C. R. WEST[3], J. W. GRAU[1];
[1]Texas A&M Univ., College Station, TX; [2]Army Inst. of Surgical Res., JBSA Fort Sam Houston, TX; [3]Cell. and Psychological Sci., Univ. of British Columbia, Kelowna, BC, Canada

Abstract: Prior work has shown that engaging pain fibers after spinal cord injury (SCI) by applying noxious electrical stimulation or the irritant capsaicin increases tissue loss and impairs long-term recovery (Grau et al., 2017, *J Neurotrauma*, *34*, 1873). These adverse effects have been related to a breakdown in the blood-spinal cord barrier and the infiltration of blood (hemorrhage). Noxious electrical stimulation also induces an acute increase in blood pressure. The current study examined whether this rise in blood pressure is causally related (necessary) to pain-induced tissue loss and impaired recovery. Male Sprague-Dawley rats received a contusion injury at the T11-12 vertebrae. The next day, animals were given an intraperitoneal (IP) injection of the alpha-1-adrenoreceptor agonist, prazosin (3 mg/kg), or vehicle. Thirty minutes later, noxious electrical stimulation (6 minutes of variable intermittent shock) was applied to the tail. Blood pressure and heart rate were assessed before/after drug treatment and for a period of 3 hrs after shock. A 1-cm region of tissue encompassing the injury site was collected and assessed for hemorrhage using the Drabkin's assay. Prazosin attenuated the shock-induced rise in systolic blood pressure and hemorrhage. We then assessed whether drug treatment attenuates the adverse effect noxious stimulation has on behavioral recovery. No effect was observed. Additional assays suggest that prazosin may have been ineffective because there was a rebound in blood pressure 24 hrs after treatment. Additional studies are being conducted to assess whether a drug (carvedilol) that blocks both beta-1 and alpha-1 adrenergic activity has a protective effect.

Disclosures: **D.T. Johnston:** None. **K.E. Hudson:** None. **C.R. West:** None. **M.M. Strain:** None. **M.K. Henwood:** None. **G.N. Fauss:** None. **J.W. Grau:** None.

Poster

136. Spinal Cord Injury: Responses and Repair

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 136.02/I14

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Department of Defense (SC170241)
Craig H Neilsen Foundation

Title: Pain-induced hemorrhage after spinal cord injury: Pentobarbital anesthesia and local application of lidocaine prevent hemorrhage when given before, but not after, noxious stimulation

Authors: *J. DAVIS, M. K. HENWOOD, C. C. COX, R. E. BAINE, J. W. GRAU;
Texas A&M Univ., College Station, TX

Abstract: Nociceptive stimulation after spinal cord injury (SCI) can increase tissue loss (secondary injury) and impair long-term recovery (Grau et al., 2017 J Neurotrauma 34, 1873). This effect depends, in part, on communication with rostral (brain-dependent) systems. Supporting this, nociceptive stimulation does not induce hemorrhage in animals that have undergone a rostral transection (Reynolds et al., 2017 J Neurotrauma, 34, 1200 1208). Likewise, inhibiting neuronal communication with the brain by slowly infusing lidocaine rostral to injury (T2) blocks nociception-induced hemorrhage and the impairment of long-term recovery. Administration of systemic anesthetic (pentobarbital) has the same effect (Davis et al., 2017 J Neurotrauma 34, A-121). The present study examines whether these treatments are effective if given immediately after noxious stimulation. In all experiments, Sprague-Dawley rats received a contusion injury at the T11-12 vertebrae and, 24 hours later, were exposed to 6 min of noxious stimulation applied to the tail.. Three hours after shock, rats were sacrificed and one cm of tissue was collected enveloping the injury site. The extent of hemorrhage was assessed by measuring the absorbance of light at the absorbance peak for hemoglobin (420nm) and western blotting targeting hemoglobin. As expected, nociceptive stimulation increased the extent of hemorrhage in vehicle treated (awake) rats. This effect was not observed in rats that were pretreated with pentobarbital (50mg/kg i.p.). Inhibiting neural function at the site of injury with lidocaine had the same effect. Likewise, inhibiting communication with the brain by applying lidocaine rostral to injury (at T2) blocked nociception-induced hemorrhage when given prior to shock treatment. All three treatments had no effect when applied immediately after noxious electrical stimulation. Additional studies are being conducted to assess the effect of anesthesia on capsaicin-induced hemorrhage.

Disclosures: J. Davis: None. **M.K. Henwood:** None. **C.C. Cox:** None. **R.E. Baine:** None. **J.W. Grau:** None.

Poster

136. Spinal Cord Injury: Responses and Repair

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 136.03/I15

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant NS104422

Title: Role of blood pressure in pain-induced hemorrhage after spinal cord injury: Brain systems may drive an increase in blood pressure, but this effect was not sufficient to induce hemorrhage

Authors: *G. N. K. FAUSS[1], M. M. STRAIN[3], Y.-J. HUANG[1], J. A. REYNOLDS[1], J. A. DAVIS[2], M. K. HENWOOD[1], C. R. WEST[4], J. W. GRAU[1];
[1]Texas A&M Inst. for Neurosci., [2]Psychological and Brain Sci., Texas A&M Univ., College Station, TX; [3]Burn and Soft Tissue Injury, US Army Inst. of Surgical Res., Fort Sam Houston, TX; [4]Kinesiology, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Spinal cord injuries (SCI) are commonly comorbid with a number of debilitating conditions that lead to impaired functional recovery. Previous work has shown that pain input caudal to injury expands the area of tissue loss (secondary injury) and impairs long-term recovery (Grau et al., 2017, *J Neurotrauma, 34*, 1873). Interestingly, pain input (provided by noxious electrical stimulation or capsaicin) has no effect if communication with the brain is blocked by means of a rostral (T2) transection. Here we examine whether brain processes fuel hemorrhage by driving an increase in blood pressure. Male Sprague-Dawley rats received a contusion injury at the T11-12 vertebrae. Eighteen hr later animals received a spinal transection at T2 or a sham surgery. Six hours later, pain fibers were engaged by applying noxious electrical stimulation (shock) or capsaicin caudal to injury. Blood pressure and heart rate were assessed prior to noxious stimulation and for a period of 3 hrs after. A 1-cm region of the spinal cord that encompassed the injury was then collected and assessed for hemorrhage. Both forms of nociceptive stimulation induced hemorrhage, but only shock treatment produced an increase in blood pressure and heart rate. These cardiovascular effects were blocked by a spinal transection. Next, we assessed whether inducing a rise in blood pressure increases hemorrhage in contused rats that received a rostral transection and noxious shock. Treatment with the adrenergic agonist norepinephrine induced a robust increase in heart rate and blood pressure but did not, in combination with shock treatment, increase the area of hemorrhage. The results suggest that inducing a rise in blood pressure may be neither necessary nor sufficient to drive nociception-induced hemorrhage after SCI.

Disclosures: G.N.K. Fauss: None. **M.M. Strain:** None. **Y. Huang:** None. **J.A. Reynolds:** None. **J.A. Davis:** None. **M.K. Henwood:** None. **C.R. West:** None. **J.W. Grau:** None.

Poster

136. Spinal Cord Injury: Responses and Repair

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 136.04/I16

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH NS104422
DoD SC170241

Title: Role of blood pressure in pain-induced hemorrhage after spinal cord injury: Effect of shock and capsaicin treatment on blood pressure, behavioral performance, and hemorrhage over time

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Abstract: In trauma resulting in spinal cord injury, accompanying injuries (polytrauma) provide pain (nociceptive) input. Our laboratory has shown that this can increase the area of tissue loss (secondary injury) and impair long-term recovery (Grau et al., 2017, *J Neurotrauma, 34*, 1873). In a typical experiment, male Sprague-Dawley rats receive a contusion injury at the T11-12 vertebrae, and noxious stimulation (variable intermittent shock or the irritant capsaicin) is applied caudal to injury 24 hrs later. Both treatments increase hemorrhage at the site of injury and undermine long-term behavioral recovery. The present study examined whether these treatments also induce an increase in blood pressure/heart rate and how the effects of noxious stimulation unfold over time. Rats received a contusion injury and a day later were treated with noxious electrical stimulation or capsaicin. Blood pressure and locomotor function were monitored for a period of 3, 24, or 192 hrs. At the end of the observation period, a 1-cm region of the spinal cord encompassing injury site was collected and hemorrhage was assessed using the Drabkin assay. Both shock and capsaicin impaired locomotor function and increased hemorrhage, with robust effects observed 3-24 hr after treatment. While noxious electrical stimulation produced an increase in acute blood pressure, capsaicin treatment had relatively little effect. Additional work is being conducted to evaluate the acute effect of capsaicin treatment on blood pressure.

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Poster

136. Spinal Cord Injury: Responses and Repair

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 136.05/I17

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Swedish Research Council
Swedish Brain Foundation
Knut and Alice Wallenberg Foundation
Hållsten Foundation

Title: Defining the origin of fibrotic scar tissue at single cell resolution

Authors: *D. HOLL[1], W. HAU[1], D. OLIVEIRA DIAS[1], S. SAVANT[1], M. AMIRY-MOGHADDAM[2], C. GORITZ[1];
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Abstract: Central nervous system lesions often lead to long lasting functional deficits due to poor regeneration. Following spinal cord injury, one major obstacle for axonal regeneration and recovery is the formation of a fibrotic scar. The primary source of stromal cells that form the fibrotic scar is a discrete subpopulation of perivascular cells, termed type A pericytes. Combining fate mapping of type A pericytes with single cell transcriptome profiling, we investigated the origin of fibrotic scar tissue at single cell resolution. Our study revealed heterogeneity within the scar-forming type A pericyte population, identifying two distinct subpopulations, one large and one small subset accounting for 90% and 10% of the scar forming pericytes, respectively. The small subset distinctively expressed Col1a1. Fate mapping of Col1a1^{+}perivascular cells after spinal cord crush injury showed partial fibrotic scar contribution in comparison with type A pericyte-derived scar tissue. These results indicate that both subsets contribute to fibrotic scar formation after crush injuries. Characterization of the two scar-forming perivascular populations using immunohistochemistry and immune electron microscopy showed distinct characteristics between both subsets, with the Col1a1 subset being mainly associated with larger vessels while the larger subset of scar forming cells is associated with smaller vessels and encapsulated by a basal lamina. Injury induced gene expression changes of scar forming pericytes affected fibrosis associated pathways and provide relevant information about the fate changing mechanisms driving a perivascular cell towards a myofibroblast. Utilizing the gene expression profile of the distinct populations allows us to follow the fate of the different pericyte subtypes and their specialized function during fibrotic scar formation. Our data further define the cellular origin of fibrotic scar tissue and provide insights into the transcriptional changes following central nervous system injury at single cell resolution.

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Poster

136. Spinal Cord Injury: Responses and Repair

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 136.06/I18

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Swedish Research Council
Swedish Brain Foundation
Knut and Alice Wallenberg Foundation
Hållsten Foundation

Title: Vascular dynamics during scar formation after spinal cord injury in 3D

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Abstract: Injury to the central nervous system (CNS) induces a wound healing reaction and consequently scar formation. Part of the endogenous repair process is the re-vascularization of injured tissue mediated through angiogenesis and vascular remodulation. Besides the importance for tissue revascularization, sprouting vessels also bring scar forming pericytes into the lesion, a process important for the regain of tissue integrity but consequently leading to fibrotic scar formation. Using tissue clearing in combination with light sheet and confocal microscopy, we elucidated the vascular dynamics following spinal cord injury.
Blood vessels were filled by perfusion of fluorescent-labelled albumin in gelatin. In addition, we labelled pericytes using the PDGFRb-EGFP and Glast-CreER^{T2} x tdTomato mouse lines. Using tissue clearing and image rendering, we were able to reconstruct the whole vasculature in a mouse spinal cord segment including their associated perivascular cells. In the uninjured spinal cord, scar-forming type A pericytes were present on all infiltrating vessels until a certain number of bifurcations. To investigate vascular dynamics following injury, we examined the vasculature in the lesioned segment between 1 and 7 days after spinal cord injury. Our results indicate that the first vessel sprouts appear in the lesion around 3 days after injury. From day 7 post lesion we found functional blood vessels. Scar-forming pericytes entered the lesion between 3 and 7 days post injury associated with sprouting vessels. Our results suggest angiogenic sprouting as part of the mechanism leading to fibrotic scarring.

Disclosures: **W. Hau:** None. **C. Goritz:** None.

Poster

136. Spinal Cord Injury: Responses and Repair

Location: Hall A

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Program #/Poster #: 136.07/I19

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Swedish Research Council
Swedish Brain Foundation
Knut and Alice Wallenberg Foundation
Hållsten Foundation

Title: Myelin damage induces pericyte-derived fibrotic scarring

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Abstract: Central nervous system (CNS) injury often severs axons. The lack of adequate regeneration in the adult CNS following traumatic injury is attributed to an inflammatory response, myelin debris and the formation of an inhibitory scar, which prevents re-growing axons to cross the lesion and re-innervate downstream targets. However, is unclear if these factors are independent from each other. We previously identified a discrete population of pericytes, termed type A pericytes, as the major source of scar-forming fibroblasts that constitute the fibrotic scar (Goritz et al., Science, 2011). Recently we established that pericyte-derived fibrosis limits axonal regeneration and functional recovery after spinal cord injury (Dias et al., Cell, 2018).
Here we investigate the mechanisms leading to fibrosis following lesions to the CNS. We report that white matter lesions lead to more fibrotic scarring compared to grey matter lesions. Using the lysolecithin demyelination model, we show that myelin damage is sufficient to evoke fibrotic scarring. Furthermore, syngenic injection of myelin debris into gray matter spinal cord regions mimics white matter-damage induced fibrotic scarring and shows that myelin debris is the determinant factor for pericyte-derived fibrosis. The number of fibrotic pericytes recruited in response to myelin debris linear correlated to the number of $MAC2^{+}$ immune cells at the lesion side. Reduction of monocyte infiltration in $CCR2^{-/-}$ mice also reduced fibrotic pericyte recruitment to a similar extend. Together, our results show that fibrotic tissue-forming pericytes are co-recruited from the blood vessel wall by infiltrating monocytes, a process triggered by myelin debris. Our study links the three axon growth inhibitors pericyte-derived scarring, myelin debris and infiltrating immune cells together in the process of fibrotic scar formation.

Disclosures: **J. Kalkitsas:** None. **D. Oliveira Dias:** None. **C. Goritz:** None.

Poster

136. Spinal Cord Injury: Responses and Repair

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 136.08/I20

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Swedish Research Council
Swedish Brain Foundation
Knut and Alice Wallenberg Foundation
Hållsten Foundation

Title: A perivascular origin of fibrotic scar tissue across diverse central nervous system lesions

Authors: *D. O. DIAS[1], J. KALKITSAS[1], Y. KELAHMETOGLU[1], C. P. ESTRADA[2], J. TATARISHVILI[3], A. ERNST[1], Z. KOKAIA[3], O. LINDVALL[3], L. BRUNDIN[2], J. FRISÉN[1], C. GÖRITZ[1];
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Abstract: Lesions to the central nervous system often result in long lasting functional deficits due to poor tissue regeneration and the formation of a permanent scar. Scar tissue is required to regain tissue integrity but blocks axon regeneration and prevents functional recovery. Despite the extensive knowledge on scar-forming astrocytes, less attention has been drawn to the fibrotic, or stromal, component of the scar.We previously demonstrated that, in the injured mouse spinal cord, the primary source of fibroblast-like stromal cells that accumulate at the non-neural lesion core of the scar is a discrete subpopulation of perivascular cells lining the microvasculature, termed type A pericytes. Attenuation of pericyte-derived fibrotic scarring promotes axon regeneration and ameliorates sensorimotor recovery after spinal cord injury. Here, we set out to investigate the cellular origin of scar-forming fibroblasts following various pathologies and traumatic lesions to the brain and spinal cord. Using inducible *in vivo* lineage tracing, we investigated the contribution of type A pericytes to scar formation following penetrating and non-penetrating lesions to the spinal cord, traumatic brain injury, experimental autoimmune encephalomyelitis and transient middle cerebral artery occlusion-induced ischemic stroke. In addition, we explored the contribution of type A pericytes to tumor stroma in a glioblastoma model. In all lesion models, except striatal ischemic stroke, type A pericytes generated progeny that dissociated from the blood vessel wall and gave rise to fibroblast-like stromal cells. The magnitude of pericyte recruitment from the vasculature was dependent on the extent and type of lesion. We conclude that pericyte-derived fibrotic scar tissue formation is a conserved mechanism in response to different central nervous system lesions and identify type A pericytes as a novel therapeutic target to interfere with stroma formation.

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Poster

136. Spinal Cord Injury: Responses and Repair

Location: Hall A

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Program #/Poster #: 136.09/I21

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Grantin-Aid for Scientific Research on Innovative Areas Adaptive Circuit Shift to T.I. (Project no. 26112008)

Title: The biomarker of motor recovery after spinal cord injury in macaque monkey assessed by resting-state electrocorticography

Authors: *T. KAWASAKI[1,2], R. YAMAGUCHI[3], Z. C. CHAO[1], M. MITSUHASHI[1,4], S. UENO[1], Y. YAMAO[2], T. KIKUCHI[2], K. YOSHIDA[2], S. MIYAMOTO[2], T. ISA[1];
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Abstract: Spinal cord injury (SCI) inflicts severe permanent damage on motor and somatosensory functions but accumulating evidence has shown that impaired movements could recover considerably through rehabilitative training in some cases. Our previous studies showed that the rehabilitative training following the SCI induces neuronal plasticity in the central nervous system not only in the spinal cord but also in the cerebrum in macaque monkeys (Isa, Ann Rev Neurosci 2019). However, the electrophysiologically-measured resting-state brain activity after the SCI has not been carefully examined. To investigate the change in the resting-state brain network structure associated with recovery of motor functions, we chronically implanted the multi-channel electrocorticogram (ECoG) electrodes (18 channels each) on the bilateral the primary motor (M1), primary somatosensory (S1) and premotor (PM) cortices of the macaque monkey, and longitudinally monitored brain activity before and after the sub-hemisection of the spinal cord at C4/C5. In the current model, the monkey started reaching after 20 days following the injury. However, the recovery stopped at the coarse power grip and the precision grip did not fully recover. First, we calculated the Granger causality to investigate connectivity between each electrode using all data in awake and anesthetized conditions. And then, we applied Parallel factor analysis (PARAFAC) to extract structured information from high-volume datasets of cortical activation or corticocortical interactions. We identified a distinct brain network: the interhemispheric connections from contra-lesional PM/M1 to ipsi-lesional

PM/S1 cortices in beta frequency band (peak is around 28 Hz). In the awake condition, the strengths of this network structure increased after Day 18 post-injury almost simultaneously as the monkey started reaching. On the other hand, there was no remarkable dynamic changes after SCI in the anesthetized condition. These results suggested that the change in the resting-state brain activity after SCI under the awake condition would be different from that recorded under the general anesthesia. The interhemispheric connection in beta frequency band at resting-state condition might become a biomarker of the motor functional recovery after SCI.

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Poster

136. Spinal Cord Injury: Responses and Repair

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 136.10/I22

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Grantin-Aid for Scientific Research on Innovative Areas Adaptive Circuit Shift to T.I. (Project no. 26112008)

Title: Functional brain network for recovery of hand functions after spinal cord injury

Authors: *R. YAMAGUCHI[1], T. KAWASAKI[2,3], Z. C. CHAO[2], M. MITSUHASHI[2,4], S. UENO[2], T. ISA[1,2];
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Abstract: Spinal cord injury (SCI) causes long-term devastating loss of physical functions in the patients. Elucidating the neuronal mechanism of recovery from SCI is expected to contribute to development of better therapeutic strategies. We previously showed that dexterous hand movements recovered in 1-3 months after the SCI limited to the lateral corticospinal tract in the dorsolateral funiculus (DLF) at the C4/C5 cervical segments in macaque monkeys. We also found that the motor-preparation-related interhemispheric interactions from the contralesional premotor cortex (PM) to the ipsilesional PM and primary motor cortex (M1) at the α and low-β bands is enhanced during early recovery period in the DLF lesion model (Chao et al., 2018). On the other hand, expansion of lesion size would cause severe impairment in dexterous hand movements compared with the DLF lesion. The brain activity associated with recovery from the larger lesion would be different from that after the DLF lesion. In this study, we chronically implanted the multi-channel ECoG electrodes in bilateral PM/M1/S1 (primary sensory) and

longitudinally monitored the cortical activity during the reach and grasp task before and after the sub-hemisection. After Day 16 post-injury, the monkey started reaching but could hardly move his digits. The monkey started grasping after Day 32. Since then digit movements became smoother and faster as the days passed. But the recovery stopped at the coarse power grip and the precision grip did not recover. The cortical electrical stimulation through each ECoG electrode (at 3 mA, 3 shocks at 20 Hz) was tested. Muscle twitches started being induced in proximal muscles from the contralesional PM/M1 after around Day 16. Twitch responses gradually spread from the proximal to distal muscles including digits almost simultaneously as the monkey started grasping and became inducible from ipsilesional PM/M1. The interhemispheric interaction became facilitatory. These results suggested that disinhibition spread across bilateral global cortical network along with the recovery. We calculated the Granger causality (GC) to evaluate the connectivity between each pair of ECoG channels. GC between bilateral PM/M1 at the high-γ band increased during the grasping after Day 32. These results suggested that global disinhibition in the cortical networks might be related to the recovery of grasping-related commissural interaction between bilateral PM/M1 at high-γ band and contributed to the recovery after the sub-hemisection. However, lack of commissural interaction at the α and low-β bands observed in case of DLF lesion might explain the limited recovery in case of sub-hemisection.

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Poster

136. Spinal Cord Injury: Responses and Repair

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 136.11/I23

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Spatiotemporal histopathological pattern of the porcine spinal cord after SCI

Authors: *E. B. OKON[1], X. ZHAO[1], S. DALKILIC[1], S. BASNAYAKE[1], A. ALLARD BROWN[1], R. M. CHAN[1], F. STREIJGER[1], B. K. KWON[1,2];
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Abstract: Pigs are similar to humans in anatomy, physiology and immunological responses, and thus may serve as a useful large animal preclinical model to study potential cellular- and drug mediated- treatments for human spinal cord injury (SCI). Understanding the histology of the injured porcine spinal cord is important for the proper characterization and efficient use of such animal models. Therefore, in this study we investigated the spatial and temporal histopathological changes after a contusion/compression injury using female Yucatan pigs.

Before SCI, and at 1 and 12 weeks following injury, a 30-mm spinal cord segment centred at the injury epicentre was collected and prepared for cutting on the cryostat at 20 µm. Cross-sections from the epicentre, 5 mm from the epicentre and 15 mm from the epicentre were immunostained for myelin basic protein (MBP), neurons, axons, astrocytes, and chondroitin sulphate proteoglycans (CSPGs), as well as with Wisteria Floribunda Lectin (WFA) for visualization of the perineuronal nets (PNNs) and Eriochrome Cyanine R for a gross estimation of the spread of white and grey matter sparing. Immunofluorescence labeling of the white matter of the spinal cords from SHAM animals revealed a typical pattern of nerve fibers and myelin rings surrounded by CSPG-positive endoneurium. In the ventral, and lateral horns of the grey matter, neuronal cells bodies were predominantly surrounded by PNN. At 1 and 12 weeks after SCI, the structural integrity of myelin, axons and CSPGs in the white matter, and PNN was clearly disrupted at the lesion epicentre, with massive macrophage infiltration in between regions of myelin debris. No labeling of neuronal cell bodies and astrocytes was observed in the damaged grey matter at 1 week. At 12 weeks after injury, the lesion epicenter was characterized by isolated patches of CSPG-positive tissue, embedded with individual non-myelinated and myelinated nerve fibers and astrocytes. At 5 mm, a densely packed GFAP-positive astroglial scar was clearly distinguishable. At 15 mm rostral to the injury site, immunoreactivity for astrocytes and CSPGs was still high, and the regular, dense cytoarchitectural organization, which was observed in the spinal cord of the SHAM animals, was still impaired.
The present data extends our investigations into the pattern of changes that affect the myelin and axonal architecture of the porcine spinal cord after SCI, which is crucial for the design and execution of pre-clinical SCI studies and for the most appropriate use of cellular and drug treatments.

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Poster

136. Spinal Cord Injury: Responses and Repair

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 136.12/I24

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Characterization of gut microbiome changes in a porcine model of traumatic SCI

Authors: *M. WEBSTER[1], S. TIGCHELAAR[1], M. S. KEUNG[1], B. MCCONEGHY[2], F. STREIJGER[1], S. SINHA[2], S. FISK[1], C. MORRISON[1], C. NISLOW[2], B. K. KWON[1,3];
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Abstract: While much of SCI research has focused on neuroprotection and repair, the consequences of SCI on remote organs and systems is not well understood. SCI causes significant adverse effects on a variety of organ systems, such as the gastrointestinal (GI) tract, kidney, bladder, and liver, affecting their function and compromising quality of life of SCI patients. In the GI tract alone, patients experience delayed gastric emptying, abdominal pain, and deficits in gut motility. GI complications are responsible for 11% of hospitalizations in the SCI population. Importantly, 30% of patients living with SCI consider bowel disorders to be a greater concern than bladder or sexual dysfunction. Despite these significant effects of SCI on GI function and vested interest by SCI stakeholders, limited studies have focused on a better understanding of the acute and chronic effects of SCI on GI function. The gut microbiome is a significant determinant of health and is critical for the development and maintenance of cellular metabolism, digestion, nutrient absorption, and immune system development. Gut microbes also regulate normal development and disease pathogenesis in the central nervous system. While current studies characterizing the changes in gut microbiota are limited to small animal, rodent models of SCI, we sought to evaluate the changes in gut microbiota in a large animal, porcine model of contusion SCI. Pigs represent an ideal model to study SCI-related changes in microbiota due to their similarities in anatomy, genomic makeup, and microbiome composition. Female Yucatan minipigs underwent a T2 or T10 spinal cord injury using a weight drop contusion impactor, followed by compression for 5 minutes. Fecal samples were obtained weekly for 5 weeks prior to SCI in order to characterize baseline porcine microbiota composition. Following SCI, fecal samples were collected daily for 14 days, followed by weekly sampling for 10 weeks. Post-SCI microbiota were compared to non-SCI SHAM operated animals. Behavioural analyses were conducted weekly for 12 weeks to quantify hindlimb recovery, and at 12 weeks, spinal cords were collected for histological analysis. Using 16s ribosomal RNA gene sequencing, we identified the dysregulation of gut microbiota implicated in CNS trauma in fecal samples collected from pigs with SCI. By characterizing the gut dysbiosis following SCI in pigs, we hope to utilize this large animal model for further investigation into the effects of antibiotic use following SCI, chronic inflammation, and the therapeutic potential of probiotics or fecal transplants.

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Poster

136. Spinal Cord Injury: Responses and Repair

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 136.13/I25

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Continuous optical monitoring of spinal cord hemodynamics during the first 7 days post-injury in a porcine model of acute spinal cord injury

Authors: *A. CHEUNG[1], L. TU[1], F. SHAHRAGARD[1], N. MANOUCHEHRI[1], K. SO[1], M. WEBSTER[1], S. FISK[1], S. S. TIGCHELAAR[1], F. STREIJGER[1], A. MACNAB[2], B. KWON[1,3], B. SHADGAN[1,4];
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Abstract: Introduction: Current clinical guidelines recommend augmenting the mean arterial pressure (MAP) in acute spinal cord injury (SCI) patients to increase spinal cord perfusion and potentially improve neurologic function. However, it is difficult for clinicians to hemodynamically manage acute SCI patients without real-time physiologic information about the effect of MAP augmentation within the injured cord. A non-invasive method for monitoring physiologic parameters inside the injured spinal cord would greatly optimize the hemodynamic management of acute SCI. We developed an implantable optical sensor, based on Near Infrared Spectroscopy (NIRS), for non-invasive real-time monitoring of regional spinal cord tissue oxygenation and hemodynamics after acute SCI. *In this study, we investigated the feasibility and validity of using a customized NIRS sensor to continuously monitor spinal cord oxygenation and hemodynamics during the first 7 days post-injury in a porcine model of acute SCI.*
Methods: Six Yucatan mini-pigs weighing between 25-31 kg underwent a dorsal laminectomy at the T5 to L1 levels and received a weight-drop T10 contusion-compression injury. A multi-wavelength NIRS system with a custom-made miniaturized optical sensor was placed directly onto the dura at T9 to non-invasively measure tissue oxygenation and hemodynamics within the spinal cord. Using NIRS, the spinal cord tissue oxygenation percentage (TOI%) and concentrations of oxygenated (O_2Hb), deoxygenated (HHb) and total hemoglobin (THb) were monitored before and after SCI. To validate the NIRS measures, an invasive intraparenchymal (IP) combined PO2/blood flow (SCBF) sensor was inserted directly into the spinal cord adjacent to the NIRS sensor at T11. Episodes of MAP alterations and hypoxia were performed acutely after injury, 2 days post-injury, and 7 days post-injury to simulate the types of hemodynamic changes SCI patients experience after injury.
Results: Non-invasive NIRS monitoring identified changes in spinal cord hemodynamics and oxygenation levels during episodes of MAP alterations and hypoxia throughout the first 7 days post-injury. Changes of O_2Hb and TOI% followed similar patterns of oxygenation changes measured by the IP PO_2 sensor, and changes of THb showed strong correlations with IP SCBF.
Conclusions: Our novel NIRS sensor is feasible as a non-invasive technique to monitor real-time changes in spinal cord oxygenation and hemodynamics 7 days post-injury. Further development of this method would allow a clinically applicable device spine surgeons could place on the dura at the time of surgical decompression to monitor spinal cord tissue hemodynamics post-injury.

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Poster

136. Spinal Cord Injury: Responses and Repair

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 136.14/I26

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Bridging biobanking and SCI research: From biological specimen to biomarkers

Authors: *N. MANOUCHEHRI[1], K. DONG[1], F. STREIJGER[1], L. A. M. MATZKE[2,3], T. E. TARMIN[2], S. DEE[2], A. ALUDINO[7], A. TSANG[7], L. REICHL[7], L. PARKER[8], F. SAMADI[1], C. FOURNIER[1], A. YUNG[4], P. KOZLOWSKI[1,4], C. LAULE[1,5], W. MOORE[1,2], P. WATSON[2], B. K. KWON[1,6];
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Abstract: A key obstacle to medical research involving acute spinal cord injury is the difficulty in obtaining sufficient amounts of sample to power their analyses. Despite the tragic prevalence of this type of injury, many potential donors are missed due to logistic problems in identifying and consenting them within the acute window.
Biobanks are repositories of biological samples that are used as a source for medical research. Their growth and prevalence have been driven by the needs of the research community for higher-powered studies and the emergence of high-throughput analytical technologies such as microarrays. An attractive aspect of these biobanks is they can provide researchers with material that would otherwise be too resource intensive to obtain on their own.
In 2016, the International Collaboration on Repair Discoveries (ICORD) announced the launch of ISCIB, a project for establishing the first spinal cord injury (SCI) biobank in Canada. ISCIB'S major goal is to enhance the distribution of high-quality, well-characterized human biospecimen to investigators and to increase awareness of the value of sample donation among the public. ISBC provides access to the collections of five partner sites, as well as additional resources, including medical records and clinical data sets and sample quantity metrics.
ISCIB is a novel translational research resource for future investigative purposes and international collaborations on biomarker analysis, which we believe can play a significant role

in predicting injury severity and providing insights into personalized medicine. To date, the bank has accumulated samples from over 150 acute SCI participants of AIS grades A, B, and C and their associated clinical data, with well over 20,000 combined serum & CSF aliquots as well as 9 post-mortem human spinal cords. Since 2018, ISCIB collects human post-mortem spinal cord tissue from SCI patients who donated their tissue. This is a valuable resource for research and has the potential to change our understanding of the microscopic changes that occur after traumatic SCI.
The biobank is a novel translational research resource for future investigative purposes and international collaborations on biomarker analysis, which we believe can play a significant role in predicting the severity of injury and providing insights into personalized medicine.

Disclosures: **N. Manouchehri:** None. **K. Dong:** None. **F. Streijger:** None. **L.A.M. Matzke:** None. **T.E. Tarmin:** None. **S. Dee:** None. **A. Aludino:** None. **A. Tsang:** None. **L. Reichl:** None. **L. Parker:** None. **F. Samadi:** None. **C. Fournier:** None. **A. Yung:** None. **P. Kozlowski:** None. **C. Laule:** None. **W. Moore:** None. **P. Watson:** None. **B.K. Kwon:** None.

Poster

136. Spinal Cord Injury: Responses and Repair

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 136.15/I27

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Urodynamics and telemetric monitoring of bladder function in minipigs

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Abstract: Background: The current gold standard for objectifying dysfunction of the lower urinary tract (LUT) is urodynamics testing. While animal models have been widely used for preclinical urodynamic investigations, there are limitations in the interpretation due to the one-time, non-physiological filling of the bladder. LUT studies would benefit greatly with longitudinal recording in non-anaesthetized and unrestrained animals with normal physiological filling of the bladder. Therefore, we explored a method for long-term recording of bladder function using a fully implantable wireless telemetry system in a minipig model.
Methods: A wireless telemetric transmitter was implanted into female Yucatan minipigs. Intravesical (Pves) and intra-abdominal pressures (Pabd) were obtained from the device by surgically implanting fine-wire pressure leads into the dome of the bladder and the abdominal

cavity, respectively. The detrusor pressure (Pdet) was calculated by subtracting Pabd from Pves. Electromyography (EMG) leads were inserted beneath the pubic bone, alongside the urethra, to measure the activity of the external urethral sphincter (EUS). Wires were passed subcutaneously and connected to the device located in a nearby subcutaneous pocket. Telemetric recordings were performed while the animals were in their pens and were repeated up to 2 times weekly for 2-hour sessions, for a total of 12 weeks without anesthesia or restraint. In addition, urodynamic studies with concurrent telemetric recording was performed.
Results: During the entire 12 weeks, the animals showed no observable negative effects related to the surgery or implants. The telemetric recordings during natural voiding were stable over 12 weeks and demonstrated visible bladder contractions with consistent changes in Pdet values. Changes in EMG activity were observed at the start and at the end of the void, possibly signifying EUS activity. Telemetric recording also demonstrated comparable pressure traces with urodynamics during an infusion evoked void. Furthermore, voiding detrusor contractions were observed in some (but not all) animals; which was contrary to natural voids. Currently, we are analyzing the telemetric and urodynamic data from spinal cord injured animals.
Conclusions: Telemetric monitoring is a validated and feasible methodological approach to monitor physiologically-relevant bladder function in awake, freely moving uninjured minipigs.

Disclosures: **M.S. Keung:** None. **M. Webster:** None. **F. Streijger:** None. **S. Fisk:** None. **K.N. Chen:** None. **N. Manouchehri:** None. **S. Tigchelaar:** None. **K. So:** None. **L. Stothers:** None. **A. Kavanagh:** None. **B.K. Kwon:** None.

Poster

136. Spinal Cord Injury: Responses and Repair

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 136.16/I28

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Evaluating the congruency between intraparenchymal and subdural intraspinal pressure in a porcine model of acute spinal cord injury

Authors: *A. ALLARD BROWN[1], M. RIZZUTO[1], K. T. KIM[1], K. SO[1], N. MANOUCHEHRI[1], M. WEBSTER[1], S. FISK[1], D. E. GRIESDALE[2], M. S. SEKHON[3], F. STREIJGER[1], B. K. KWON[1,4];
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Abstract: For decades, clinical practice guidelines for hemodynamic management have focused largely upon augmenting the mean arterial pressure (MAP) to improve blood flow and limit

secondary ischemic damage following spinal cord injury (SCI). Recently, however, clinical evidence has shown that the spinal cord perfusion pressure (SCPP), calculated as the difference between the MAP and the intraspinal pressure (ISP), is a better measure of blood flow and, importantly, is more closely associated with neurologic outcome than MAP. To fully capitalize upon the approach of SCPP monitoring for optimizing hemodynamic management, we must understand the factors that influence the measurement of ISP at the injury site. Here, we explored the **method** by which ISP is measured - either within the injured spinal cord or within the subdural space, how the relationship between these two ISP measurements evolved with **time,** and how relative **location** from the injury site influenced ISP measurements.
For this study, we used our pig model of a contusion-compression SCI at T10. Pressure sensors were placed 2-mm rostral and caudal from the injury site. At each location, one sensor was inserted inside the injured spinal cord ("intraparenchymal ISP"; ISP_{IP}) and another outside the cord within the subdural space ("subdural ISP"; ISP_{SD}). Pressure measurements were continuously recorded for up to 10 hours post-SCI. Hourly ultrasound images were captured to track and quantify spinal cord swelling and to compare such changes with changes in ISP.
Our preliminary data demonstrated that when the spinal cord swells to fill the entire intrathecal space, there is a strong positive correlation between ISP_{IP} and ISP_{SD} rostral to the injury site. Conversely, at this point there is no close correlation between ISP_{IP} and ISP_{SD} on the caudal side of the injury. Throughout the entire post-injury monitoring period, a clear positive correlation was observed between rostral and caudal ISP_{IP}; the strength of the correlation was not dependent on the amount of cord swelling within the intrathecal space. No positive correlation was found between rostral and caudal ISP_{SD}.
Our results suggest that the measurement of pressure within the spinal cord can be achieved with a subdural catheter, but that the accuracy of this measurement is dependent upon the cord swelling to fill the entire intrathecal space. Factors that influence the swelling (e.g. injury severity, location), and the relative size of the spinal cord to the thecal sac will dictate whether localized compression by the dura will occur.

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Poster

136. Spinal Cord Injury: Responses and Repair

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 136.17/I29

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Minnesota Spinal Cord and Traumatic Brain Injury Grant
North American Spine Society

Title: Segment specific orientation of the dorsal and ventral spinal roots in human

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Abstract: The understanding of the spinal cord functional neuroanatomy is essential for diagnosis, treatment, and management of multiple neurological and neurosurgical conditions. Previous computer simulation and animal studies suggest that location and segment-specific orientation of the spinal roots may influence the efficacy of epidural electrical stimulation (EES). Surprisingly, till now there is no information available on dorsal and ventral roots anatomy and their segment-specific orientation in human. In this study we collected main anatomical measurements of the dorsal and ventral roots in relation to the spinal cord and vertebral bone from nine (5 male and 4 female) adult alcohol-formaldehyde solution fixed cadavers. Spinal nerve roots from C1-L5 were extracted and meticulously dissected, with the following parameters evaluated under the microscope (Table).

Dorsal and ventral roots	**Spinal cord structures**	**Vertebra bones**
Number of dorsal and ventral rootlets Root diameter Rostral rootlet angle Caudal rootlet angle	Transverse diameter Width across dorsal columns Rostral rootlet to caudal rootlet length Segment length Intervertebral foramen to rostral and caudal rootlet distance	Midvertebrae foramen length Vertebral bone length Intervertebral foramen diameter Intervertebral foramen length

Evaluation of the dorsal and ventral roots angles demonstrated less variability in rostral compared to the caudal rootlet angles across all segments. Dorsal and ventral rootlets were oriented mostly perpendicular to the spinal cord midline at cervical level, while at the thoracic level they had mainly parallel orientation, and at the lumbar level they demonstrated gradual shift from parallel to perpendicular orientation. The number of rootlets for both dorsal and ventral roots was the highest for cervical (8.4 and 8.3) and lumbar (7.9 and 5.5) and the lowest for thoracic segments (4.9 and 4.4). Similarly, width across dorsal columns at cervical (0.69 cm) and lumbar segments (0.52 cm) was the highest; while at thoracic segments (0.5 cm) was the smallest. To our knowledge, this is the first detailed analysis of the segment-specific dorsal and ventral spinal roots' spatial orientation conducted for human. These results provide important background for correlation between anatomy of spinal cord structures with bone landmarks, which may improve surgical procedures, i.e. dorsal rhizotomy, intrathecal injections, and electrodes placement for spinal cord stimulation. Results of this study will also help to further optimize electrode selection based on angular variations of dorsal and ventral rootlets for spinal cord neuromodulation procedures.

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Poster

136. Spinal Cord Injury: Responses and Repair

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 136.18/I30

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Minnesota Spinal Cord and Traumatic Brain Injury Grant
North American Spine Society
Morton Cure Paralysis Fund

Title: Sparse re-connection across complete spinal cord injury influences sub-lesional spinal network excitability during epidural electrical stimulation enabled locomotor activity in rats

Authors: *R. ISLAM[1], A. SIDDIQUI[1], C. CUELLAR[1], J. SILVERNAIL[1], B. KNUDSEN[1], D. CURLEY[1], B. CHEN[1], T. LATYPOV[2], N. AKHMETOV[2], S. ZHANG[1], P. SUMMER[1], J. NESBITT[1], P. GRAHN[1], N. MADIGAN[1], M. YASZEMSKI[1], A. WINDEBANK[1], I. LAVROV[1];
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Abstract: Spinal cord injury (SCI) has lifelong devastating impacts on function and quality of life. Experimental approaches to repair neural tissue after SCI have investigated pharmacologic, cellular, and biomaterial agents, most of which have demonstrated limited potential with little-to-no functional recovery observed. Evidence suggests approximately 90% of humans diagnosed with complete loss of motor function due to SCI have limited anatomical connectivity across the injury (i.e., discomplete SCI). In these subjects, clinical trials of epidural electrical stimulation (EES) have enabled spinal sensorimotor network excitability and partial voluntary control over previously paralyzed motor functions. In this regard, elucidating the exact mechanisms of EES-enabled facilitation of locomotor activity in an animal model with limited connectivity may further advance EES therapy in SCI subjects and open the possibility of combining EES with neuroregenration therapies.To address this need, we developed a rodent model of complete thoracic spinal cord transection (T9) that combines EES, locomotor training, and tissue regeneration, via drug-eluting biodegradable multi-channel scaffolds seeded with Schwann cells that over express glial cell line-derived neurotrophic factor (GDNF) implanted at the site of SCI. A comparative analysis of gait recovery across multiple modalities including BBB score, kinematics and electromyography recorded in hind limb muscles, was performed for 7 weeks after SCI. Scaffold implanted rats underwent a re-transection at week 6 to determine extent of recovery. Starting at 2 weeks after SCI, significant improvement in EES-facilitated stepping (40

Hz, 1-2.5V) was observed in animals implanted with scaffolds compared to no-scaffold group. No significant difference in stepping ability was observed between groups with and without scaffolds in the absence of EES. After re-transection through the scaffold at week 6, EES-enabled motor function was reduced, still remaining higher compared to control group that received SCI with no regenerative intervention. These findings suggest *sub-functional connectivity across SCI influences excitability and functional reorganization of spinal networks that interact with EES to enable motor function in discomplete SCI.* These results also demonstrate the establishment of new experimental pre-clinical platform to study the spinal cord regeneration and neuroplasticity and potential of combining neuromodulation and neuroregeneration therapies to achieve functional recovery after severe SCI.

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Poster

136. Spinal Cord Injury: Responses and Repair

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 136.19/I31

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Minnesota Spinal Cord and Traumatic Brain Injury Grant
North American Spine Society

Title: Comparison of lumbar spinal cord anatomy between small and large animal models vs. human: Clinical and electrophysiological implication

Authors: *P. BASA[1], A. MENDEZ[1], R. ISLAM[1], N. LACHMAN[2], P. J. GRAHN[3], A. J. WINDEBANK[1], I. A. LAVROV[1,4,5];
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Abstract: Spinal cord epidural electrical stimulation (EES) is commonly used procedure to control chronic pain with growing number of potential applications. Multiple preclinical and recent clinical studies led to encouraging results on recovery of motor functions after spinal cord injury with EES. Studies performed on small and large animal models suggest that location and orientation of dorsal roots in relation to electrical field play a critical role in outcome of EES. These findings inspired an idea of correlation between spinal cord anatomy and motor evoked potentials (MEP) in different animal models vs. human. In this study we collected lumbar spinal cord anatomical measurements from small (rats, n=6) and large (white swine, n=7) animal

models and compared them with measurements on human cadavers (male, n=5 and female, n=4). EES-evoked MEP recorded from selected hind limb muscles were retrieved from our previous studies with following comparative analysis of electrophysiological and anatomical data. *Focusing on dorsal roots' anatomy as a main target for EES,* we found significant variation in lumbar segments' rostral angles for human and caudal angles for pig, while no variations in dorsal root angles was observed in rats. *Rostral angles* in human gradually decreased from L1 to L5 (169^{o}-160^{o}) and had no significant variations in rats and pigs. *Caudal angles* exhibited more parallel alignment along the midline across all lumbar segments in human and rats (13^{o}-15^{o} and 23^{o}-17^{o}), compared to pig, where more perpendicular alignment was found at L1-L3 segments (138^{o}-124^{o}) with gradual transition to parallel orientation at L4-L6 segments (56^{o}-34^{o}). EES-induced MEP recorded from pigs showed higher variation across L1-L6 segments with the highest amplitudes with EES delivered over the dorsal root entry zone compared to stimulation between the roots. Compared to pig, intersegmental variation of MEP in rat and human was not significantly different across lumbar segments. The results of this study indicate on remarkable variation in dorsal roots angles in commonly used rat and pig models compared to human. Observed difference in electrophysiological results could be related with specific dorsal roots orientation with minimal intersegmental space in rat and human and gradual increase in amplitude from rostral to caudal segments could be related with a higher number of dorsal root fibers in the caudal segments and also more parallel orientation of rootlets. These findings may guide the interpretation of EES-related outcomes and electrophysiological differences across multiple models and facilitate selection of the optimal pre-clinical model for neuromodulation studies.

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Poster

136. Spinal Cord Injury: Responses and Repair

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 136.20/I32

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Merit Review Award # B1005-R/1I01RX001005-01A2, from the United States (U.S.) Department of Veterans Affairs Rehabilitation Research and Development Service (RR&D)

Title: Iron chelator therapy reduces secondary spinal cord injury

Authors: *F. J. THOMPSON[1,2,3], J. HOU[1,2], S. TSUDA[1,2], R. NELSON[1], D. PLANT[1], K. BUCKLEY[1], I. ANWAR[1], J. GODIN[1], A. SADEESHKUMAR[1], V. BAEZ[1], R. J. BERGERON[4], P. BOSE[1,2,5];

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Abstract: Cervical spinal cord injury (C-SCI) is a devastating injury that can result in life-long locomotor and spasticity disabilities. SCI involves a cascade of pathophysiological events that evolve over time, and the objective of the current research is to minimize these events and their subsequent impact on quality of life. Contusion SCI causes micro-vessel shear injury, blood spinal cord barrier (BSCB) dysfunction, and bleeding. Iron deposited by diffuse micro-bleeds fuels inflammation through reactive oxygen species, and multiple inflammatory pathways, which further induce progressive disabilities. There is also an urgent need to reduce these risk factors for long-term progressive disabilities, and to develop effective therapies that have excellent potential for translation. The current studies tested the preclinical evaluation of the safety and efficacy of a potent iron chelator, NaHBED, to remove microbleed-induced iron, a powerful catalyst of inflammation, and to upregulate neural and vascular trophic agents to protect and heal injured neural and vascular tissues. These studies were conducted in a clinically relevant rodent model of C-SCI, in which we reported enduring motor disabilities. SCI was produced using a 200 kdynes force protocol that we reported previously. NaHBED treatment was initiated PO Day-0 and continued through PO Wk-2 by SQ injection of 50 mg/kg/day for 2 weeks (n=7). The control animals received equal volume SQ injections of saline (n=5). The outcome measures for motor (e.g. spasticity, gait functions) were conducted in saline control and NaHBED treated groups. Our data revealed long-term spasticity and gait disabilities following experimental moderate C-SCI, and significant reductions in these disabilities by NaHBED treatment. C-SCI-NaHBED treated animals showed marked reduction of iron staining (Perls' staining) and SWI MRI showed significant removal of iron/bleeds at the injury epicenter compared to saline treated SCI controls. *Ex-vivo* DTI MRI revealed better spared/regenerative capacity of the injured dorsal corticospinal and reticulospinal tracts. Immonohistochemistry studies in the C-SCI animals showed patterns of a) iron deposition and disruption of BSCB, b) increased expression of markers for inflammation, and c) loss of regulatory noradrenergic neuromodulator and trophic factors. However, tissues from the NaHBED-treated C-SCI animals exhibited robust normalization of each of these markers. These preliminary results suggest that C-SCI related motor disabilities can be attenuated by NaHBED iron chelator therapy by inhibiting neuroinflammation and inflammation-mediated neuronal cell death.

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Poster

137. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 137.01/I33

Topic: D.06. Auditory & Vestibular Systems

Support: NIH R01-MH060163-16

Title: A cortical spiking model with differential short-term plasticity onto parvalbumin and somatostatin interneurons reproduces *in vivo* results of sensory adaptation in auditory cortex

Authors: *M. J. SEAY[1], R. G. NATAN[2], M. N. GEFFEN[3], D. V. BUONOMANO[1];
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Abstract: Cortical responses to sensory stimuli are strongly modulated by temporal context. Typically, tuned responses decrease as a stimulus is repeated, a phenomenon referred to as sensory adaptation. Recent studies have demonstrated that the inhibitory parvalbumin (PV) and somatostatin (SOM) interneurons differentially contribute to sensory adaptation (e.g., Natan et al., 2015), an effect that may result from distinct short-term plasticity (STP) at synapses formed by the two cell types—with repeated excitatory synaptic currents to PV and SOM units exhibiting short-term depression and facilitation respectively. While previous computational approaches to adaptation have focused on firing rate models (Natan et al, 2015; Philips et al, 2017), here we developed a spiking model of auditory cortex that incorporates known cellular, synaptic, and circuit characteristics of PV and SOM neurons. The spiking model accounted for and reproduced sensory adaptation, with modeled pyramidal unit firing rates exhibiting qualitative fits to the averaged experimental data and some single units. Inactivating model PV or SOM units produced a similar pattern of firing rate alterations compared to experimental optogenetic manipulations. The model also generated predictions that led to novel analyses of the considerable variability among individual units in the experimental dataset. For example, the model predicted that a unit's evoked firing latency would be positively correlated with its degree of SSA, and this prediction was confirmed. Building on this finding, we clustered the experimental units according to a set of key features including latency, frequency tuning, SSA, and optogenetic effect size, revealing three clusters. These three clusters could be accounted for by adjusting parameters in the model including the STP at excitatory synapses onto PV and SOM units. Finally, we addressed a contentious question in the study of sensory cortical microcircuits: whether sensory-driven excitatory input onto SOM units is primarily feedforward or feedback. We found that feedforward architectures overestimated the effect of inactivating SOM neurons, and that a feedback architecture better captured the experimental data. Our results indicate that the differential STP of excitatory input to distinct interneuron subtypes enriches the representational capacity of sensory cortex for temporal structure. We predict that specifically disrupting STP at excitatory-to-SOM synapses will weaken sensory adaptation, and that much of the temporal context sensitivity of cortical neurons is governed by STP.

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Poster

137. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 137.02/I34

Topic: D.06. Auditory & Vestibular Systems

Support: SNF professorship
ERC starting Grant

Title: Auditory response plasticity in thalamic circuits

Authors: *J. A. TAYLOR[1], M. HASEGAWA[1], J. AMORIM FREIRE[1], M. THEODORE[1], T. LU[2], J. GRÜNDEMANN[1];
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Abstract: Associative learning is a fundamental feature of developed nervous systems guaranteeing an animal's survival. However, its distributed coding mechanisms across brain circuits is poorly understood. Here we developed a miniature microscope-based deep brain calcium imaging approach to monitor the neural activity of large neuronal populations of auditory thalamus (medial geniculate body, MGB) in freely moving mice upon associative learning using an auditory fear conditioning paradigm. We tracked populations of MGB neurons across days and find that neuronal responses to auditory stimuli are plastic upon fear learning, both in relation to the conditioned stimulus as well as on the level of frequency tuning. Furthermore, MGB neurons exhibit functional cell classes similar to those previously identified in downstream amygdala circuits (e.g. fear cells, extinction cells). Our data identifies MGB as a site for neuronal plasticity in associative fear learning upstream of the basolateral amygdala that might drive plasticity in downstream limbic brain areas. More generally, using a large-scale imaging approach, we demonstrate that auditory thalamus plays a role in experience-dependent plasticity that goes beyond a classic thalamic relay function.

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Poster

137. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 137.03/I35

Topic: D.06. Auditory & Vestibular Systems

Title: Olfactory cue congruency modulates neuronal responses in primary auditory cortex

Authors: *O. D. GILDAY, A. MIZRAHI;
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Abstract: Sensory cortex is driven by both bottom-up and non-bottom-up information. Among others, non-bottom-up modulations consist of contextual, attentional, motivational and expectation driven effects. These might arise from local circuit computations, top-down modulation, neuro-modulatory or other sources. Here, we tested the contribution of olfactory-cue congruency as expectation cues for sound representation in the primary auditory cortex (A1). Using in vivo two-photon calcium imaging, in awake head-restrained mice, we recorded auditory responses from L2/3 neurons. We used specific olfactory stimuli as cues that precede specific sounds. We exposed mice to odor-sound pairs such that there was a one-to-one mapping between specific odors and sounds. After several dozen exposures of congruent pairs, we added a small fraction of incongruent trials in which the odor-sound pairing does not match the original one. We compared neuronal responses to the same sound in congruent vs incongruent trials. We show a small sub-population (~7%) of neurons in A1 that respond stronger to sounds when the olfactory cue is incongruent (when the sound is less expected). These findings show an example of expectation-driven modulation learned through a short experience. We are currently in search for the source of this modulation, expecting it to be outside of auditory cortex, since it is non-auditory in nature.

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Poster

137. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

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Program #/Poster #: 137.04/I36

Topic: D.06. Auditory & Vestibular Systems

Support: Canadian Institutes of Health Research (CIHR)

Natural Sciences and Engineering Research Council of Canada (NSERC)

Title: Identity recognition across different emotional expressions - An fMRI study in visual and auditory modalities

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Abstract: It is an important social skill to recognize previously encountered persons when their facial expression or speaking tone (prosody) changes. Specific brain areas have been identified involved in storing neural signatures of individual facial identities, which remain distinct even when the facial expression changes. Parallel studies on voice revealed identity-sensitive areas in which the neural activity patterns were distinct among different speakers. However, it remains unknown whether there are brain regions capable of encoding identity information when the speech prosody changes. The present study aims to investigate how facial and vocal identities are represented across multiple expressions in the brain. Seventeen healthy adults (11 female, mean age ± SD: 21.6 ± 3.1) completed a functional magnetic resonance imaging (fMRI) experiment in a 3T MRI scanner, in which they performed an ongoing identity recognition task. Faces with three expressions (anger, fear, and happy), or neutral-content speech clips in the same three prosodies, produced by multiple actors, were repeatedly presented in 2 audio and 2 visual blocks. Participants were asked to determine whether the currently presented individual had been encountered in any of the previous trials, regardless of changes in their expression. Behavioral results revealed that the overall voice recognition accuracy (0.71 ± 0.23) was significantly lower than the facial recognition accuracy (0.78 ± 0.30) ($t(16) = 4.75$, $p < 0.001$). Reaction times (RTs) were submitted to a repeated-measure ANOVA with modality, block and repetition number as within-subject factors. It yielded significant effects of modality ($F(1,16) = 208.1$), block ($F(1,16) = 6.1$), and repetition ($F(5,80) = 7.0$) ($ps < 0.001$). Moreover, RTs reduced linearly with repetition ($F(1,16) = 10.8$, $p=0.005$). fMRI data showed that activity in right occipital fusiform gyrus, right cingulate gyrus/superior frontal gyrus, and right anterior insula decreased linearly over identity repetitions in visual blocks, and left occipital fusiform gyrus displayed the same linear decrease of activity in audio blocks. Behavioral RT reductions in both modalities indicate an implicit identity memory formed over repetitions despite expression changes. Imaging results primarily suggest that the occipital fusiform gyrus may be identity sensitive, in line with the facilitated responses. Altogether, although voice recognition was generally worse than facial recognition across emotions, our findings at both behavioral and neural levels are in support of successful implicit identity recognition of human faces and voices, across different emotional expressions.

Disclosures: H. Xu: None. **J.L. Armony:** None.

Poster

137. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 137.05/I37

Topic: D.06. Auditory & Vestibular Systems

Support: NSF 2016226887
NIDCD DC012557
DARPA TNT 16-A0-00-006862

Title: Auditory representation in cortex during perceptual learning

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Abstract: During perceptual learning, animals improve their ability to discriminate between sensory stimuli. This behavioral improvement occurs at variable rates across animals. Previous work in the auditory system showed that perceptual learning reliably enhances cortical representations of task-relevant stimuli in trained animals. These neural changes have predominantly been observed outside of the behavioral context and regardless of learning rate. However, our lab recently found that engagement in behavior alters auditory cortical responses. Neural and behavior discrimination over perceptual learning are correlated. How do sensory representations change over auditory perceptual learning?
To address these questions, we assessed neural dynamics over learning during behavior within each mouse. We developed an appetitive, head-fixed auditory perceptual learning two-alternative forced-choice task for mice. Animals learned to lick a left lick port for tones of a chosen frequency (usually 11 or 13 kHz) and right for tones of other frequencies to obtain water rewards. This allowed us to probe both auditory acuity and categorical responses over perceptual learning. Animals improved their discrimination between center and surround frequencies over the course of weeks (9-21 days), but continued to make errors on frequencies close to the center frequency. Bilateral muscimol infusions in auditory cortex of trained mice substantially reduced behavioral performance. We performed two-photon calcium imaging of excitatory neurons, inhibitory neurons, and cholinergic axons in auditory cortex throughout learning, to assess neural activity both during this behavior and passive listening. In the behavioral context, many excitatory neurons exhibited a categorical response to the auditory stimuli, not simply encoding the frequency of the stimulus, but rather the behavioral meaning. This categorical response was present early in behavioral learning, but was broader, mirroring the behavioral performance.

Disclosures: K.A. Martin: None. **R.C. Froemke:** None.

Poster

137. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

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Program #/Poster #: 137.06/I38

Topic: D.06. Auditory & Vestibular Systems

Support: TCU SERC Grant 180535
TCU SERC Grant 180536

Title: Auricular vagus nerve stimulation as a method for driving brain plasticity in learning a novel orthography

Authors: *V. J. THAKKAR, A. S. ENGELHART, N. MATTOX, G. PECORARO, Z. RICHARDSON, T. M. CENTANNI;
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Abstract: Achieving reading fluency is a difficult task for individuals with dyslexia and for adults learning to read in a new orthography. Cervical vagus nerve stimulation (cVNS) is capable of driving neural plasticity and is in clinical trials for a variety of neurological disorders, suggesting this mechanism may be capable of improving fluency in reading as well. However, an invasive approach is not practical for a reading intervention. Recent work suggests auricular vagus nerve stimulation (aVNS) as a non-invasive alternative, since it projects to similar brain regions as cVNS and requires only small amounts of electrical stimulation to the left cymba concha. The aim of the current study was to evaluate the efficacy of aVNS in improving fluency of novel letter-sounds. We recruited young adults that were typically-developing (TD) or with dyslexia. Participants were randomized into one of four experimental groups (computer training, sham stimulation, active earlobe stimulation, or active stimulation to the cymba concha). Participants then completed five 30-minute training sessions, learning 2-3 Hebrew letter-sound combinations each day and receiving aVNS to the left ear during multi-sensory auditory and visual feedback. After the training period, participants were assessed on Hebrew versions of three standard English measures: letter identification, rapid naming, and timed pseudo-word reading. Finally, saliva samples were obtained to investigate the role of the Val66Met SNP in BDNF (brain derived neurotrophic factor) on aVNS efficacy. Results showed that all participants learned letter-sound combinations well within five days. The control groups (computer, sham, and earlobe) did not differ on any of the dependent measures, so those groups were collapsed together. In TD individuals, those in the active group performed significantly better on both the rapid naming task and the timed pseudo-word task, as compared to those in the control groups. We will discuss these findings in the context of a potential future treatment for dyslexia and implications of genetic variants on aVNS efficacy.

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Poster

137. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 137.07/I39

Topic: D.06. Auditory & Vestibular Systems

Support: Fyssen Foundation

Title: Neural activity in the auditory cortex during memory formation of auditory patterns

Authors: ***H. KANG**[1], R. AUKSZTULEWICZ[1], H. AN[1], M. L. SUTTER[2], J. W. SCHNUPP[1]; [1]Dept. of Biomed. Sci., City Univ. of Hong Kong, Hong Kong, Hong Kong; [2]Univ. of California Davis, Davis, CA

Abstract: Learning and memory processes through repetitive exposure to target sounds are one of key factors for efficient auditory processing. Fast and robust memory formation of initially random, complex sounds as well as neural correlates of such memory in normal hearing humans have been previously reported (Agus et al., 2010; Andrillon et al., 2015; Luo et al., 2013). However, further in-depth neural recordings are required to identify neural mechanisms underlying memory formation. The present study aims at investigating the neural basis of learning and memory for initially random auditory patterns by recording neural activity in rodents. We examined seven female Wistar rats (8-11 weeks) using electrocorticography (ECoG) recording over the auditory cortex (AC) under anaesthesia. Random sparse tone clouds (cloud duration: 1 s; tone duration: 20 ms; 16 tones total) were used as stimuli. Tone clouds were either random for the whole duration (random tone clouds, T), or 0.25 sec. frozen random segment immediately repeated four times (repeated tone clouds, RT). While each stimulus type was generated afresh for 100 trials within one test block, one target RT (reference repeated tone clouds, RefRT) kept its characteristics and re-appeared for another 100 trials, presented in a randomised order. Five different RefRTs were generated for each test block, and two test blocks with the same RefRT were consecutively played. Greater neural responses for RefRT were observed compared to RT and T during sound presentation. To verify whether this difference is not simply due to unbalanced number of new patterns between RefRT and other stimulus types, we analysed the signal-to-noise ratio (SNR) of response amplitudes during the sound presentation relative to silence for each stimulus type and trial. Greater SNR for RefRT compared to RT and T started to emerge after about 10 trials. This effect remained robust until the end of the test block and continued from the first trial of the following test block containing the same RefRT. In line with previous human behaviour and neuroimaging studies, this finding indicates a fast formation of neural activity in the AC specific to re-occurring sound patterns.

The results also establish an animal model of macroscopic neural correlates of memory formation and provide a useful basis for further micro- and mesoscopic neural recordings in individual neurons, impossible to achieve in humans, which will enable a deeper understanding of neural mechanisms of perceptual memory formation.

Disclosures: H. Kang: None. **R. Auksztulewicz:** None. **H. An:** None. **M.L. Sutter:** None. **J.W. Schnupp:** None.

Poster

137. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 137.08/I40

Topic: D.06. Auditory & Vestibular Systems

Support: ERC consolidators grant to A.M (#616063)
Gatsby Charitable Foundation

Title: Neuronal correlates of perceptual learning in the auditory cortex

Authors: *I. MAOR, A. MIZRAHI;
The Edmond and Lily Safra Ctr. for Brain Sci., The Hebrew Univ., Jerusalem, Israel

Abstract: Auditory perceptual learning of pure tones causes tonotopic map expansion in the primary auditory cortex (A1), but the mechanism of this plasticity and the function it sub-serves is unclear. To study perceptual learning in mice we developed an automated training platform called the 'Educage', which was used to train mice on a go/no-go auditory discrimination task to their perceptual limits. Following weeks of training with reward and punishment, mice could discriminate among pure tones (upto 3% octave apart) or natural sounds (upto 10% frequency modulated). To study mechanisms of plasticity, we then recorded spiking responses of excitatory neurons in L2/3 in expert versus naïve mice. Our recordings show distinct signature of plasticity following perceptual learning of pure tones and natural sounds. While learning-induced overrepresentation of the learned tones, in accordance with previous literature, perceptual learning of natural sounds induced 'sparsening' and decorrelation of neuronal representation. To study the possible contributions of inhibitory circuits we recorded the signature of plasticity of local inhibitory neurons using two-photon targeted patch. To date, we measured spiking responses from parvalbumin- and somatostatin- positive neurons, often side by side to their neighboring excitatory neurons. We are currently analyzing the contribution of each neuronal subtype to unravel local circuit mechanisms underlying learning induced plasticity in A1.

Disclosures: I. Maor: None. **A. Mizrahi:** None.

Poster

137. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 137.09/I41

Topic: D.06. Auditory & Vestibular Systems

Support: Swiss National Science Foundation Grant 168602

Title: Optogenetic up- and downregulation of basal forebrain activation impacts distinct aspects of auditory learning

Authors: *A.-L. KLAASSEN[1,2], K. THOMAS[1], M. HARVEY[1], B. RASCH[2], G. RAINER[1]; [1]Dept. of Medicine, Univ. of Fribourg, Fribourg, Switzerland; [2]Dept. of Psychology, Univ. of Fribourg, Fribourg, Switzerland

Abstract: To study the functional contribution of the basal forebrain (BF) projection system on learning behavior, we developed an auditory discrimination paradigm in freely moving rats. We employed classical music as it is a spectrally rich and dynamic stimulus that may more closely approximate ecologically important sounds than pure tones. Rats learned to discriminate segments of different Fourier-amplitude-matched classical music pieces using an operant conditioning paradigm (go/nogo variable interval 30s schedule with 2 S+ and 2 S- auditory stimuli). We have preliminary data suggesting that rats readily learn this task in about 14 days. Interestingly, rats demonstrated evidence of generalization, correctly responding to other segments of the learned classical music pieces that they had never heard before.
In separate wild type animal groups, we optogenetically up- or downregulated neural activity (ChR2, Arch) in the BF ventral pallidum nucleus using non cell-type specific viral constructs, focusing on effects of BF network activation. During the entire learning period, one of the two music pairs (S+, S-) was accompanied by optogenetic activation (ChR2: 40Hz, Arch: continuous light). Each animal thus served as its own control, allowing a sensitive estimation of the impact of optogenetic stimulation on learning behavior. Upregulation of BF activity reduced learning rate, and animals exhibited additional locomotor as well as increased false alarm responses in the operant task (n=4 animals). Downregulation of BF activity on the other hand reduced task participation and decreased rates of operant responses, but the learning rate was unimpaired (n=6 animals).
Taken together, over-activation of BF increases general arousal and animals fail to focus appropriately on the auditory stimulus and therefore learn poorly. Under-activation of BF reduces operant responding, while maintaining learning rate at least in the range tested here. Our findings suggest that BF activation in healthy animals may represent a local optimum between learning capacity and behavioral resource allocation.

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Poster

137. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 137.10/I42

Topic: D.06. Auditory & Vestibular Systems

Title: Effect of novel acoustic environment exposure on lateralized processing patterns

Authors: *B. FUREST CATALDO[1], B. CABEZAS[1], J. OVETSKY[1], L. YANG[2], D. VICARIO[1];
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Abstract: The neural representation of speech has been extensively studied with the goal of determining what necessary functions of speech and language are subserved by each hemisphere. Similarly, second language processing raises additional questions about the function of each hemisphere since lateralized activity is known to differ depending which language is heard: first or second languages. In the Zebra finch, a songbird whose vocalizations are learned during development, neural responses to conspecific vocalizations are higher in auditory areas in the right hemisphere than in the left. Our laboratory used electrophysiological recordings to assess the pattern of lateralization in birds exposed to a heterospecific acoustic environment for different periods of time. We observed that the novel acoustic environment induced transient shifts in the pattern of lateralization. The goals of the current study were: 1) implement a novel methodology that enables the chronic assessment of lateralized activity, and 2) determine the dynamic time course of lateralization with this longitudinal method. Adult male Zebra finches were surgically implanted with a custom-made epidural electrode array over the caudal telencephalon, isolated, and randomly assigned to be exposed to recordings of a heterospecific (HETENV) or a control conspecific (CONENV) acoustic environment. Event related potential (ERP) recordings were obtained (every 1-3 days over a ~3 week period) in response to playbacks of novel conspecific female calls. A lateralization index (LI), calculated from the response magnitudes of ERPs over each hemisphere, measured the relative strength of activity between hemispheres. The CONENV birds showed right-biased LI, as expected from previous work. In contrast, neural activity in birds that had ~4 days of HETENV exposure, showed a change to a left-biased LI and then, after ~14 days, returned to the initial right-biased LI. This pattern is consistent with earlier work, and suggests that auditory representations can undergo transient dynamic updating in response to immersion in a novel acoustic environment.

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Poster

137. Auditory Processing: Adaptation, Learning, and Memory

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Program #/Poster #: 137.11/I43

Topic: D.06. Auditory & Vestibular Systems

Support: DARPA Grant BAA-16-24

Title: Behavioral and neural correlates of changes in arousal state evoked by vagal nerve stimulation in mice

Authors: *L. J. BODDINGTON[1], L. N. COLLINS[1], R. C. FROEMKE[2], M. J. MCGINLEY[3], D. A. MCCORMICK[1];
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Abstract: Stimulation of the vagus nerve is used for treatment of some forms of epilepsy and depression, although the mechanisms of these effects are not well known. Many forms of epilepsy are state-dependent, occurring more prominently at some states (e.g. drowsiness, transition between sleep and waking, etc.) than others. We hypothesized that vagal nerve stimulation (VNS) may alter the state of the brain, perhaps through the activation of ascending sensory or neuromodulatory pathways. Our lab has also previously demonstrated that task performance on an auditory detection task is optimized during an intermediate state of arousal (McGinley et al., Neuron, 2015), therefore we hypothesized that VNS may be a useful tool to modulate arousal states and in turn influence sensory processing and task performance. To test this hypothesis and investigate the mechanisms underlying the effect of VNS on brain state, we performed wide-field imaging of the dorsal and lateral surfaces of the brain (encompassing multiple sensory cortices) in Thy-1GCaMP awake, behaving mice. We monitored pupil diameter, face movements, and locomotion while periodically delivering VNS. Our data demonstrate that VNS can reliably and dose-dependently increase arousal (as indicated by pupil dilation), alongside widespread cortical activation (calcium signals) centered around somatosensory areas. VNS is known to activate brainstem structures that project to regions of the brain involved in regulating arousal, therefore current work aims to investigate the mechanisms underlying stimulation-evoked activation of cortical networks. We use 2-photon mesoscopic imaging to examine how VNS influences neuromodulatory axon and excitatory/inhibitory activity in cortical regions strongly activated by stimulation. Ongoing work is underway to determine whether this stimulation-evoked increase in arousal alters the ability of the mouse to learn, retain, and perform auditory detection and discrimination tasks.

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Poster

137. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 137.12/I44

Topic: D.06. Auditory & Vestibular Systems

Support: NSF-IOS-BSF

Title: Synaptic zinc contributes to contrast adaptation in auditory cortex

Authors: *P. CODY[1], T. TZOUNOPOULOS[2];
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Abstract: Phenomena of sensory adaptation have been extensively described but understanding of the underlying synaptic mechanisms trails behind. A common function of sensory adaptation is to maintain a signal percept amid changing contexts. This is, at least in part, accomplished via contrast gain control, a process in which instantaneous firing-rate gain compensates for changes in background stimulus contrast, or variance. In auditory cortex this process modulates the sensory evoked response to the sound level contrast within which a signal is presented: the signal response amplitude increases with decreasing variance in sound level of background noise. We assay this phenomenon with 2-photon calcium imaging in layer (L) 2/3 of mouse primary auditory cortex (A1) to investigate the biological mechanisms involved in this specific form of sensory adaptation termed contrast gain control. Consistent with previous studies, A1 L2/3 principal neurons adapt their sound evoked responses to the sound level contrast and duration of the background noise preceding the signal. We find that modulation to contrast was limited to A1 L2/3 principal neurons displaying monotonically increasing sound level tuning curves, termed monotonic neurons. Contrast modulation also depended on the location of the signal sound frequency within neuronal receptive fields as well as within the context frequency bandwidth. Because synaptic zinc has emerged as a cell-specific modulator of response gain and tuning in A1 L2/3 neurons, we tested whether it contributes to contrast adaptation. We found that upon zinc chelation, responses were no longer greater in low contrast relative to high contrast; high contrast responses increased while low contrast responses remained consistent. Synaptic zinc thus contributes to elevated low contrast responses via inhibition of responses in high contrast. These findings reveal that synaptic zinc has a physiologically relevant impact on sound perception related to sensory adaptation and begin to approach a synaptic mechanism driving contrast adaptation in auditory cortex.

Disclosures: P. Cody: None. **T. Tzounopoulos:** None.

Poster

137. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

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Program #/Poster #: 137.13/J1

Topic: D.06. Auditory & Vestibular Systems

Support: NIDCD F30DC017351-01

Title: Neuromodulation enhances plasticity in a rodent model of cochlear implant use

Authors: *E. G. GLENNON[1], J. MULTANI[1], M. A. SVIRSKY[1,2,3,4], R. C. FROEMKE[1,2,3,5,6]; [1]Dept. of Neurosci. and Physiol., [2]Neurosci. Inst., [3]Dept. of Otolaryngology-HNS, [4]Ctr. for Neural Sci., [5]Skirball Inst., NYU Sch. of Med., New York, NY; [6]Howard Hughes Med. Inst., Chevy Chase, MD

Abstract: Rates of auditory perceptual learning and asymptotic speech perception performance with cochlear implants are highly variable across patients. Adaptation to cochlear implants is believed to require neuroplasticity within the central auditory system. However, mechanisms by which behavioral training enables plasticity and improves outcomes are poorly understood. Here we investigate the hypothesis that neural mechanisms that promote plasticity in the rodent auditory system are key to optimizing cochlear implant usage, and might be especially helpful in cases of poor performance. We focus on noradrenergic modulation of rat auditory cortex by the locus coeruleus, which can enable robust and long-lasting neural and behavioral changes.
We developed a new surgical approach for cochlear implantation in adult rats. Our approach allows insertion of an 8-channel electrode array covering up to 360 degrees in the cochlea and allows rats to freely behave while using the implant to perform auditory tasks. Rats are trained on a go/no-go task, and self-initiate trials to respond to a target tone. Previously, we showed in normal hearing animals that this task requires auditory cortex, and that this task is sensitive to cortical modulation and plasticity.
Here we examined the effect of pairing locus coeruleus stimulation with an auditory stimulus on auditory learning when the animal has to relearn a tone identification task using a cochlear implant. Initial training was done using acoustic stimuli in normal hearing animals. Animals were then bilaterally deafened and unilaterally cochlear-implanted. Next, animals were retrained on the auditory task with the new target delivered by intracochlear electrical stimulation. Prior to each daily behavioral training session for the new target, one group of rats underwent a 5-10 min pairing session. Pairing accelerated learning with cochlear implants compared to animals that did not receive it. We then conducted multi-unit recordings in the auditory cortex to assess activation of the cortex by the cochlear implant. Animals that had been trained with the cochlear implant had more effective activation of the cortex, and those that underwent pairing had a sharper representation of the target cochlear implant channel. We used fiber photometry to monitor

activity of noradrenergic locus coeruleus neurons. During auditory learning, normal hearing animals display dynamic locus coeruleus activity, specifically during the acquisition of the new meaning of reward relevant tones. These studies indicate that neuromodulation can play a powerful role in shaping outcomes with cochlear implant use and training.

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Poster

137. Auditory Processing: Adaptation, Learning, and Memory

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Topic: D.06. Auditory & Vestibular Systems

Support: DARPA D15AP00101

Title: Short-term effects of vagus nerve stimulation on auditory learning and stimulus-specific activity in auditory cortex

Authors: *J. LAI, S. V. DAVID;
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Abstract: Previous studies of vagus nerve stimulation (VNS) have shown that chronic stimulation can facilitate central plasticity, e.g. enhancing the rate of motor rehabilitation following stroke or producing stimulus-specific changes in auditory cortical selectivity. VNS is believed to trigger release of neuromodulators, including norepinepephrine and acetylcholine, which may mediate the associated plasticity. Most previous work has studied the effects of chronic VNS over many days. To study short-term VNS effects, we measured its effects on learning and auditory cortical activity following brief, acute periods of stimulation. We implanted cuff electrodes onto the vagus nerve of ferrets and trained them by classical conditioning to associate one specific target sound (T1) with a reward and another target sound (T2) with no reward. T1 and T2 were changed every 2 days (200-250 trials/day), typically after reward associations were learned. When T1 and T2 were paired with VNS (1 s duration, 30 Hz, 200 us biphasic pulses, 0.4-2 mA, VNS onset 100-150 ms before T1/T2 onset), rates of learning the reward association increased on day 1, regardless of task difficulty. In contrast, animals' learning rates were lower when VNS occurred randomly during the silence after T1/T2 presentation (non-paired condition). Afferent VNS pathways involve nuclei that mediate arousal, which is reflected by changes in pupil size. A phasic pupil dilation was observed for several seconds following VNS, suggesting an increase in arousal that may support the greater learning efficiency. To measure effects of VNS on cortical activity, we recorded neurophysiological single- and multi-unit activity in primary auditory cortex of passively listening animals pre- and

post-VNS. Neural responses in a subpopulation of neurons were decreased in the condition after pairing VNS with the best frequency tone. Neural activity was also positively correlated with pupil size. Regressing out effects of pupil-indexed arousal decreased the response difference of post- versus pre-VNS. However, significant reduction of neural responses remained in post-VNS. This outcome contrasted with previous findings, which have generally reported enhancement of stimulus-specific responses after long-term VNS. This difference may reflect the short timescale of VNS (20 times per tone, i.e. about 10 min) in our study. Taken together, the results of this study support a role for VNS in auditory learning and help establish VNS as a tool to facilitate neural plasticity.

Disclosures: J. Lai: None. **S.V. David:** None.

Poster

137. Auditory Processing: Adaptation, Learning, and Memory

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Conte Center 5P50MH109429 (RTK and JJL)

Title: Auditory predictions in cortical and subcortical temporal regions

Authors: *A. TZOVARA[1], T. FEDELE[2], J. SARNTHEIN[3], J. LIN[4], R. T. KNIGHT[5]; [1]Univ. of California, Berkeley, Berkeley, CA; [2]Higher Sch. of Econ., Moscow, Russian Federation; [3]Klinik fur Neurochirurgie, Universitatsspital Zurich, Zurich, Switzerland; [4]Dept. of Neurol., Univ. of California, Irvine, Irvine, CA; [5]Univ. of California Berkeley, Berkeley, CA

Abstract: Our brains have the ability to extract structure from the environment and form automatic predictions given past sensory experience. Although predictive circuits have been identified in wide-spread regions of the cortex, i.e. temporal and prefrontal areas, other regions, such as the hippocampus and amygdala, are also sensitive to violations of established auditory rules. However, the extent to which these structures also support the formation of predictions remains under-explored.
Here, we designed an experiment consisting of repeated presentations of pure tones, the majority of which were drawn from a Gaussian distribution around a mean frequency (500 Hz). Deviant sounds occurred on the tail of the distribution (2000 Hz) and were presented either in a temporally predictable or unpredictable way. We presented the auditory stimuli to a group of 12 epileptic patients, with implanted depth electrodes undergoing presurgical monitoring for pharmacologically resistant epilepsy. Patients were instructed to watch silent movies and ignore

the stream of sounds. We extracted intracranial electroencephalography (EEG) responses to sounds from contacts in the hippocampus, amygdala and temporal cortex, in the range of 1-40 Hz.
Temporal cortical areas showed strong deviance responses across contacts and patients. These consistently peaked at latencies starting at ~100 ms post stimulus onset ($p_{corr} < 0.05$), in accordance to previously reported evidence based on non-invasive electrophysiology. Subcortical contacts in the hippocampus and amygdala also showed consistent deviance responses at latencies peaking at ~ 200 ms post stimulus onset ($p_{corr} < 0.05$). Importantly, deviance effects were modulated by predictability within the hippocampus, but not temporal cortical areas, and mainly for low-frequency responses in the theta range (< 8 Hz).
In summary, we used intracranial EEG recordings to provide direct electrophysiological evidence that cortical and subcortical brain regions are sensitive to violations of auditory rules. Our results expand the neural network of regularity extraction, by showing that subcortical brain regions of the temporal lobe, including the hippocampus and amygdala, participate in the detection of statistical regularities and are sensitive to implicitly formed auditory predictions.

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Poster

137. Auditory Processing: Adaptation, Learning, and Memory

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PSL Data Science Grant

Title: Opposite valences learning shapes neuronal selectivities in auditory cortex through inhibitory networks

Authors: ***J.-F. LEGER**[1], X. LIU[1], A. LOURDIANE[1], C. VENTALON[1], L. BOURDIEU[1], Y. BOUBENEC[1], S. A. SHAMMA[3,2], S. WOLF[1], S. COCCO[1], R. MONASSON[1];
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Abstract: Listening is a complex process in which we not only hear and perceive incoming audible sounds but also recognize and interpret them according to their learned meaning. At the first stage of cortical auditory processing, in the auditory cortex A1, sensory representation is modulated based on whether or not behavioral relevance is attached to the sounds. The

mechanisms allowing for these modulations are not yet clearly dissected despite their importance to understand the basis of auditory memory formation. This work addresses the following questions: is there a general pattern of changes in A1 when a sound becomes behaviorally relevant? How the learning strategy, with either negative or positive reinforcement, influences sound representation in A1. Can we identify different circuits recruited during these opposite motivations auditory learning? We first explore these issues with CBA mice that learn to perform two tasks with the same acoustic discrimination but with differential reward valence. By taking advantage of the imaging capability of two-photon microscopy, we follow the same GCaMP6f expressing neurons in A1 throughout successive learning. Awake head-fixed recordings provide a rich observation of A1 activity in its layer 2/3. In parallel, another group of CBA mice are recorded with permanently implanted linear Silicon probes in A1, allowing chronic recordings of A1 deeper layers (L4/5/6). With these chronic recordings, we observed the network reshaping that occurs during learning. Systematic patterns appear, with post-learning reinforcement of some cells that have pre-learning selectivity already pointing toward the target sound. By contrast, the cells surrounding these reinforced neurons are mainly inhibited during presentation of the target sound, leading to an increased contrasted response to the target after learning. This general pattern is found after both positive and negative reinforcement, although the detail of the identity of the inhibited cells can vary with varying learning strategy. By applying approaches inspired from statistical physics to analyze our recordings and infer the underlying functional connectivity, we find that learning is accompanied by a strong reorganization of the couplings between cells *within* A1. In particular, negative couplings seem to contribute to the patterns of contrasted response observed after learning. We will present preliminary results obtained using mice with PV+ neurons expressing td-Tomato to selectively identify them in our optical recordings. This work will improve our understanding of the various circuits involved in learning associated with opposite values.

Disclosures: **J. Leger:** None. **X. Liu:** None. **A. Lourdiane:** None. **C. Ventalon:** None. **L. Bourdieu:** None. **Y. Boubenec:** None. **S.A. Shamma:** None. **S. Wolf:** None. **S. Cocco:** None. **R. Monasson:** None.

Poster

137. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 137.17/J5

Topic: D.06. Auditory & Vestibular Systems

Support: ADRC pilot P50AG005146

Title: Deficits in central auditory processing in mouse models of Alzheimer's disease

Authors: *K. A. FOGELSON, J. LAWLOR, Z. ZHU, K. V. KUCHIBHOTLA;
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Abstract: Alzheimer's disease (AD) is a form of dementia that is characterized by the progressive loss of cognitive capacity, including the loss of executive functions and memory. There is growing evidence that hearing loss and dementia are tightly linked, with recent studies showing that hearing loss is independently associated with accelerated cognitive decline. However, the neural mechanisms linking hearing loss to AD-related pathologies and subsequent cognitive decline remain unknown.

We will test the hypothesis that amyloid pathology impacts feedforward sensory processing and short-term sensory memory formation in primary auditory cortex (A1). We performed *in vivo* two-photon calcium imaging in awake, head-fixed APPswe/PS1dE9 (APP/PS1) mice to investigate how AD pathology impacts central auditory processing. APP/PS1 mice express chimeric mouse/human amyloid precursor protein (APP) and mutant presinilin-1 (PS1). These transgenes target neurons of the central nervous system, and lead to the rapid accumulation of soluble amyloid beta and subsequent plaque deposition in cortical and hippocampal areas.

Our study uses the fine-scale tonotopic arrangement of excitatory neurons in A1 as an assay for feedforward sensory integration. With two-photon calcium imaging, we have the spatial and temporal resolution to monitor the activity of hundreds of neurons simultaneously with single-cell resolution. Preliminary data collected from 11 mice (7 APP+, 4 WT) suggests clear differences in the processing of feedforward sensory information, namely differences in the tonotopic arrangement of neurons in A1 between WT and APP/PS1 mice.

In contrast to tonotopy which is largely inherited from sub-cortical structures, the auditory cortex plays an active role in the formation of sensory memories, including in stimulus-specific adaptation (SSA). SSA is the reduction in firing rate to a repeated stimulus which does not generalize to an alternative rare and infrequent stimulus. We aim to characterize SSA to determine how AD pathology impacts the formation of sensory memories.

Future work will investigate how AD pathology affects sensorimotor learning. Learning rates will be monitored in APP/PS1 mice trained on a go/no-go stimulus recognition task. To probe the neural computations that are critical for the selection of auditory cues during behavior, excitatory networks in A1 will be monitored using two-photon calcium imaging while animals perform the task. This study hopes to elucidate neural mechanisms of how amyloid beta pathology impacts central auditory processing, and thus, mechanistically link hearing loss, AD-related pathologies and cognitive impairments.

Disclosures: K.A. Fogelson: None. **J. Lawlor:** None. **Z. Zhu:** None. **K.V. Kuchibhotla:** None.

Poster

137. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 137.18/DP06/J6

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: D.06. Auditory & Vestibular Systems

Title: cellular mechanisms of auditory surprise in faithful computer replica of cortical microcircuit

Authors: *O. AMSALEM[1], J. KING[2], M. REIMANN[2], E. MULLER[2], H. MARKRAM[2], I. SEGEV[1];
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Abstract: The nervous system is notorious for its strong response to a "surprise" – an input that deviates significantly from an expected stimulus. One manifestation of such a surprise-response is the stimulus-specific-adaptation (SSA), whereby the neuronal response is reduced for a repeated stimulus (the "standard") but not (or less so) for a rare stimulus (the "deviant"). We explored the mechanisms underlying SSA in auditory cortex using a dense computer-generated neocortical network of a ~0.3 mm^3 composed of ~31,000 cells and ~36 million synapses. This circuit includes the physiological characteristics of excitatory and inhibitory synapses and the spikes patterns for the 55 cell types comprising this 6-layered circuit. We simulated SSA by activating 574 tonotopically-mapped thalamic afferents impinging on this network; the response of each axon was fitted to experimental results in the rat. Without parameter tuning, SSA and other related auditory signals, emerged in this circuit as found experimentally. We uncovered three-key mechanisms underlying the emergence of SSA: synaptic depression (which is the typically assumed to be the key mechanism for SSA), spike frequency adaptation (SFA) and network connectivity. The relative contribution of each of these mechanisms was explored, and its underpinning was explained. We concluded that the fine-scale reconstructed cortical microcircuit provides a powerful explanatory and predictive tool for uncovering emergent cortically-based phenomena.

Disclosures: O. Amsalem: None. **I. Segev:** None. **H. Markram:** None. **J. King:** None. **E. Muller:** None. **M. Reimann:** None.

Poster

137. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 137.19/J7

Topic: D.06. Auditory & Vestibular Systems

Support: Wellcome trust

Title: Mapping experience-dependent changes in sensorimotor representations across mouse auditory cortex

Authors: *S. A. M. PICARD, Y. WEISSENBERGER, A. J. KING, J. C. DAHMEN;
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Abstract: Ongoing activity in sensory cortex is not only determined by incoming sensory signals, but it is also influenced by a variety of contextual variables, such as an animal's movements and behavioural state. Many of these variables have been assumed to reflect a general cortical state shift that is relatively unaffected by experience, such as locomotion-induced gain changes in visual and auditory cortex. However, recent work has suggested that a subset of movement-related signals in auditory cortex may reflect the learned anticipation of the specific acoustic consequences of the animal's actions, such as its vocal output or the sounds of its footsteps. How these cortical sensorimotor representations are topographically organised, and how they change as a function of experience is currently unclear. Here, a closed-loop auditory locomotion paradigm for head-fixed mice was developed, in which running speed was directly mapped onto sounds ordered along a perceptually uniform frequency axis. We performed chronic two-photon imaging experiments on Camk2a-Cre;Ai95d mice expressing the calcium indicator GCaMP6f in excitatory neurons, allowing us to track hundreds of neurons in layer 2/3 over weeks of exposure to a novel auditory environment. This revealed a variety of contextual modulations of auditory responses across different cortical subfields. Some of these modulatory effects were found to change over time as a function of experience. Results point towards a role for secondary auditory cortex in linking movements to sound, even when the relation between motor action and auditory feedback is arbitrary and newly learned.

Disclosures: S.A.M. Picard: None. **Y. Weissenberger:** None. **A.J. King:** None. **J.C. Dahmen:** None.

Poster

137. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 137.20/J8

Topic: D.06. Auditory & Vestibular Systems

Support: DARPA TNT Program (N66001-17-2-4008)

Title: Vagus nerve stimulation-enhanced auditory perceptual training in the common marmoset (Callithrix jacchus)

Authors: *M. S. OSMANSKI, ***X. SHEN**, S. D. KOEHLER, X. WANG;
Biomed. Engin., Johns Hopkins Univ., Baltimore, MD

Abstract: Vagus nerve stimulation (VNS) has previously been shown to trigger neural plasticity and enhance auditory discrimination task performance in rodents. The goal of this study is to investigate if pairing VNS with an associated auditory perceptual task improves discrimination performance in marmoset monkeys, a highly vocal non-human primate with a similar hearing range as humans. In addition, this project also aims to determine the most effective VNS-paired training paradigm for enhanced auditory perceptual learning. We implanted a chronic cuff electrode on the left cervical vagus nerve in two marmosets, which were then tested on two heterospecific vocalization discrimination tasks: a macaque vocalization task relying on temporal and spectral cues and a Mandarin Chinese phoneme task relying on pitch cues. Three VNS protocols were evaluated: 1) VNS paired with every sound stimulus presentation, 2) VNS presented only when the subject responded to the target stimulus correctly, and 3) VNS presented during a 10-minute stimulation session before each behavioral task session ("priming"). Learning rates were measured to assess the effectiveness of VNS on the auditory perceptual learning. Results showed that VNS improved the discrimination performance relative to a control group of marmosets that received no VNS. Furthermore, VNS priming protocol appeared to show a more prominent effect on task learning compared to VNS during correct trials, while there was a seemingly destructive effect when VNS was paired with every stimulus presentation. These findings suggest potential benefits and side effects of VNS on perceptual learning.
Research support: DARPA TNT Program (N66001-17-2-4008)

Disclosures: M.S. Osmanski: None. **X. Shen:** None. **S.D. Koehler:** None. **X. Wang:** None.

Poster

137. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 137.21/J9

Topic: D.06. Auditory & Vestibular Systems

Support: BBSRC project grant to MC

Title: Implicit memory for regularities in rapid sound sequences

Authors: R. BIANCO[1], C. BOLGER[1], M. HU[1], P. HARRISON[2], M. PEARCE[2], *M. CHAIT[1]; [1]UCL, London, United Kingdom; [2]Queen Mary Univ., London, United Kingdom

Abstract: Sensitivity to acoustic statistical regularities is fundamental to many aspects of auditory perception and cognition. Recent work using repeated noise paradigms demonstrated robust, long-term auditory implicit memory for 'frozen' reoccurring white noise. Participant performance suggested that they become sensitive to an idiosyncratic, local spectral feature within the noise samples. Here we focus on implicit memory for *sequences* of auditory events - a specific arrangement of twenty different 50ms tone pips. Using behavioural methods, modelling and EEG in humans we ask (1) whether listeners can remember complex sequences of acoustic events (2) the limits on this memory (3) whether it requires active involvement with the sequence.

Building on previous work from our lab (Barascud et al, 2016), participants monitored novel rapid sequences of tone-pips for a transition from a random to a regular frequency pattern (repeating cycles of twenty 50 ms tone-pips). On half of the trials, sequences were random throughout; the other half contained a transition from a random to a regularly repeating pattern at a random time partway through the sequence. Most of the regular patterns were generated anew for each trial. Unbeknownst to the participants, several regular patterns (different for each listener) sparsely reoccurred across trials (every ~2.5 minutes) . Compared with novel sequences, response time (RT) to reoccurring regularities became substantially faster within only a few reoccurrences, reflecting rapid learning of sequence structure. This benefit persisted after 24 hours, and up to 7 weeks; it was robust to doubling the number of regularities to memorise, and to subsequent memorization of other patterns. Similar learning also occurred during passive exposure (i.e when naive listeners were listening passively, without performing a transition detection task).

Overall, we show that as regularities reoccur they are implicitly stored in memory, progressively up-weighted and more quickly retrieved to resolve the identity of sensory signals. EEG measurements from passively listening participants exposed to recurring patterns are also provided to demonstrate a progressive change in brain responses with sequence structure learning.

Barascud, N., Pearce, M.T., Griffiths, T.D., Friston, K.J**, Chait, M** (2016). Brain responses in humans reveal ideal observer-like sensitivity to complex acoustic patterns. *PNAS* 113 (5), E616-E625. doi:10.1073/pnas.1508523113

Disclosures: R. Bianco: None. **C. Bolger:** None. **M. Hu:** None. **P. Harrison:** None. **M. Pearce:** None. **M. Chait:** None.

Poster

137. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 137.22/J10

Topic: D.06. Auditory & Vestibular Systems

Title: Neural activity in mouse auditory cortex is influenced by context, behavior, and expectation

Authors: *N. J. AUDETTE[1], D. M. SCHNEIDER[2];
[1]Ctr. for Neural Sci., [2]New York Univ., New York, NY

Abstract: Hearing is not simply about detecting sounds. To benefit from hearing, animals must perceive and respond to sounds in a way that is sensitive to behavioral context by integrating acoustic input with variables including environmental features, prior associations, and ongoing movement plans. Recent experiments have shown that movement, reward-relevance, and predictability can independently affect neuronal activity at the earliest stage of cortical processing. However, it remains unclear how these contextual variables interact with one another to influence sensory responses at the single neuron and population levels in the primary auditory cortex. To investigate this question, we developed a simple lever-press behavioral paradigm in which mice learn to make highly-stereotyped, reward-driven forelimb movements accompanied by auditory feedback. The relationships among movement, sound, and reward can be experimentally controlled to produce a variety of contexts and associations. Trained mice altered their behavior in response to tones that were omitted or presented at unexpected times, indicating that they learned the expected relationship between movement and sound. We then used dense multi-unit array recordings in performing mice to measure neural population activity in response to sensory stimuli with varying contextual properties. We find that average sound-evoked neural responses in primary auditory cortex are suppressed during self-generated movements compared to passive listening. Movement-based suppression was strongest for predictable tones while tones that deviated from an expected frequency evoked relatively larger neural responses. Surprisingly, strong neural suppression was present even when predictable tones were tightly linked with rewarded outcomes. Despite this population-level suppression, single-neuron analysis revealed that a subset of neurons was preferentially activated by expected tones, raising the possibility that predictability might sparsen - rather than dampen - neural responses to

expected sounds. This closed-loop, lever-based behavior provides an experimental platform for studying how internal and external variables augment auditory processing, behavior, and perception.

Disclosures: N.J. Audette: None. **D.M. Schneider:** None.

Poster

137. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

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Program #/Poster #: 137.23/J11

Topic: D.06. Auditory & Vestibular Systems

Support: Whitehall Foundation Research Grant, Grant No. 2018-08-88
NARSAD Young Investigator Grant, Grant No. 27668

Title: Longitudinal stability and dynamics of neuronal ensemble representations in the auditory cortex

Authors: *H. SURI, G. ROTHSCHILD;
Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: Humans and other animals rely on familiarity with common sensory cues in the environment to guide behavior, while adapting to behaviorally relevant changing conditions. Neuronal sensory representations in the mammalian neocortex balance between these needs for providing a coherent and stable representation of the world, while reorganizing in response to salient changes in the environment. In the auditory cortex, neurons exhibit robust, consistent and selective responses to sound stimuli, and hence form a stable representation of sounds, at least on the timescale of acute recording experiments. On the other hand, studies have demonstrated that auditory cortical neurons can exhibit experience-dependent plasticity, for example following sound-guided learning. However, little is known about the longitudinal degree of ensemble-level stability or plasticity of auditory cortical sound representations under baseline conditions, in the absence of guided learning. To address this question, we carried out chronic two-photon calcium imaging in the auditory cortex of awake mice to derive the response properties of identified neuronal ensembles to simple and complex sounds across days. Our results suggest a surprising degree of dynamics in the longitudinal sound representations within local neuronal ensembles in the auditory cortex. Different neurons exhibited significant increases or decreases in responses in a sound-specific manner. Moreover, across days some sound-unresponsive neurons became responsive while some responsive neurons became unresponsive. Together, our results suggest that the neuronal representation of sounds in the auditory cortex is surprisingly dynamic.

Disclosures: H. Suri: None. **G. Rothschild:** None.

Poster

137. Auditory Processing: Adaptation, Learning, and Memory

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Program #/Poster #: 137.24/J12

Topic: D.06. Auditory & Vestibular Systems

Support: Whitehall Foundation Research Grant, Grant No. 2018-08-88
NARSAD Young Investigator Grant, Grant No. 27668

Title: Heterogeneity of locomotion-induced modulation of neuronal activity in the auditory cortex

Authors: *C. A. P. VIVALDO, G. ROTHSCHILD;
Psychology, Univ. of Michigan, Ann Arbor, Ann Arbor, MI

Abstract: Locomotor activity has a substantial influence on incoming sensory input and on the manner by which this input is processed. In the visual modality, locomotion has been shown to increase responses to visual stimuli in the visual cortex. Interestingly, within the auditory modality locomotion has been associated with inhibited spontaneous and sound-evoked responses in pyramidal cells, with the strongest inhibition observed in response to reafferent, self-generated sounds. However, previous studies have focused on the effect of locomotion on the activity of single cells, while the effect of locomotion on local network dynamics in the auditory cortex remains largely unknown. To address this knowledge gap we used two-photon calcium imaging to monitor activity in local neuronal ensembles in the auditory cortex of awake head-fixed Thy1-GCaMP6f mice that were free to stand, walk or run on a treadmill. Using this approach we analyzed single-cell and network response properties of excitatory pyramidal neurons to different sounds under different behavioral states. Our preliminary results demonstrate substantial heterogeneity of locomotion-related modulation of neural activity within local neural ensembles. Neighboring neurons exhibited differential and often divergent influence by locomotion: while some neurons exhibited reduced activity during locomotion as expected, responses of other neurons within the same ensemble were often strongly enhanced. Furthermore, heterogeneity in the effect of locomotion was observed on both spontaneous and sound-evoked responses, and for neural responses to different types of sound stimuli. These results may offer clues to the nature of network-level sound processing that underlie the perception of hearing during locomotion.

Disclosures: C.A.P. Vivaldo: None. **G. Rothschild:** None.

Poster

137. Auditory Processing: Adaptation, Learning, and Memory

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Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant F32DC015160
NIH Grant R01DC014452

Title: Maladaptive plasticity along the central auditory pathway leads to aberrant loudness perception following acoustic trauma

Authors: *B. D. AUERBACH, K. RADZIWON, R. SALVI;
Univ. at Buffalo, Buffalo, NY

Abstract: Neuronal gain control—the ability of neurons to adjust the slope of their input-output functions— is an essential mechanism employed across sensory systems to maintain perceptual stability in the face of a constantly changing sensory environment. A price to this plasticity, however, is the potential for maladaptive encoding in the face of abrupt or extreme changes to sensory input. Using a series of novel behavioral paradigms in combination with acute and chronic electrophysiological recordings, we demonstrate that typically adaptive gain control mechanisms in the central auditory pathway can lead to over-amplification of sound-evoked activity and excessive loudness perception following hearing loss. We found that acoustic trauma causes aberrant thalamo-cortical connectivity, decoupling cortical intensity coding from subcortical drive. The resulting cortical hyperactivity was strikingly correlated with changes to perceived loudness. These results provide novel insight into the nature of auditory perceptual disorders associated with hearing loss and the circuit mechanism underlying experience-dependent plasticity.

Disclosures: B.D. Auerbach: None. **K. Radziwon:** None. **R. Salvi:** None.

Poster

138. Human Auditory Processing I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 138.01/J14

Topic: D.06. Auditory & Vestibular Systems

Support: NIH R01-DC04290
Wellcome Trust WT092606AIA
European Research Council ERC CoG-MECHIDENT
University of Iowa Clinical and Translational Science Program UL1-RR024979

Title: Anterior temporal lobe disconnection disrupts auditory cortical oscillatory neural responses to speech in the human brain

Authors: *Z. KOCSIS[1,3], R. L. JENISON[4], B. MCMURRAY[2], A. E. RHONE[1], M. E. SARRETT[2], P. E. GANDER[1], K. V. NOURSKI[1], M. STEINSCHNEIDER[5], R. M. CALMUS[3], H. KAWASAKI[1], J. D. GREENLEE[1], C. K. KOVACH[1], T. D. GRIFFITHS[3], M. A. HOWARD, III[1], C. I. PETKOV[3];
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Abstract: Understanding the impact of surgical disconnection on neural responses in the human brain has the potential to advance models of normal neurophysiology and its disruption by pathology. We present data from four patients who underwent surgical disconnection of the anterior temporal lobe as part of the procedure to treat intractable epilepsy. In two patients, we obtained intraoperative electrocorticographic (ECoG) recordings pre- and post-resection while the patient was lightly sedated, but awake and responsive during a speech-sound perceptual prediction task. In two of the patients, we were also able to obtain pre- and post-operative magnetic resonance imaging (MRI) including T1 and T2 structural and diffusion-weighted scans. Time-frequency analyses of data recorded from auditory cortex (Heschl's gyrus or superior temporal gyrus) demonstrated an enhancement of the high-gamma response to speech sounds. We also observed changes in the timing and magnitude of the neurophysiological signal at lower frequencies, notably beta, which we interpret within the context of the predictive coding framework. Post-operative T1- and T2-weighted structural MRI scans were used to identify the surgical lesion. Probabilistic diffusion tensor imaging (DTI) tractography using seeds in the anterior temporal lobe confirmed disconnection of the temporal pole from areas caudal to the resection, including auditory cortex. Disruption of functional connectivity between disconnected and intact cortex was confirmed with state-space Granger Causality analyses of the task-based neurophysiological data, and they revealed changes of inter-regional connectivity within the intact cortex. These rare datasets provide first impressions of the crucial impact on speech processing in human auditory cortex following anterior temporal lobe disconnection.

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Poster

138. Human Auditory Processing I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 138.02/J15

Topic: D.06. Auditory & Vestibular Systems

Support: Rothberg Research Award in Human Brain Imaging
Greater Milwaukee Foundation

Title: The role of auditory scene analysis in auditory object perception: Neuroimaging evidence

Authors: *G. GURARIY[1], R. RANDALL[2], A. S. GREENBERG[3];
[1]Psychology, Univ. of Wisconsin, Milwaukee, Milwaukee, WI; [2]Music, Carnegie Mellon Univ., Pittsburgh, PA; [3]Dept. of Psychology, Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: A central challenge of auditory perception involves the extraction and subsequent grouping of acoustic features known as Auditory Scene Analysis (ASA). The resulting percepts can be referred to as “auditory objects”. Music is one example of an auditory object - an emergent property of sound contingent upon the organization of its constituent features. Previously, we have shown that perceived musicality of an auditory sequence varies according to its high-level organizational features (Randall & Greenberg, 2016). Here, we aimed to explore the neural mechanisms mediating ASA and auditory object perception. Participants performed judgments of perceived musicality on randomly generated pure-tone sequences as well as manipulated versions of each sequence that contained low-level changes in either amplitude or timbre. Manipulations of low-level features affected auditory object perception as evidenced by changes in musicality ratings of altered sequences. Next, fMRI was used to measure neural activation in response to the auditory sequences rated most & least musical (musicality manipulation), and the altered versions of each sequence (ASA manipulation). Using these data, we generated the following two separate, but partially overlapping, networks: (1) a music processing network (via independent music localizer), and (2) an ASA network (via contrast of base sequences vs. ASA manipulated sequences). To better understand the functional properties of these networks we employed Representational Similarity Analysis. A Representational Dissimilarity Matrix (RDM) was computed for every ROI on the basis of neural activation in response to each condition. Next, a series of computational models were constructed based on hypothetical auditory processes that ranged in sensitivity to low-level features (such as timbre or amplitude) and perception of musicality. Additionally, an RDM was generated on the basis of the behavioral musicality ratings. In order to test which model best accounts for the activity of each region, RDMs from every ROI were correlated to each of the computational models as well as to the behavioral RDM. In the resultant overlapping regions, areas near primary auditory cortex displayed significant correlations with low-level ASA models involved in auditory feature

extraction. Furthermore, an area in right IPS was significantly correlated with subject behavior (musicality ratings). The existence of shared neural mechanisms that correlate with behavioral ratings and underlie both ASA and music perception suggests that low-level features of auditory stimuli play a direct role in auditory object perception.

Disclosures: G. Gurariy: None. **R. Randall:** None. **A.S. Greenberg:** None.

Poster

138. Human Auditory Processing I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 138.03/J16

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant DC012379

Title: Lexical tone processing in human superior temporal gyrus

Authors: *Y. LI[1], C. TANG[1], J. LU[2], J. WU[2], E. F. CHANG[1];
[1]Dept. of Neurolog. Surgery, Univ. of California, San Francisco, San Francisco, CA; [2]Dept. of Neurolog. Surgery, Huashan Hospital, Fudan Univ., Shanghai, China

Abstract: In tone languages such as Mandarin Chinese, the pitch trajectory of a syllable distinguishes word meanings. Previous studies have identified cortical regions that are involved in the process of lexical tone perception. However, it remains unclear how the specific acoustic features that underlie tone differences are represented in human non-primary auditory cortex. To understand the auditory cortical representation of lexical tones, we used electrocorticography to record neural activity from Mandarin-speaking participants while they passively listened to natural, continuous Mandarin speech. We found that select neural populations in the superior temporal gyrus (STG) have activity patterns that differentiate lexical tones. Specifically, using representation similarity analysis, we found that the patterns of neural activity in these populations preserve the acoustic differences between lexical tones, while compressing the pitch variance within each tone. Next, using encoding models, we show that these activity patterns can be explained by neural tuning for the speaker-normalized pitch features of relative pitch height and pitch change. Furthermore, we show that these responses are largely language-independent. By having our Mandarin-speaking participants listen to English speech, we could fit encoding models using neural responses to English. These models revealed similar tuning for pitch features as the Mandarin models and were also able to predict responses to Mandarin tones. These results show that neural activity in STG supports categorical perception of lexical tones, and that the representation of lexical tones in STG is based on a language-general encoding of speech related pitch.

Disclosures: **Y. Li:** None. **C. Tang:** None. **J. Lu:** None. **J. Wu:** None. **E.F. Chang:** None.

Poster

138. Human Auditory Processing I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 138.04/J17

Topic: D.06. Auditory & Vestibular Systems

Title: Human speech cortex encodes amplitude envelope as transient, phase-locked responses to discrete temporal landmarks

Authors: *K. KOJIMA[1], Y. OGANIAN[2], C. CAI[3], A. FINDLAY[3], E. F. CHANG[2], S. S. NAGARAJAN[3];
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Abstract: The slow temporal amplitude envelope of speech reflects acoustically and perceptually relevant information about speech temporal structure and content. It is well-established that the phase of neural activity in the delta-theta bands is aligned to the phase of the speech amplitude envelope during listening. This has been taken as evidence for continuous entrainment of endogenous low-frequency oscillations to the speech envelope, possibly driven by phase-reset at some landmark event in the envelope, such as local peaks in the envelope (peakEnv) or times of rapid increases in amplitude (peakRate). We recently showed using direct electrocorticography that local neural populations in speech cortical areas selectively encode peakRate events in continuous speech. However, it leaves open whether phase-locking of low-frequency oscillatory activity observed with M/EEG reflects these transient responses. Alternatively, it might reflect the phase-reset of endogenous oscillatory activity by landmark events. We predicted that if it reflects transient responses, phase-locking would 1) diminish between acoustic edge events and 2) cover a frequency range reflective of the temporal structure of the speech stimulus envelope. In contrast, if phase-alignment reflects phase-reset of ongoing oscillatory activity, it should continue for several cycles between consecutive acoustic edges and its frequency range should be independent of stimulus envelope dynamics. To contrast these predictions, we recorded neural activity using MEG while participants (n = 6) listened to regularly-paced and 1/3-slowed continuous speech. We analyzed the phase of neural activity in the delta-theta band over bilateral temporal regions, aligned to peakEnv and peakRate. Phase-locking was increased when neural activity was aligned to peakRate events, more than it was aligned to peakEnv events, replicating our intracranial results. Crucially, phase-locking in lower frequency bands increased for slowed speech compared to regular speech. Finally, phase-locking peaked after peakRate events and diminished within a single cycle. This pattern of phase-locking is suggestive of an underlying transient response, rather than continuous oscillatory entrainment.

These data confirm and extend our previous intracranial findings to low-frequency activity and provide a link between results from intracranial electrophysiology and non-invasive MEG recordings. Taken together, our results demonstrate that the speech envelope induces a series of evoked responses at times of rapid increases in the speech amplitude envelope, rather than continuous alignment of intrinsic oscillatory activity.

Disclosures: K. Kojima: None. **Y. Oganian:** None. **C. Cai:** None. **A. Findlay:** None. **E.F. Chang:** None. **S.S. Nagarajan:** None.

Poster

138. Human Auditory Processing I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 138.05/J18

Topic: D.06. Auditory & Vestibular Systems

Support: Fonds Erasme (Brussels, Belgium)

Title: Cortical tracking of speech-in-noise in patients suffering from auditory obscure dysfunction

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Abstract: Patients encountering difficulties when listening to speech in noise without any observable peripheric hearing impairment is a common clinical situation known as obscure auditory dysfunction or King Kopetzki syndrome (KKS). Despite a prevalence exceeding 5% of the adult population, KKS pathophysiology remains largely unknown. When adults free of KKS listen to speech in a multi-talker background noise, their auditory cortex tracks the attended speech stream rather than the global auditory scene. In this magnetoencephalography (MEG) study, we aimed at assessing how the tracking is impacted by KKS. Specifically, we expected a disruption of the preferential tracking in KKS patients. MEG signals were recorded from 14 KKS patients and 14 controls paired for age, sex and educational level (mean age: 30 (21-41), sex ratio (F/M): 6/8). All were native French-speakers. Subjects were asked to listen to 5 different recordings of stories in French. Different levels of multi-talker background noise were randomly added to four of the recordings leading to the following signal to noise ratio conditions: No Noise, +10, +5, 0 and -5 dB. For each subject, we evaluated the coherence between MEG signals and the temporal envelope of 1) the global auditory scene (attended stream + multitalker background), 2) the attended speech stream (attended stream) and 3) the multitalker

background. Coherent sources were reconstructed via minimum norm estimation. Both groups demonstrated a cortical selective representation of the attended stream. Statistically significant coherence was observed between MEG signals originating from the auditory system and the attended stream at < 1 Hz, 1-4 Hz, and 4-8 Hz in all SNR conditions. Patients displayed similar coupling at < 1 Hz and 1-4 Hz but displayed lower coherence at 4-8 Hz in all SNR conditions. We have shown that KKS patients display a selective tracking (against our initial hypothesis) that is reduced at 4-8 Hz. As 4-8 Hz corresponds to syllable rate, our results indicate that KKS patients' difficulties to understand speech in noisy conditions might be linked to difficulties extracting syllables from connected speech.

Disclosures: M. Vander Ghinst: None. **M. Bourguignon:** None. **M. Niesen:** None. **V. Wens:** None. **S. Goldman:** None. **X. De Tiège:** None.

Poster

138. Human Auditory Processing I

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Program #/Poster #: 138.06/J19

Topic: D.06. Auditory & Vestibular Systems

Support: Vannevar Bush Faculty Fellowship program by the ONR to A.O. (N00014-16-1-3116)

Title: Spatiotemporal dynamics of sound representations in the human brain

Authors: *M. X. LOWE[1], Y. MOHSENZADEH[1], B. LAHNER[1], S. TENG[1], I. CHAREST[2], A. OLIVA[1];
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Abstract: How are sounds represented in the human brain? Here we use similarity-based fusion of human magnetoencephalography (MEG) and functional magnetic resonance imaging (fMRI) responses from sounds of human voices, animals, objects, and spaces to resolve the spatiotemporal dynamics of sound processing in the human brain. First, using a whole-brain searchlight approach we elucidate with millisecond and millimeter resolution the progression of sound representations from primary to non-primary temporal auditory areas within the first one hundred milliseconds post-stimulus onset. Second, we reveal the categorical structure of these representations using multidimensional scaling, and highlight the pronounced distinction of human voices. Using multivariate analyses, we then compare and contrast the spectral frequencies of these representations to dissociate multiple regions in the auditory stream. Finally, we reveal the subsequent unfolding of representations in several multisensory regions and examine overlap and distinctions in their temporal and representational space when compared with auditory cortex. Together, our results support a hierarchical framework of cortical sound

representations across space and time in the human brain and reveal how the structure of these representations is shaped by sound category and sound frequency content. We thus provide an integrated spatial, temporal, and informational account of human auditory processing.

Disclosures: M.X. Lowe: None. **Y. Mohsenzadeh:** None. **B. Lahner:** None. **S. Teng:** None. **I. Charest:** None. **A. Oliva:** None.

Poster

138. Human Auditory Processing I

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Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 138.07/J20

Topic: D.06. Auditory & Vestibular Systems

Support: BBRF NARSAD YI 26282
CMU BrainHUB

Title: Hyper-sensitivity to pitch is related to poorer prosody processing in adults with autism

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Abstract: Individuals with autism typically experience sensory abnormalities that include hyper-sensitivity to simple stimuli. Autism is also characterized by difficulties interpreting social communication. It is not known whether the sensory abnormalities contribute to the communication deficits. In the current study, we measured change detection to tones that differ in pitch and to emotional utterances using electroencephalography (EEG). Pitch is a key feature that distinguishes prosody (the emotional intonations in language). If pitch is processed abnormally in autism, then this could impact prosody processing adversely. Seventeen adults with autism and matched neurotypical controls took part in two experiments. For the first experiment, participants heard tones presented at either 1046.5Hz (C6), 1108.7Hz (C#6), or 1244.5Hz (D#6) and each tone was either repeated three or nine times before the pitch of the tone changed (a roving pitch paradigm). For the second experiment, participants heard two utterances (different speakers) of delight or frustration repeated three or six times before the speaker or the emotion changed. For both experiments, participants monitored a central fixation cross and responded when they saw the fixation cross change color. Event-related potentials (ERPs) were measured from fronto-central and central electrodes. Adults with autism exhibited larger mismatch negativity (MMN) to the change in pitch but smaller P3a to the change in

emotional utterance compared to neurotypical controls. MMN was also larger with greater pitch change (e.g. C6 to D#6) and the change in emotional utterance from delight to frustration was larger than the reverse. Together, this suggests that the hyper-sensitivity to pitch in autism might negatively impact the processing of prosodic sounds, and so the auditory hyper-sensitivity could contribute to the social communication deficits associated with autism.

Disclosures: **S.M. Haigh:** None. **P. Brosseau:** None. **C. Lele:** None. **S.M. Eack:** None. **D.I. Leitman:** None. **D.F. Salisbury:** None. **M. Behrmann:** None.

Poster

138. Human Auditory Processing I

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Topic: D.06. Auditory & Vestibular Systems

Support: R01 DC004290-11
NIDCD
WT091681MA, Wellcome Trust
NIH Grant UL1-RR024979

Title: Oscillatory correlates of auditory working memory in human intracranial EEG

Authors: *J. I. BERGER[1], P. E. GANDER[1], S. KUMAR[2], K. V. NOURSKI[1], M. I. BANKS[3], H. OYA[1], H. KAWASAKI[1], M. A. HOWARD, III[1], T. D. GRIFFITHS[2,4];
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Abstract: Working memory is the capacity to hold and manipulate behaviorally relevant information in mind in the absence of ongoing sensory input. Here we explored the hypothesis that working memory for tones requires a network of oscillatory activity in auditory cortex, frontal cortex, and hippocampus, and examined the form of such activity in neuronal ensembles. We recorded local field potentials from six human subjects undergoing invasive monitoring for presurgical localization of epileptic foci. The subjects were implanted with depth electrodes along the axis of Heschl's gyrus (HG) containing primary cortex in its posteromedial portion, subdural electrodes over temporal and frontal cortex, and depth electrodes targeting hippocampus. Following a visual alert, subjects were presented with a pair of tones belonging to two different categories. A visual cue informed the subjects which tone to keep in mind. A 3 s retention period was followed by a tone which could be the same or different from the tone held in mind. The subjects made a same/different judgement. A total of 160 trials (80 each of 'Low' and 'High' tone retention) were presented. We measured averaged event-related potentials,

carried out time-frequency analysis using wavelet transforms and examined phase-based functional connectivity.
During retention, a sustained increase (compared to rest period) in power in the beta band (15-20 Hz) was observed in the lateral part of HG. Increase in power in the gamma band (60-100 Hz) was observed in the posterior portion of superior temporal gyrus and in inferior frontal gyrus. In the hippocampus, power increase in low frequencies (less than 10 Hz) in the retention period was observed. Functional connectivity analyses revealed interactions among nodes of the auditory/frontal/hippocampal network.
The data demonstrate a network of brain regions during auditory working memory that includes auditory, frontal, and hippocampal cortex and is consistent with the network shown in our previous fMRI study (Kumar et al, J Neurosci 2016 36:4492-4505). The findings serve as a foundation for analyses of effective connectivity to test the hypothesis that the auditory cortex activity during retention is driven by the activity in inferior frontal gyrus or hippocampus.

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Poster

138. Human Auditory Processing I

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Topic: D.06. Auditory & Vestibular Systems

Support: R01 DC004290-11, NIDCD
WT091681MA, Wellcome Trust
University of Iowa Clinical and Translational Science Program's NIH UL1-RR024979

Title: Mapping of the cortical tinnitus network: Direct cortical recordings and electrical stimulation induced suppression

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Abstract: Tinnitus occurs when peripheral hearing damage leads to changes in ongoing brain activity. These secondary central mechanisms of tinnitus are poorly-understood, partly because relevant experimental evidence is almost entirely indirect, meaning it does not reflect the real-time perception of tinnitus, and/or does not provide a direct measure of neural activity. Previously we reported on a test of this hypothesis in a human neurosurgical subject, who had an extensive array of electrocorticography and depth electrodes placed for the localization of epilepsy (Sedley, Gander et al, Curr Biol 2015 25:1208-14). Tinnitus loudness was modulated with residual inhibition using noise, and quantified with real-time ratings. Here we report an experimental replication in a second neurosurgical subject (47yrs, F) with broadly comparable tinnitus and intracranial recording.
Similar findings in both subjects were obtained: 1) Suppression of tinnitus correlated with widespread reductions in delta (1-4 Hz) oscillatory power throughout most of auditory cortex, and extending to temporal, parietal, limbic and motor areas. These areas also showed changes in inter-regional delta phase coherence with tinnitus suppression. 2) Theta (4-8 Hz), alpha (8-12 Hz), and high beta (20-28 Hz) power was similarly suppressed in most of these areas. 3) Gamma (28-144 Hz) power increased during tinnitus suppression throughout auditory cortex and in posterior temporal, inferior parietal, sensorimotor and parahippocampal areas. In the second subject, a novel finding was observed during electrical stimulation of Heschl's gyrus which elicited reductions in tinnitus loudness comparable to those induced by sound. The change in tinnitus perception from stimulation occurred without alteration to other external auditory perception.
These findings support the definition of the brain networks critically involved in tinnitus perception, which will be necessary to create effective tinnitus management strategies and possible cures.

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Poster

138. Human Auditory Processing I

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Program #/Poster #: 138.10/J23

Topic: D.06. Auditory & Vestibular Systems

Support: NIH R01-DC04290
NIH R01-GM109086
NIH UL1-RR024979

Title: Cortical responses to auditory novelty across task conditions as revealed by intracranial recordings

Authors: *K. V. NOURSKI[1], M. STEINSCHNEIDER[2], A. E. RHONE[1], H. KAWASAKI[1], M. A. HOWARD, III[1], M. I. BANKS[3];
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Abstract: Elucidating changes in predictive coding across attentional and arousal states is a major focus in neuroscience. The local/global deviant paradigm (Bekinschtein et al, PNAS 2009 106:1672-7) engages auditory predictive coding over short (local deviance, LD) and long (global deviance, GD) time scales, and has been used to assay disruption of auditory predictive coding upon loss of consciousness. Previous work (Nourski et al, J Neurosci 2018 38:8441-52) has examined effects of propofol anesthesia on short- and long-term novelty detection. GD effects were suppressed at subhypnotic doses of propofol, suggesting that they may be more related to attention than consciousness per se. The present study addressed this hypothesis by comparing cortical responses to auditory novelty between passive and active task conditions in awake listeners. Subjects were adult neurosurgical patients undergoing chronic invasive monitoring for medically intractable epilepsy. Sequences of five 100 ms vowels separated by 50 ms silent intervals were presented to subjects as they watched a silent TV program and attended to its content (passive task) or pressed a button in response to GD target stimuli (active task). Intracranial recordings were made from core and non-core auditory, temporo-parietal auditory-related, prefrontal and sensorimotor cortex. Task performance was measured as sensitivity index, hit rate and reaction times. Cortical activity was measured as averaged auditory evoked potentials (AEPs) and high gamma (70-150 Hz) event-related band power. The onset of the stimuli and LD elicited robust AEPs in all studied brain areas in both passive and active experiments. The active task was associated with an increase in the fraction of sites with AEPs to stimulus onset and the LD effect in prefrontal cortex. High gamma responses to stimulus onset and LD were localized predominantly to the auditory cortex in the superior temporal plane and had a comparable spatial extent between the two conditions. In contrast, GD effects were greatly enhanced during the active task in auditory cortex on the lateral superior temporal gyrus, auditory-related, prefrontal and sensorimotor cortex. The prominence of GD effects was associated with individual subjects' task performance. The data demonstrate distinct attention-related effects on responses to auditory novelty across the cortical processing hierarchy. The results motivate closer examination of effective connectivity underlying attentional modulation of cortical sensory responses, and serve as a foundation for examining changes in sensory processing associated with general anesthesia, sleep and disorders of consciousness.

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Poster

138. Human Auditory Processing I

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Program #/Poster #: 138.11/J24

Topic: D.06. Auditory & Vestibular Systems

Support: DFG Grant GU593/5-1

Title: Effects of task and arousal level on the processing of peri-threshold tones investigated with MEG, EEG, and pupillometry

Authors: L. DOLL[1], *A. R. DYKSTRA[2], A. GUTSCHALK[1];
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Abstract: Conscious perception of weak stimuli is state-dependent and likely influenced by selective attention and arousal level. We studied these factors using a multi-block tone-in-noise detection experiment. In blocks 1 and 3, participants detected weak but supra-threshold amplitude modulation (AM) of continuous white noise; in block 2, they detected peri-threshold tones embedded in the noise. Cortical activity was measured with simultaneous MEG/EEG, and pupil dilation was used as a proxy for locus coeruleus activity (related to arousal). Mean hit rate for tone-in-noise detection in block 2 across listeners (N=11) was 41% (range 27-52%); mean hit rate for AM detection (blocks 1 and 3) was 74% (range 42-96%). Hit rates were significantly correlated with pre-stimulus pupil size. None of the listeners noted the presence of the peri-threshold tones in block 1; five did in block 3. In block 2, auditory cortex activity between 150 and 250 ms was present irrespective of tone detection, but much larger for detected tones. No such activity was observed for the same tones when they were task-irrelevant (blocks 1 and 3). Later (400-600 ms) activity in posterior cingulate cortex was observed for hits but neither for misses nor for non-target conditions (both for peri-threshold tones and noise AM, in respective blocks). Significant transient pupil dilation was only observed for targets (either peri-threshold tones or noise AM in respective blocks) and was larger for hits than misses. These results are consistent with roles of arousal and selective attention in biasing detection of peri- threshold tones: (1) Higher arousal levels as indexed by pupillometry were associated with higher detection probability. (2) Significant AC activity was only evoked by peri-threshold tones when they were task-relevant, possibly reflecting conscious perception of the tones. However, this conclusion is limited by the post-hoc report of tone detection in block 3 by a subgroup of participants, which was not paralleled by AC activity in the average response. Enhanced AC and pupil responses for missed peri-threshold tones in block 2 may either be related to a conservative response criterion or to attentional effects operating on non-perceived stimuli.

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Poster

138. Human Auditory Processing I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 138.12/J25

Topic: D.06. Auditory & Vestibular Systems

Title: Minor critical notes evoke attention-related erp components in musicians over non-musicians

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Abstract: Musical training is required for individuals to correctly label musical modes using the terms “major” and “minor”, while no training is required to label these modes as “happy” or “sad”. In spite of the high accuracy of non-musicians in the happy/sad labeling task, previous research suggests there are differences between musicians and non-musicians in the neural response to the critical note---the note (the third of the relevant key) that defines a melody as major or minor. A previous electrophysiological study revealed a late positive component to the first critical note of a minor melody but not the first critical note of a major melody in trained musicians. This effect was not present in non-musicians. The current study extends this finding using high-density EEG in a sample of young adults to examine the neural correlates of the second critical note in a melody. Further, we evaluated whether the minor critical note elicited the early right anterior negativity response (ERAN) in minor vs. major modes. While there was no evidence of an LPC response to the second critical note in either group, we did see a strong ERAN response in the inferior frontal gyrus in musicians in response to the first critical note in the minor mode. These findings support the hypothesis that musical training is needed to recognize the critical note in a minor scale and that the minor critical note may be processed as an unexpected stimulus in the context of the melody.

Disclosures: T.M. Centanni: None. **A.R. Seisler:** None. **A.R. Halpern:** None. **M.J. Wenger:** None.

Poster

138. Human Auditory Processing I

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Topic: D.06. Auditory & Vestibular Systems

Support: NIDCD 5F31DC015956 to IMB
NSF GRFP to AK
Vanderbilt Institute for Clinical and Translational Research VR52433 to AJD

Title: Cochlear implant users experience repetition induced musicality for speech and environmental sounds

Authors: *A. KASDAN[1], I. M. BUTERA[1], A. J. DEFREESE[3], J. ROWLAND[4], M. BURCHESKY[3], M. T. WALLACE[1], R. H. GIFFORD[2];
[1]Vanderbilt Brain Inst., [2]Hearing and Speech Sci., Vanderbilt Univ., Nashville, TN; [3]Dept. of Audiol., Vanderbilt Univ. Med. Ctr., Nashville, TN; [4]Princeton Univ., Princeton, NJ

Abstract: The speech-to-song illusion is a robust effect wherein repeated speech induces the perception of music. This effect was first reported by Deutsch et al. (2011), and in recent studies by Rowland et al. (2018) and Simchy-Gross & Margulis (2018) has been further applied to non-speech stimuli (i.e. environmental sounds) to show that repetition of auditory material induces the perception of music (the sound-to-music effect). To date, all studies exploring this effect have focused on listeners with normal hearing (NH). Here, we aim to test the saliency of the sound-to-music effect in a cohort of adult cochlear implant (CI) recipients. Because CIs provide good temporal resolution yet poor spectral resolution, users can excel at understanding speech in quiet while experiencing substantial difficulty with speech in noise as well as the perception of music. Since prior work from Rowland et al. (2018) shows that removing spectral information reduces but does not eliminate perception of the sound-to-music effect, we hypothesize that CI users *will* perceive the illusion yet to a lesser extent than NH controls. Thus far, we have tested 3 CI users and 5 NH controls using repeated speech as well as non-speech (i.e. water droplet) stimuli. Participants listened to a single presentation of each stimulus and made a judgement in response to the question "how much does this sound like music to you?" on a scale of "not at all like music" to "exactly like music" (measured on a continuous scale from 0 to 1). The participants then listened to 16 repetitions of the same sound file and made a post-repetition musicality judgement. On average, NH controls experienced the effect as a 4x increase in pre- to post-repetition ratings for speech (from 0.07 to 0.30) and 2.3x increase for the non-speech condition (from 0.22 to 0.51). Interestingly, CI users experienced the effect as a 21x increase in musicality for speech (ratings from 0.01 to 0.21) and 7x for the non-speech condition (ratings from 0.06 to 0.41). The magnitude of CI users' final post-repetition ratings was lower than for

NH controls for both speech (0.21 v. 0.30) and non-speech (0.41 v. 0.51) conditions. Qualitative self-reports following this experiment suggest that CI users often perceive rhythmic elements, and it is likely that CI users rely more on the rhythmic properties of the stimulus for music perception instead of melodic/prosodic elements. In contrast, NH controls typically classified their percepts as more melodic in nature. These preliminary results give insight into how the auditory system processes repetition-induced musicality and suggest that short repetitions may provide a promising way for CI users to better experience and enjoy music.

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Poster

138. Human Auditory Processing I

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Program #/Poster #: 138.14/J27

Topic: D.06. Auditory & Vestibular Systems

Support: DFG Grant PU590/1-1
CIHR Grant FDN143217

Title: Musical training enhances cortical phaselocking while listening to continuous natural speech

Authors: *S. PUSCHMANN, M. REGEV, S. BAILLET, R. J. ZATORRE;
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Abstract: There is increasing evidence that musical training can benefit the perception of speech in noise (Coffey et al., 2017). Previous electrophysiological work suggests that this "musician advantage" is related to a superior sensory encoding of low-level auditory information in musicians, leading to more robust and more distinct cortical representations of speech (Du & Zatorre, 2017; Parbery-Clark et al., 2012). However, whether musical training also benefits speech processing beyond early sensory encoding is still largely unknown.
We used magnetoencephalographic (MEG) source imaging and an inter-subject phase-locking analysis to test whether musical training benefits the functional coupling between auditory cortices and higher-order brain regions during the perception of natural speech. MEG data were obtained from 20 individuals (11 female; age: 21±3 years) with a varying degree of musical training (duration: 0-18 years) while listening to a continuous audio story (duration: 15 minutes) without background noise.
While listening to continuous speech, subjects showed robust inter-subject phase-locking

between both auditory cortices and a broad ensemble of brain regions of the adjacent temporal, frontal and parietal lobes, in both hemispheres ($p<.05$, FDR). This effect was consistent for the delta (i.e., 1-4 Hz), theta (4-8 Hz) and alpha (8-12 Hz) bands. Musical training was associated with increased alpha-band phase-locking between bilateral auditory cortex and the dorsal and ventral speech processing streams (Spearman correlation; $p<.05$, FDR). Importantly, musical training enhanced auditory cortex phase-locking to both ipsi- and contralateral brain regions, indicating a more bilateral processing of speech information in musically trained individuals. Our results therefore suggest that the previously reported "musician advantage" in speech-in-noise processing may not only arise from a more robust low-level encoding of speech information, but also from a stronger functional coupling between the auditory cortex and higher-order brain areas involved in speech processing.

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Du Y, Zatorre RJ. 2017. Musical training sharpens and bonds ears and tongue to hear speech better. Proc Natl Acad Sci USA. 114:13579–13584.

Parbery-Clark A, Tierney A, Strait DL, Kraus N. 2012. Musicians have fine-tuned neural distinction of speech syllables. Neurosci 219, 111-119.

Disclosures: S. Puschmann: None. **M. Regev:** None. **S. Baillet:** None. **R.J. Zatorre:** None.

Poster

138. Human Auditory Processing I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 138.15/J28

Topic: D.06. Auditory & Vestibular Systems

Support: EMBO IG 3028
TUBA GEBIP 2015
BAGEP

Title: Attentional modulation of articulatory and semantic representations during multi-speaker natural speech perception

Authors: I. KIREMITCI[1], O. YILMAZ[1], A. G. HUTH[2], U. KELES[1], *T. CUKUR[1]; [1]Bilkent Univ., Ankara, Turkey; [2]UT Austin, Austin, TX

Abstract: Humans can easily focus on a certain speaker's speech stream in crowded environments. Auditory attention is thought to play a key role in this remarkable ability (Regev, C Cortex, 2018; Brodbeck, C Biology, 2018). Yet, it remains unclear across what levels of speech features and where in the brain attentional selection occurs. To address this question, we

recorded whole-brain BOLD responses during a cocktail-party task. We measured voxel-wise selectivity for spectral, articulatory and semantic features of natural speech. We then examined the attention modulation in selectivity for these features across cortex.
Natural stories told by a male and a female speaker were overlaid to simulate a cocktail-party environment, and subjects were asked to attend to either speaker in different runs. We fit voxel-wise models that best predicted measured BOLD responses in terms of spectral, articulatory and semantic features (Huth, Nat, 2016). Cortical voxels involved in language perception were identified as voxels significantly predicted by spectral, articulatory or semantic models during passive story listening. Language-related functional ROIs were localized using an automatic atlas-based parcellation (Destrieux, Neuroimage, 2010). Modulation in feature selectivity was measured for each model; and an overall attention index was taken as the weighted-average of modulations across models based on their prediction scores.
We find that the attention index is smallest at Heschl's Gyrus (attIdx=0.18, $p<0.05$) in the left hemisphere and at Planum Temporale (attIdx=0.11, $p<0.05$) in the right hemisphere. There is a consistent increase in attention index across a dorsal pathway starting at HG, ending at BA44 (attIdx=0.59, $p<0.05$) and BA6 (attIdx=0.68, $p<0.05$); and on a ventral pathway starting at HG, ending at BA45 (attIdx=0.65, $p<0.05$) and pSTS (attIdx=0.74, $p<0.05$) in the left hemisphere. Moreover, we find that attentional modulations are dominated by changes in articulatory but not spectral selectivity in HG and PT; by changes in articulatory selectivity in BA6, and by changes in semantic selectivity in BA45 and pSTS. These results suggest that: 1) Attention enhances the representation of the attended stream with an increasing strength towards higher language areas on dorsal and ventral pathways. 2) Attentional selection is primarily mediated by modulation of articulatory selectivity in earlier areas, whereas modulation of articulatory and semantic selectivity dominates towards later levels of the dorsal and ventral hierarchies respectively. 3) Early auditory regions maintain spectral representations of both attended and unattended streams.

Disclosures: **I. Kiremitci:** None. **O. Yilmaz:** None. **A.G. Huth:** None. **U. Keles:** None. **T. Cukur:** None.

Poster

138. Human Auditory Processing I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 138.16/J29

Topic: D.06. Auditory & Vestibular Systems

Support: DFG Grant GU593/5-1

Title: Neural correlates of tone-sequence awareness probed with pre- and post-stimulus cues in human listeners

Authors: K. GAERTNER, *A. GUTSCHALK;
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Abstract: Previous studies suggested that a long-latency negativity in auditory cortex is closely coupled to perceptual awareness of tones under informational masking, hence termed the awareness-related negativity (ARN). An alternative interpretation of this ARN is that it reflects enhanced processing subsequent to the detection of the pre-defined target. To further explore the role of the ARN, we present two MEG experiments in which the target can only be identified based on a cue presented before or after the multi-tone scenes. In Experiment 1, scenes comprising 9 different, non-synchronous tones each repeated 5 times with random inter-tone intervals, were presented to 20 participants. The target tone was indicated by a cue that was either placed before or after the scene, and participants were asked to indicate if the tone was part of the scene or not. Hit rates were significantly higher (96% vs 76%) and false-alarm rates lower (4% vs 16%) for pre- compared to post-stimulus cues. MEG showed no difference between hit and miss trials for the post-stimulus cues, but strong enhancement of negative source activity in auditory cortex for hit trials in the pre-stimulus cue condition (75-275 ms). In Experiment 2, to ensure that listeners perceived the whole target stream, random tone sequences ("melodies") were presented in the presence of a random multi-tone masker to 14 participants. Participants were required to indicate if the post-stimulus cue (a repetition of the target sequence or another random tone sequence) was present in the masker interval, or not (hits: 47%, false alarms: 7%). MEG results in Experiment 2 showed stronger negative source activity in auditory cortex for hit compared to miss trials, despite the post-stimulus cue used. These results suggest that the ARN may be related to the perception of auditory streams in the presence of a multi-tone masker, but make it unlikely that it is related to the perceptual awareness of the single tones. Possibly, attention is generally required to perceive auditory streams in this setting.

Disclosures: A. Gutschalk: None. **K. Gaertner:** None.

Poster

138. Human Auditory Processing I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 138.17/J30

Topic: D.06. Auditory & Vestibular Systems

Support: NWO 016.Vidi.185.137
P50 MH109429

Title: Rhythmic facilitation of temporal prediction: Testing the neural entrainment hypothesis

Authors: *L. IEMI[1], I. TAL[2], A. BRESKA[3], J. SAMAHA[4], E. M. MERRICKS[5], C. A. SCHEVON[6], G. M. MCKHANN[7], C. E. SCHROEDER[8], S. HAEGENS[9];

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Abstract: The neural entrainment hypothesis proposes that rhythmic streams of salient sensory stimuli can produce phase-reset of ongoing neural oscillations, such that the high-excitability phase of intrinsic oscillations becomes aligned with the occurrence of task-relevant events. One consequence of such entrainment is that responses to the task-relevant events can be amplified relative to event-related responses occurring out of phase with the entrained oscillation. Entrainment also represents a mechanism through which the brain may instantiate temporal predictions and support active perception in general. We tested this hypothesis in a series of experiments using psychophysics, and either magnetoencephalography (MEG) or electrocorticography (ECoG) in human subjects. We used an experimental paradigm that specifically dissociates periodicity of sensory stimulation (thought to drive neural entrainment) from temporal predictions (which do not require periodicity). We found that, compared to aperiodic streams that provide a comparable amount of temporal prediction, periodic isochronous streams (at a delta-band frequency) were followed by: (1) increased phase-alignment of low-frequency activity, including frequencies harmonically unrelated to the stimulation rate, and (2) by faster (not better) responses in subsequent target discrimination. Critically, our results suggest that periodic stimulus streams lead to a general phase reset, rather than frequency-specific neural entrainment of ongoing oscillations, and benefit reaction times, rather than perceptual accuracy. Therefore, these results seem to call into question the hypothesis of neural entrainment as it is currently formulated in the literature.

Disclosures: L. Iemi: None. **I. Tal:** None. **A. Breska:** None. **J. Samaha:** None. **E.M. Merricks:** None. **C.A. Schevon:** None. **G.M. McKhann:** None. **C.E. Schroeder:** None. **S. Haegens:** None.

Poster

139. Vestibular System and Balance

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 139.01/J31

Topic: D.06. Auditory & Vestibular Systems

Title: Morphological misalignment of vestibular organs predicts motion sickness susceptibility

Authors: *T. HARADA[1], T. SUGAWARA[2], M. FUKUNAGA[3], N. SADATO[3], S. LAUREYS[4], H. SAKAI[1];
[1]Toyota Central R&D LABS., INC., Tokyo, Japan; [2]Toyota Central R&D LABS., INC., Aichi, Japan; [3]Natl. Inst. for Physiological Sci., Aichi, Japan; [4]Univ. of Liège, Sart Tilman, Belgium

Abstract: It is thought that motion sickness results from a conflict among different sensory modalities, such as the visual and vestibular senses. However, little is known about the impact that sensory conflicts within a sensory modality have on motion sickness. Here, we hypothesized that the morphological misalignment of vestibular organs is a cause of motion sickness. To examine this hypothesis, we performed structural magnetic resonance imaging (MRI) of the inner ear in 36 adults susceptible to motion sickness (MSS group) and age/sex-matched adults resistant to motion sickness (MSR group). For each participant, motion sickness susceptibility was confirmed by a questionnaire, and vestibular misalignment was indexed as an angle between the interocular axis and the vector connecting the normal vectors of each corresponding pair of left and right semicircular canals (SCs). Additionally, each participant underwent a resting-state functional MRI (fMRI) of the brain to identify functional connectivity associated with vestibular misalignment. The results show that the misalignment index of the horizontal SC was greater in the MSS group as compared to that of the MSR group. Furthermore, the resting-state fMRI analysis revealed that the misalignment index of the horizontal SC was significantly and positively correlated with functional connectivity between the left parieto-insular vestibular cortex and right anterior insular cortex, which is an area involved in the production of nausea and vomiting. These findings are consistent with our hypothesis, and thus, suggest that sensory conflicts within a sensory modality, as well as among sensory modalities, are a cause of motion sickness.

Disclosures: **T. Harada:** A. Employment/Salary (full or part-time):; TOYOTA CENTRAL R&D LABS., INC. **T. Sugawara:** A. Employment/Salary (full or part-time):; TOYOTA CENTRAL R&D LABS., INC.. **M. Fukunaga:** None. **N. Sadato:** None. **S. Laureys:** F. Consulting Fees (e.g., advisory boards); TOYOTA CENTRAL R&D LABS., INC. **H. Sakai:** A. Employment/Salary (full or part-time):; TOYOTA CENTRAL R&D LABS., INC..

Poster

139. Vestibular System and Balance

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 139.02/J32

Topic: D.06. Auditory & Vestibular Systems

Title: Impact of haltere removal on gravitational perception

Authors: A. RAZMI[1], *J. L. FOX[2];
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Abstract: Found on the fly metathorax, halteres are mechanosensory organs known to aid in stabilization during flight. Information from the halteres is sent to wing-steering and head movement motoneurons, allowing for postural adjustments to outward stimuli. Some flies oscillate halteres during walking, such as flesh flies (Sarcophagidae), while others, including long-legged flies (Dolichopodidae) do not. In flesh flies subjected to a free fall while stationary, halteres mediate the fly's response to the sudden gravitational shifts. However, the utility of these organs in flies that do not oscillate halteres during walking, or in flies responding to sudden changes in gravity while walking, has yet to be explored and could shed insight into the role of halteres in gravitational perception. In this study, wild-type, adult, stationary Dolichopodidae (varying species) and wild-type, adult, walking *Sarcophaga bullata* were observed. The behavior of intact flies was compared to behavior of flies with their halteres removed. Each fly was placed in a clear plastic container suspended 2 cm above a surface and filmed using a high-speed videography camera as the container fell. For Dolichopodidae, there was no significant difference in median body velocity (relative to the falling container) during the fall between intact and haltereless flies (n=9 flies; 5 trials intact, 5 trials ablated per fly; $p>0.05$), suggesting that flies that do not oscillate halteres while walking do not use halteres to respond to shifts in gravity. Similarly, the median body velocity of intact and haltereless *Sarcophaga bullata* (n=10) during the fall was not significantly different ($p>0.05$). These data suggest that any sensory information from oscillating halteres during shifts in gravity is only supplemental as opposed to necessary for behavioral adaption; for example, sensory input from changing leg loads during walking may be more beneficial to the fly than input from the halteres.

Disclosures: J.L. Fox: None. **A. Razmi:** None.

Poster

139. Vestibular System and Balance

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 139.03/J33

Topic: D.06. Auditory & Vestibular Systems

Support: Natural Sciences and Engineering Research Council of Canada RGPIN-2016-05211

Title: Questioning the lasting effect of galvanic vestibular stimulation on postural control

Authors: *M. NOORISTANI, M. MAHEU, L. BEHTANI, M.-S. HOUDE, F. CHAMPOUX; Univ. De Montréal, Montreal, QC, Canada

Abstract: *Background:* Noisy galvanic vestibular stimulation (nGVS) has been shown to enhance postural stability, and this effect has been observed to persist for several hours post-stimulation. However, these effects were observed without proper control (sham condition) and

the possibility of experimental bias has not been ruled out. The lasting effect of nGVS on postural stability therefore remains in doubt.
Objective: To investigate the effect of nGVS on postural stability using a control (sham) condition to confirm or infirm the possibility of experimental bias.
Methods: 28 participants received either nGVS or a sham stimulation. Static postural control was examined before stimulation, immediately after 30 minutes of nGVS and one-hour post-stimulation.
Results: A significant improvement of sway area ($p=0,05$), sway velocity ($p<0,05$) and path length ($p<0,05$) was observed following nGVS, as previously shown. However, a similar improvement was also observed in the control group and there was no significant difference between groups ($p>0,05$), suggesting that the observed postural enhancements could be due to a learning effect.
Conclusion: This finding suggests the presence of experimental bias in nGVS effect on postural stability and highlights the need to use a sham condition in the exploration of nGVS effect so as to disentangle the direct effect of the electrical stimulation from a learning effect. Furthermore, numerous parameters and populations need to be explored in order to confirm or infirm the presence of a real long-lasting effect of nGVS on postural stability.

Disclosures: **M. Nooristani:** None. **M. Maheu:** None. **L. Behtani:** None. **F. Champoux:** None. **M. Houde:** None.

Poster

139. Vestibular System and Balance

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 139.04/J34

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant R01 DC002390

Title: Using vestibular prosthesis to restore yaw rotation perception in primates with bilateral vestibular hypofunction

Authors: *K. WIBOONSAKSAKUL[1], C. C. DELLA SANTINA[2], K. E. CULLEN[1];
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Abstract: The vestibular system senses movement of the head and provides information crucial for appropriate reflexes and vestibular perception. Patients with bilateral loss of vestibular function not only experience disequilibrium and visual instability due to impaired vestibular reflexes but also suffer from loss of vestibular perception. To help improve the quality of life of these patients, one emerging approach has been centered on developing a prosthesis that senses

head rotation and translates the movement into ampullary stimulation, substituting for the damaged vestibular periphery. Recent experiments in our groups have characterized the efficacy of our vestibular prosthesis on the vestibulo-ocular reflex (VOR) and vestibulocollic reflex in nonhuman primates. However, to date, its ability to restore vestibular perception in nonhuman primates has not been explored. Here, we investigated the use of the vestibular prosthesis to restore yaw rotation perception in primates. A unilaterally vestibular-deficient rhesus monkey was trained to distinguish between passive left and right yaw rotations in a two-alternative forced-choice recognition task. The whole-body motion stimulus consisted of single cycles of sinusoidal acceleration at 1 Hz. The monkey then underwent prosthetic implantation on the normal side, resulting in a bilateral vestibular hypofunction (VOR gain < 0.15 bilaterally). Perceptual performance was quantified before and after prosthetic implantation by computing the vestibular perceptual threshold via fitting a Gaussian cumulative distribution psychometric function to the binary responses. The perceptual threshold in darkness worsened from 43.5 deg/s to >385 deg/s after implantation, indicating very little residual vestibular function consistent with the VOR results above. When prosthetic modulation was turned on, using the natural head-velocity-to-stimulation-rate mapping function [Sadeghi et al. 2008], the threshold improved significantly to 85 deg/s ($p < 0.01$). Additionally, using a more sensitive stimulation mapping (2.5 times steepness increase), the perceptual threshold improved even further ($p < 0.01$) to 50 deg/s. Though different, these two thresholds corresponded to comparable stimulation rate of ~170 pulse/s, suggesting a common neuronal threshold underlying the observed improvement in yaw rotation perceptual performance. Future work will investigate the neural correlates of the perceptual improvement, in addition to employing a comparable psychophysical-based methodology in implanted patients.

Disclosures: K. Wiboonsaksakul: None. **C.C. Della Santina:** None. **K.E. Cullen:** None.

Poster

139. Vestibular System and Balance

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 139.05/J35

Topic: D.06. Auditory & Vestibular Systems

Support: Natural Sciences and Engineering Research Council of Canada RGPIN-2016-05211

Title: The effect of ageing on multisensory weighting for postural control

Authors: *L. BEHTANI, M. MAHEU, M.-S. HOUDE, F. CHAMPOUX;
Univ. of Montreal, Montreal, QC, Canada

Abstract: INTRODUCTION: Falls represent a leading cause of injuries and death among older adults, and can become a bigger society health concern considering the aging of the population. Therefore, aging of the sensory systems involved in maintaining posture is an important phenomenon to study for the prevention of falls among elderly. The vestibular system consists of three semicircular canals and two otolith organs within the inner ear. The vestibular system is an absolute reference to other sensory systems involved in postural control, but becomes less reliable with age, leading to higher risk of falls. Current evidence suggests that reduced vestibular semicircular canal function is the major driver of increased postural sway in the elderly, particularly under limited sensory conditions. Unfortunately, it is still unclear how the vestibular otolith function contributes to increased falls in the elderly.
OBJECTIVE: Our aim was to investigate the effect of age-related loss of otolith function on static postural control in older adults as compared to younger adults.
METHODOLOGY: We tested a total of 30 healthy community-dwelling old and young adults in four postural conditions using a force platform. We used wavelet analysis for the postural measures to identify the relative weight of vestibular cues. We assessed otolith function by using Vestibular Evoked Myogenic Potential (VEMP), and then correlated them with the force platform measures.
RESULTS: The older group had significantly lower postural control under challenging postural conditions as compared to the younger group ($p = 0.001$). Wavelet analysis revealed a significant weight shift to vestibular cues among the elderly, since they seem to rely more on vestibular cues than the younger group ($p = 0.007$) when responding to somatosensory perturbations during quiet standing. Moreover, VEMP significantly correlated with postural measures ($r = -0.490$, $p = 0.004$) and could be useful for predicting postural control.
CONCLUSION: Our findings demonstrate how age-related otolith vestibular loss contributes to poorer postural control in the elderly and suggests a vestibular-postural measure (VEMP) for fall prevention programs.

Disclosures: **L. Behtani:** None. **M. Maheu:** None. **M. Houde:** None. **F. Champoux:** None.

Poster

139. Vestibular System and Balance

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 139.06/J36

Topic: D.06. Auditory & Vestibular Systems

Support: Faculty research grant of Yonsei University College of Medicine (3-2018-0182)

Title: Data analysis methods for measurement of postural instability during virtual reality immersion

Authors: *E. SON, J. KIM, I. KIM, M. HONG;
Dept. of Otorhinolaryngology, Yonsei Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: Computerized dynamic posturography (CDP) is widely used as a method to assess functional impairments of balance. However, CDP is limited because it measures center of gravity(COG) during 10-seconds duration. Under threatening condition like standing on the high and looking down, amplitude and frequency of sway during static balance is expected to be altered, but traditional CDP measurement was inadequate to measure the changes. We designed virtual reality(VR) contents that people stand the edge of the building with 3D environments where the user can navigate, and analyzed collected data for COG sway during prolonged 30-second trials. Two conditions of VR immersion scenarios(GL, EL) were created and operated on head mount devices. GL condition is scene of field on ground level, and EL condition shows standing on a tall building edge. Ten healthy subjects were instructed to maintain up right posture during the test. Data of 3000 COG measurements was collected during test for each condition. Subjective symptoms were measured using visual analog scale and the simulator sickness questionnaire. Mean COG sway velocities for x-axis increased in EL(-0.02±0.44 deg/sec) compared to GL(0.17±0.35 deg/sec)(p=0.048), but for y-axis was comparable between GL and EL(p=0.08).In order to compare variability within each data set coefficient of variation(cv) was analyzed, there was no significant difference between GL and EL conditions in x- or y-axis directions(p=0.14 and 0.57 respectively). Mean VAS scores 0.1 for GL and 4.5 for EL respectively. Mean total SSQ scores were 3.4 for GL and 14.6 for EL, showing that even healthy subjects showed wide range of symptoms related to fear of heights. Subjective symptoms were elicited during VR contents of EL, and there was discrepancy between statistical analysis methods for COG sways in both directions. Further investigation would help our understanding of how the threatening situations such as falling down from heights influence postural control.

Disclosures: E. Son: None. **J. Kim:** None. **I. Kim:** None. **M. Hong:** None.

Poster

139. Vestibular System and Balance

Location: Hall A

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Program #/Poster #: 139.07/J37

Topic: D.06. Auditory & Vestibular Systems

Support: Utah State University Undergraduate Research and Creative Opportunities Grant

Title: Limited habituation to prolonged electrical vestibular stimulation during treadmill walking

Authors: *K. B. HANNAN[1], M. K. TODD[1], N. J. PEARSON[2], P. A. FORBES[3], C. J. DAKIN[1];
[1]Kinesiology and Hlth. Sci., [2]Utah State Univ., Logan, UT; [3]Dept. of Neurosci., Erasmus Med. Ctr., Rotterdam, Netherlands

Abstract: The vestibular system encodes motion and head orientation in space, providing information critical for postural control. A common means of probing the vestibular system is to indirectly apply an electrical current to the vestibular nerve and record the associated behavioral responses (e.g. ground reaction forces, electromyography). The interpretation of these responses, however, can be confounded by processes of habituation, in which the amplitude of vestibular-induced responses decreases during periods of prolonged electrical vestibular stimulation (EVS). Additionally, EVS often produces unwanted secondary effects (e.g., tingling, dizziness, nausea) that may affect an individual's willingness to complete the experiment. Here we investigate these stimulation-related phenomena with the aim of formally documenting the magnitude and time course of their effect during and after periods of prolonged EVS. Our aim was (1) to determine if prolonged exposure to random EVS is accompanied by habituation and (2) to document participants' subjective reports of sensations associated with EVS. Participants were randomly assigned to a treatment (n = 13) or control (n = 13) group upon providing informed consent. The treatment and control groups received continuous, random EVS (±5mA, 0-25Hz) for 4 minutes while standing quietly on a force plate at the start and end of the experimental trials. Both groups walked on a treadmill for 60 minutes between the standing trials. Only the treatment group received EVS while walking. To quantify habituation, we recorded ground reaction forces and muscle activity in the lateral soleus and medial gastrocnemius. We documented self-reported EVS-evoked sensations via verbally administered questionnaires at the start of each trial. EVS-induced postural responses and muscle activity did not change over the course of the experiment. Furthermore, no significant differences were found between the control and experimental groups. We observed similar results for the self-reported secondary effects. These results suggest little habituation to random EVS over one hour of exposure while walking. This outcome supports the use of prolonged random EVS as an experimental tool in situations with lengthy application time or with multiple conditions collected successively.

Disclosures: K.B. Hannan: None. **M.K. Todd:** None. **N.J. Pearson:** None. **P.A. Forbes:** None. **C.J. Dakin:** None.

Poster

139. Vestibular System and Balance

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Program #/Poster #: 139.08/J38

Topic: D.06. Auditory & Vestibular Systems

Support: NWO Grant #016.Veni.188.049
NSERC Grant RGPIN: 356026-13

Title: Electric vestibular stimulation induces gravity dependent inferences of linear acceleration

Authors: *P. A. FORBES[1], N. KOSHRAVI-HASHEMI[2], C. DAKIN[3], J.-S. BLOUIN[2]; [1]Dept. of Neurosci., Erasmus Univ. Med. Ctr., Rotterdam, Netherlands; [2]Univ. of British Columbia, Vancouver, BC, Canada; [3]Utah State Univ., Logan, UT

Abstract: Electrical vestibular stimulation (EVS) is increasingly used for research and clinical applications to selectively modulate vestibular activity and generate virtual sensations of motion. Varying interpretations of the responses evoked by EVS, however, have led to conflicting views whether the behavior induced by the vestibular activity arises from a signal of rotation, linear acceleration or a combination of both. Here we show that because vestibular signals are processed through an internal model of gravity, EVS-evoked responses attributed to both linear and angular motion can arise depending on the orientation of the head relative to gravity. Using a mechanistic model of visual-vestibular processing, combined with a rotational model of EVS, we hypothesized that non-zero estimates of linear accelerations arise only when the EVS rotation vector has a component orthogonal to gravity. We then compared these predictions to perceptual responses (N=18) and vestibulo-ocular reflexes (VOR) (N=20) induced by EVS in human subjects in four head orientations: one with the EVS vector parallel to gravity (head pitched down) and three with the EVS vector orthogonal to gravity (head pitched up while sitting upright, lying on the right side, and lying on the left side). With the EVS vector parallel to gravity, perceptual responses matched the prediction of rotation absent accompanying linear acceleration. In contrast, with the EVS vector orthogonal to gravity, perceptual responses were associated with linear head acceleration that was perpendicular to both the EVS vector and gravity. Notably, the inferred linear head acceleration occurred interaurally when sitting upright and superior-inferiorly when lying on either side. The evoked VORs further validated the model predictions. First, with the EVS vector orthogonal to gravity (lying side), the induced eye movements in the direction of the inferred linear acceleration (i.e. vertical VOR) were larger compared to having the EVS vector parallel to gravity (head down). Second, in half of the subjects, the phase of the VOR in lying right and left conditions was inverted, matching the predicted inversion of inferred linear acceleration across these two orientations. In subjects with non-inverted responses, we observed smaller vertical VORs, suggesting that a reduced sensitivity to EVS-evoked rotation signals may limit the inference of linear acceleration and any inversion of the VOR when lying on either side. Overall, our results resolve the controversy associated with linear or angular origins of the evoked perceptual or motor responses by considering the interaction between head rotation and gravito-inertial signals.

Disclosures: P.A. Forbes: None. **N. Koshravi-Hashemi:** None. **C. Dakin:** None. **J. Blouin:** None.

Poster

139. Vestibular System and Balance

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 139.09/J39

Topic: D.06. Auditory & Vestibular Systems

Title: Auditory vection induces postural sway in sighted and blind humans

Authors: *L. F. CUTURI[1], P. R. MACNEILAGE[2], M. GORI[3];
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Abstract: Blind individuals combine auditory information with haptic, proprioceptive, and vestibular information to achieve postural control. Auditory information is also fundamental to understand one's position with respect to the surroundings during locomotion by using allocentric cues to self-motion. Here we study the relationship between auditory self-motion cues and postural control in the absence of vision by comparing postural stability measurements before, during and after auditory vection stimulation in sighted and blind individuals. In the baseline condition, we measured postural sway (center of pressure) in the interaural and naso-occipital axis in each participant in the absence of visual or auditory stimulation. Each measurement lasted 30 s and was repeated 10 times. In the auditory vection conditions, we presented participants with stimuli simulating either clockwise or counterclockwise self-rotation in the yaw plane. These two conditions were repeated 10 times in separate blocks of trials. In each trial, we measured postural sway both during and after the auditory stimulation to test for aftereffects. The auditory stimulus was presented over binaural headphones and consisted of four environmental sounds. In the first 5 s of auditory stimulation, the sounds were static and virtually displaced around subjects' head in the four intercardinal azimuths. Subsequently, the sounds rotated at a constant velocity of 32°/s for 44 s. After the sounds were turned off, measurement continued for another 30 s. Center of pressure measurements were used to calculate root-mean-square (RMS) position as well as sway velocity. Auditory vection led to increased RMS position, compared to baseline, suggesting an impaired ability to maintain a stable upright stance in both sighted and blind participant; interestingly, while blind participants showed increased RMS both in the auditory and post-auditory phases, sighted subjects tended to return to a baseline level in the post-auditory phase. Velocity comparison showed reduced velocity of postural sway in the auditory and post-auditory phases in blind participants whereas sighted participants show reduced velocity only along the naso-occipital axis. In conclusion, auditory vection induced changes in postural control regardless of the presence of visual impairment. Interestingly, sustained postural sway in the post-sound phase suggests that blind participants experience aftereffects. This finding corroborates previous findings on the strong interconnection between the remaining senses in cases of visual impairment, providing novel insights in the context of self-motion and postural stability.

Disclosures: L.F. Cuturi: None. **P.R. MacNeilage:** None. **M. Gori:** None.

Poster

139. Vestibular System and Balance

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 139.10/J40

Topic: D.06. Auditory & Vestibular Systems

Support: This work was supported by Intramural NIH Research

Title: Differentiation and functioning of crystal cells - gravity sensors in one of the simplest metazoans, Trichoplax adhaerens

Authors: *T. D. MAYOROVA[1], C. L. SMITH[1], K. HAMMAR[2], T. S. REESE[1];
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Abstract: Trichoplax adhaerens (Placozoa), is a small marine animal that is thought to have diverged very early during metazoan evolution. Its body comprises only six morphological cell types and no nerves or muscles. Nevertheless, it exhibits different types of behavior controlled by neuropeptides and is able to chemotactically find food. Moreover, Trichoplax can react to changes in gravity; although it shows neither positive nor negative gravitaxis, it seems to be able to correct its ciliary movement with respect to the direction of the gravity vector. A specialized cell with an intracellular crystal, aptly named a crystal cell, is a likely candidate to detect gravity. The present work aims to understand how crystal cells differentiate, are activated by the gravitational force, and transmit a signal to the ciliary cells propelling the animal.
Crystal cells undergo several stages of differentiation, during which the crystal increases in size and the nucleus changes its shape from round to cup-like. Crystal growth takes place in a round vacuole flanked by mitochondria. Differentiating crystal cells with a small crystal and rounded nucleus are located deeper in from the periphery, while mature cells are found very close to the edge of the animal, which is aligned with a location of a presumptive stem cell zone. Crystal cells are eliminated presumably by apoptosis signified by the large vacuoles in its cytoplasm.
An animal put on a vertical slide maintains a constant depth, provided that it has a full set of crystal cells, while animals lacking crystals move downward. The crystal, which is located in the center of crystal cells in animals on horizontal surfaces, shifts in the direction of gravity when animals are tilted vertically. The downward shifted crystal comes into close contact with the cell membrane. We anticipated that the pressure of the crystal on the membrane might activate mechanosensitive receptors, so we tested the hypothesis that TRP channels, well known mechanoreceptors activated by cytoskeleton deformation, are involved in gravity sensing. If the movement of the crystal by gravity activates a crystal cell via TRP channels, blockers would affect the ability to maintain a constant depth on a vertical substrate. We tested this idea by applying the inhibitors of TRPA1 channels to animals on vertical slides, and the animals moved downwards in a dose-dependent manner. Our behavioral experiments along with the fact that

plasma membrane of crystal cells is underlined by a thick layer of microfilaments make TRPA1 channels candidates to be activated by changes in gravitational fields

Disclosures: T.D. Mayorova: None. **C.L. Smith:** None. **K. Hammar:** None. **T.S. Reese:** None.

Poster

139. Vestibular System and Balance

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Program #/Poster #: 139.11/J41

Topic: D.06. Auditory & Vestibular Systems

Support: MRC Grant MR/P006493/1

Title: Linking anti-saccades and postural control mechanisms in acute traumatic brain injury

Authors: *E. CALZOLARI, M. K. CHEPISHEVA, R. SMITH, H. M. RUST, B. M. SEEMUNGAL;
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Abstract: This study investigated the relationship between eye movements (saccades and anti-saccades) and postural stability in patients with acute TBI. Acute TBI patients commonly display a frontal dysexecutive syndrome, which is associated with impaired anti-saccadic function (increased error rate and response time). Our pilot data show in acute TBI patients with preserved peripheral vestibular and sensory nerve function, that most (>80%) are unbalanced. Given the recent finding linking anti-saccadic latency with balance function in Parkinson's disease (Ewenczyk et al. 2017 Neurology), we hypothesised that a similar relationship exists between anti-saccades and balance dysfunction in TBI and is mediated by frontal dysfunction. We recruited 24 acute TBI patients and 24 healthy controls. Balance was assessed as postural sway in four conditions: hard surface - eyes open (visual-proprioceptive-vestibular condition), hard surface - eyes closed (proprioceptive-vestibular), soft surface - eyes open (visuo-vestibular), soft surface - eyes closed (vestibular). The average displacement of the centre of pressure and the 95% ellipse confidence interval of the area along the mediolateral and anteroposterior directions combined were computed. All participants completed also a series of voluntary eye movements directed to a visual target (saccades) and a series of voluntary eye movements directed opposite to the visual target (anti-saccades), and all eye movement were recorded via electrooculography. Proportion of directional errors and latency (with and without directional errors) were computed for both saccades and anti-saccades. All participants had a structural and resting state MRI of the brain. Compared to controls, TBI patients showed an increased sway in all the postural conditions, with the highest sway found in the vestibular condition. Moreover, TBI patients showed higher latency of anti-saccades when directional errors where included. Interestingly, in

TBI patients, we found a positive correlation between anti-saccades latency with ($r = 0.6$, $p < 0.01$) and without ($r = 0.55$, $p < 0.05$) directional errors, and increased sway measured as average displacement of the centre of pressure in the vestibular condition as compared to the easiest postural condition. Similar correlations were found also with the 95% ellipse confidence interval of the area of sway in the vestibular condition. Our results suggest a link between altered initiation of voluntary eye movements and a poor performance in vestibular-mediated postural control in acute TBI patients, and this could be mediated by frontal dysexecutive mechanisms.

Disclosures: E. Calzolari: None. **M.K. Chepisheva:** None. **R. Smith:** None. **H.M. Rust:** None. **B.M. Seemungal:** None.

Poster

139. Vestibular System and Balance

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Program #/Poster #: 139.12/J42

Topic: D.06. Auditory & Vestibular Systems

Support: MRC Grant - MR/P006493/1
Imperial NIHR Biomedical Research Centre

Title: Impaired vestibular perception of self-motion in acute traumatic brain injury

Authors: *B. M. SEEMUNGAL[1], E. CALZOLARI[1], M. K. CHEPISHEVA[1], H. M. RUST[2], D. J. SHARP[1], A. E. J. JOLLY[1], R. SMITH[2];
[2]Brain and Vestibular Group, [1]Imperial Col. London, London, United Kingdom

Abstract: Dizziness and imbalance affect over 80 percent of traumatic brain injury (TBI) patients. Pathological vestibular activation typically causes both a prominent vestibular-ocular reflex (VOR) nystagmus and illusionary self-motion perception (‘vertigo’ or vestibular-motion perception). In patients with acute TBI we observed during vestibular activation (e.g. BPPV) a severely attenuated vertigo sensation despite preserved VOR responsivity, a disorder of vestibular-motion perception we term vestibular agnosia.
We hypothesised that i) acute TBI attenuates vertigo perception measured via laboratory tests of vestibular perception, and ii) vestibular agnosia is mediated by the disruption of vestibular-cortical networks in TBI.
This is an ongoing, prospective study. Patients with non-penetrating TBI (from mild-probable to moderate-to-severe TBI) were consented from an acute major trauma unit (time zero) and were clinically examined, completed questionnaires (symptoms, functional ability, cognition), laboratory testing and neuro-imaging. Loss of peripheral vestibular function was an exclusion criteria.
Subjects were assessed at time 0, 3 and 6 months to delineate the neural-correlates of vestibular

agnosia. Here we present the acute data.
Objective assessment during passive whole-body rotations in darkness showed normal VOR responses for all (average slow phase nystagmus onset, TBI patients = 0.67deg/s^2, controls = 0.60deg/s^2) but the TBI group showed elevated self-motion perceptual thresholds (N=26, current average = 2.91deg/s^2, range = 0.34 - 9.75deg/s^2), compared to healthy controls (current average = 0.76deg/s^2, range = 0.21 - 1.74deg/s^2). These findings were not explained by a reaction time or arousal deficit.
All patients had advanced structural and functional neuroimaging with which we will delineate the neuro-imaging correlates of vestibular agnosia.

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Poster

139. Vestibular System and Balance

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Program #/Poster #: 139.13/J43

Topic: D.06. Auditory & Vestibular Systems

Support: MRC Grant MR/P006493/1

Title: Vestibular-guided spatial orientation in healthy young and aged controls and patients with neurodegeneration

Authors: *M. K. CHEPISHEVA[1], E. CALZOLARI[1], E. BLUNT[1], P. EDISON[1], J. B. ROWE[2], B. M. SEEMUNGAL[1];
[1]Imperial Col. London, London, United Kingdom; [2]Cambridge Univ. Dept. Clin. Neurosciences, Cambridge, United Kingdom

Abstract: Navigating in the environment is essential for our daily activities. However, neurodegenerative disease patients and some healthy aged individuals, show deficits in spatial memory and spatial orientation, particularly for visually-guided navigation tasks. In the dark, vestibular cues guide orientation, particularly for angular rotations but little is known about if neurodegeneration and aging affect vestibular-guided spatial orientation (where angular head motion vestibular cues are converted by the brain to travelled angular distance). Local spatial orientation can be divided into two: EGOCENTRIC (body-referenced) or ALLOCENTRIC (referenced to stable environmental landmarks). We assessed working memory performance for vestibular-guided allocentric and egocentric spatial orientation in healthy subjects and patients with neurodegenerative disease. For the EGOCENTRIC task, participants sat on a computer-controlled, motorized rotating chair and underwent discrete passive rotations to one of six different angles (range 30-180°; steps of 30°) in the dark. Remaining in the dark, after an interval

of either 1s or 6 s, participants were asked to move the chair back to the perceived start using a joystick. The difference between the actual and the self-generated rotation was calculated. For the ALLOCENTRIC task, participants wore a virtual reality headset showing a 360° natural visual scene with time to learn during a training phase. For each trial, subjects were passively rotated to one of six different angles (range 30-180°; steps of 30°) in the dark. After the rotation, participants were required to indicate (by moving the now present virtual scene with a mouse) to which visual landmark they were facing after they rotated in the dark. The discrepancy between the actual and the perceived spatial position was recorded. Our preliminary data shows that patients and controls show similar accuracy in the egocentric task albeit with greater variability in response for larger angular rotations. This indicates that the neural transformation of vestibular inertial head velocity signals to outputs of angular distance are largely intact in patients, including those with medial temporal lobe atrophy. Our additional allocentric tasks (ongoing) will enable us to assess if patients have problems with binding egocentric estimates of travelled angle with external landmarks and whether such binding is a function of the complexity of the virtual scene (i.e. due to a working memory overload problem).

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Poster

139. Vestibular System and Balance

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Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 139.14/J44

Topic: D.06. Auditory & Vestibular Systems

Support: NIH/NIDCD Grant R01 DC017425

Title: Test-retest reliability of vestibular perceptual thresholds in yaw

Authors: ***T. L. LEE**[1], C. S. SHAYMAN[1], Y. OH[1], R. J. PETERKA[2], T. E. HULLAR[1]; [1]Otolaryngology, [2]Neurol., Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: Psychophysical testing of the vestibular system is an increasingly used method for vestibular testing. In the auditory system, perceptual testing has been shown to be highly specific, sensitive, with good test-retest reliability. However, the validity of perceptual vestibular tests remains to be established. Therefore this study characterized the potential clinical utility of yaw-axis perceptual thresholds to rotation, through the measurement of the test-retest reliability of vestibular thresholds. Fifteen human participants reporting no history of vestibular dysfunction completed yaw axis rotational chair testing for thresholds of motion perception. Participants were tested at two time intervals (baseline and again 5-14 days later) using a 2-down, 1-up adaptive psychophysical procedure. Perceptual thresholds to 1 Hz sinusoidal motion

trajectories ranged from 0.63 deg/s to 2.99 deg/s with an average of 1.49 deg/s (SD: 0.63). High intraclass correlation (ICC) was observed (ICC=0.92, 95% confidence interval: 0.77-0.97) with a minimum detectable difference of 0.45 deg/s. Given our finding of excellent test-retest reliability and previous reports linking perceptual vestibular testing to functional outcomes, vestibular perceptual testing may provide a new clinical outcome to measure and diagnose vestibular function and dysfunction.

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Poster

139. Vestibular System and Balance

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 139.15/J45

Topic: D.06. Auditory & Vestibular Systems

Support: NIH DC2390

Title: Locomotion increases the firing rate of vestibular otolith but not canal afferents in the primate

Authors: *K. E. CULLEN[1], I. MACKROUS[2], J. CARRIOT[3];
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Abstract: The role of the vestibular efferent system in the processing of information about head motion in primates has remained a mystery. The vestibular receptors receive direct innervation from centrifugally projecting efferent fibers whose somas are located the efferent cell group. Electrical stimulation of the efferent cell group produces increases in firing rate of vestibular afferents - an effect that is greater for irregularly versus regularly discharging afferents. Previous work in our laboratory has shown that primate vestibular afferent discharges are comparable during active and passive orienting head movements. However, experiments in model systems, including the toadfish and larval Xenopus, have suggested a role in the vestibular efferent system in modulating of afferent discharge during active locomotion. We thus hypothesized that the primate vestibular efferent system might specifically modulate afferent responses during active locomotion. We recorded the response of individual vestibular afferent during externally-applied head motion, active head motion while the body remained stationary and during locomotion in monkeys. Recordings were made from the two distinct populations of vestibular afferent neurons

innervating either the otoliths or the semicircular canals end organs. We found that vestibular afferents robustly encode active head motion in all conditions. First, afferent firing rates and sensitivities to head movement were comparable during passive and active head motion where the body remained stationary (all p values >0.5). Likewise, the responses of regular and irregular canal afferents and irregular otolith afferents were comparable during passive motion and locomotion (all p values >0.5). In contrast, we found that the firing rate of irregular otoliths afferents significantly increased during locomotion (58 and 98 sp/s, p = 0.02), while their sensitivity remained unchanged (gain: 74 and 75 sp/s/g, p=0.7) Taken together, our results establish for the first time that locomotion increases the firing rate of otolith but not canal afferents in primates. These findings have important implications for furthering our understanding of how self-motion experienced during everyday activity is encoded to ensure accurate behavior and perception.

Disclosures: **K.E. Cullen:** None. **I. Mackrous:** None. **J. Carriot:** None.

Poster

139. Vestibular System and Balance

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 139.16/J46

Topic: D.06. Auditory & Vestibular Systems

Support: DOD Grant
VA Grant RX

Title: Noise impacts neuronal activity in vestibular pathways

Authors: R. D. BRAUN[1], A. KUHL[1], M. HALI[1], S. KALLAKURI[1], ***A. HOLT**[1,2];
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Abstract: Introduction: The vestibular system is crucial for posture, gait, and the perception of head and body in space. Damage to this system manifests as dizziness, imbalance, and poor postural control. Recording of vestibular short evoked potentials (VsEPs) in response to linear acceleration to evoke activity in the otolith organs has been undertaken by several investigators with some using this technique to show the effect of noise on this system. On the other hand, manganese-enhanced magnetic resonance imaging (MEMRI) is commonly used to study neuronal activity that takes advantage of the paramagnetic nature of manganese which also acts as a calcium surrogate and accumulates in active neurons. These techniques offer the ability to non-invasively monitor and assess the function of vestibular neurons centrally which would allow longitudinal studies to better understand this understudied system. This study attempts to assess the effect of graded linear acceleration jerks on VsEPs as well as associated vestibular neuronal activity.

Methods: A custom-made ceramic nut was centered and secured to bregma of male Sprague Dawley rats (n=5). Each animal was anesthetized, and a screw was threaded into the ceramic nut to attach to a mechanical shaker and subjected to a jerk stimulation (either 500g/s, 2500 g/s, or 6000 g/s). Manganese chloride was administered just prior to stimulation. Typically, 200-400 jerk stimuli were delivered per trial. Each trial was repeated a total of 15 times with a ten-minute interval after every five trials. Responses were recorded (CED power 1401 data acquisition system and Spike2 software) and analyzed using custom MATLAB scripts. Animals were also subjected to baseline, 24-hour post, and 2-week post stimulation MRI to assess manganese uptake in vestibular nuclei.
Results: Although each of the jerk stimuli resulted in VsEPs, the 500 g/s stimulus did not result in a robust signal. The response latency for P1 was ~ 1 ms after the stimulus onset. Repetitive high intensity stimulation appeared to result in diminished P1 amplitudes and earlier onset. All the vestibular nuclei (VN) had significantly elevated manganese uptake following stimulation versus baseline. Manganese uptake was least in animals administered jerks of 500 g/s. Greatest manganese uptake was observed in vestibular nuclei of animals subjected to jerks of 2500 g/s and 6000 g/s.
Discussion: Our results demonstrate graded increases in manganese uptake in vestibular nuclei, particularly the spinal vestibular nucleus. Spatial differences in VN activity in response to stimuli needs to be further elucidated.

Disclosures: R.D. Braun: None. **A. Kuhl:** None. **M. Hali:** None. **S. Kallakuri:** None. **A. Holt:** None.

Poster

139. Vestibular System and Balance

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 139.17/K1

Topic: D.06. Auditory & Vestibular Systems

Support: DC006685

Title: Specialized cholinergic sensing cells discovered in semicircular canal cristae

Authors: *H. A. HOLMAN[1], L. A. POPPI[1], Y. WAN[2], R. D. RABBITT[3];
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[3]Biomed. Engin., Univ. of Utah, Salt Lake Cty, UT

Abstract: Introduction
At the center of the two vertical semicircular canal cristae lies an anatomical structure called the eminentia cruciata (EC), which is devoid of sensory hair cells, and whose function is currently unknown. We report that the EC is comprised of unique cells (denoted ECA cells) that respond

to acetylcholine (ACh) with whole-cell calcium transients, and another group of unique cells that are cholinergic. We hypothesize that these distinct cell types compose a previously unknown non-neuronal cholinergic system in the vestibular crista ampullaris that supports angular motion by hair cells and transmission by afferent neurons.

Methods

All animal experiments were approved by the University of Utah Institutional Animal Care and Use Committee. Immunohistochemistry was done using fixed tissues from wild type and transgenic mice, while calcium imaging was performed using the genetically encoded calcium indicator, GCaMP5G (G5) [1]. Reporter expression was driven by a Gad2-IRES-Cre line [2, 3]. Spontaneous and evoked G5 ($\Delta F/F$) fluorescence was recorded at a 100 ms frame rate with swept-field confocal microscopy.

Results

Specialized ECA cells respond to 100μM ACh and muscarine (Musc) with whole-cell calcium transients propagating from apex to base. These cells were observed as early as embryonic day 20, the earliest time examined, and continued to respond into adulthood (2y). The predominant ECA cell, named clino, located on the slope of the EC developed complex basolateral processes into adulthood and maintained ACh sensitivity. Calcium transients evoked by ACh and Musc in clino and other ECA cells, were reversibly blocked by atropine and inositol triphosphate (IP_3) receptor antagonist, 2-aminoethoxydiohenyl borate (2-APB).

Conclusion

Results demonstrate that newly discovered cells in the semicircular canal EC form a previously unknown local cholinergic
system in the crista ampullaris.

References

1. Gee et al., 2014
2. Taniguchi et al., 2011
3. Holman et al., 2019

Disclosures: **H.A. Holman:** None. **L.A. Poppi:** None. **Y. Wan:** None. **R.D. Rabbitt:** None.

Poster

139. Vestibular System and Balance

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 139.18/K2

Topic: D.06. Auditory & Vestibular Systems

Title: Vestibulo-sympathetic reflex in patients with bilateral vestibular loss

Authors: *O. KULDAVLETOVA[1], P. DENISE[1,2], G. QUARCK[1], M. TOUPET[3], H. NORMAND[1,2];

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Abstract: Introduction: Anatomical and physiological studies have demonstrated that the otolithic system affects sympathetic activity in humans and animals. In animals, bilateral transection of the vestibular nerve impairs the stability of blood pressure during postural changes. However, this deficiency recovers over time due to non-labyrinthine afferents. This indicate that the reflex known as vestibulo-sympathetic involves multisensory integration and is subject to plastic changes. In this study we aimed to assess the cardiovascular control during Head-Down Neck Flexion (HDNF) in a group of patients suffering from total bilateral idiopathic vestibular loss (BVL) for 7 ± 4 years. Materials and Methods: Nine adult patients (age 53.6 ± 11.5 years) with a total idiopathic BVL were recruited. Calf blood flow (CBF), mean arterial pressure (MAP), heart rate (HR) were measured with eyes closed in two lying body positions: ventral prone (VP) and lateral (LP) on the left side. Vascular resistance (CVR) was calculated as MAP/CBF. The HDNF protocol consisted in passively changing the head position: head up (HU) - head down (HD) - HU. Measurements were taken twice at each head position. Results: In ventral position CBF significantly decreased in the HD 3.65 ± 0.65 $mL.min^{-1}.100g^{-1}$ vs HU 4.64 ± 0.71; **$p < 0.002$**, while the CVR in the VP significantly raised in the HD 31.87 ± 6.93 arbitrary units vs 25.61 ± 6.36 in HU; **$p < 0.01$**. In the LP no change in CBF or CVR was found between the two head positions. MAP and HR presented no difference between HU and HD in both body positions. Age of patients did not affect the results. Discussion: Decrease in CBF of the BVL patients was similar to the decrease observed with the same HDNF protocol in normal subjects (Shortt and Ray, 1997). This suggests that after the vestibular loss a sensory compensation for the lost vestibular inputs has occurred. The compensation could originate from the integration of other sensory inputs signals such as trunk graviceptors as well as proprioceptive and cutaneous receptors. Moreover, in our patients the results were not affected by age. As the vestibulo-sympathetic reflex normally impairs with age, we might suggest that in elderly BVL patients this compensatory circuit performs better than in normal subjects.

Disclosures: O. Kuldavletova: None. **P. Denise:** None. **G. Quarck:** None. **M. Toupet:** None. **H. Normand:** None.

Poster

140. Vision: Subcortical Visual Pathways

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 140.01/K3

Topic: D.07. Vision

Title: To eat or be eaten: Is there a specific visual bias to snakes over food in humans?

Authors: *S. LI[1], J. R. KEENE[2], B. N. HARRIS[3], J. A. CARR[2];
[1]Biol Sci., [3]Biol. Sci., [2]Texas Tech. Univ., Lubbock, TX

Abstract: Snake detection theory posits that the need to avoid snakes has helped sculpt the human brain and visual processing systems. Snake-naïve human infants show a visual bias toward snake images, suggesting an innate response. In other animal species predators, including snakes, dramatically influence many aspects of foraging. Whether evidence of such a tradeoff exists in humans has not been well studied. Here we tested two questions: 1) Is there a visual bias toward images of snakes over food in humans? 2) If so, is this visual bias specific to snakes or is it seen with nonspecific, but arousing, images? Using 76 undergraduate student participants (38 men, 38 women), we carried out an eye-tracking analysis of matched pairs of normative rated food images (FOODPICS, both high and low palatability) and normative rated photographs of an open- or closed-mouth snakes (International Affective Picture System, IAPS). Participants also were tested using a stimulus set of paired high and low valence images (1-9 on IAPS valence scale) to test for the specificity of the snake images. We analyzed three metrics in balanced pairs of food and snake images: saccade latency, gaze duration, and saccade bouts. Missing data were replaced using harmonic means. Data were analyzed by a repeated measures mixed model ANOVA. There was a strong bias (Control vs. closed mouth, $p<0.001$; Control vs. open mouth, $p=0.001$) toward shorter saccade latency with the snake images relative to food images. Gaze duration and saccade bouts were significantly greater for snake images relative to food images. However, a similar trend was observed in saccade latency and saccade bouts, but not gaze duration, with high arousal non-snake images. Thus, we report support for our first question and equivocal results for our second question. Collectively our data argue for a visual bias toward snakes over food in humans. However, some aspects of the visual bias, but not others, are also seen with non-specific images eliciting arousal.

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Poster

140. Vision: Subcortical Visual Pathways

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 140.02/K4

Topic: D.07. Vision

Support: Biotechnology and Biological Sciences Research Council

Title: Enhanced circadian responses to ‘yellow’ rather than ‘blue’ light

Authors: *J. W. MOULAND, F. MARTIAL, A. WATSON, R. LUCAS, T. BROWN;
Univ. of Manchester, Manchester, United Kingdom

Abstract: Numerous studies have demonstrated that short-wavelength monochromatic light (perceived by humans as ‘blue’) evokes larger circadian responses than longer wavelength light. Such observations are now known to derive from the fact that circadian assessments of light intensity involve a ganglion cell class whose intrinsic light sensitive protein, melanopsin, is most efficient at capturing photons in the corresponding portion of the visible spectrum. By contrast, the relationship between perceived colour (as reported by the cone-based chromatic visual system) and circadian responses to light remains unknown. Given recent data indicating the mammalian circadian system does indeed receive cone-based chromatic signals, we set out to specifically determine how colour influences circadian responses in mice. Using multispectral lighting environments and mice with red shifted L-cone spectral sensitivity (Opn1mwR), we generated conditions that differed in perceived colour (i.e. the ratio of long- vs. short-wavelength sensitive cone activation) whilst providing identical brightness for rods, melanopsin and cone-dependent luminance channels. Under a variety of behavioural paradigms, we show that stimuli biased in favour of long-wavelength sensitive cone activation (appearing ‘yellow’) drive larger circadian responses than those with the opposite bias (appearing ‘blue’). This influence of colour therefore aligns with natural changes in spectral composition over twilight (where decreasing solar angle is accompanied by a relative enrichment of short wavelength light) and provides a straightforward mechanism by which animals could use colour to optimise circadian entrainment. We further confirm that the observed effects of colour specifically rely on cone-based chromatic comparisons, using animals that lack cone-based phototransduction ($Cnga3^{-/-}$). Moreover, we find that ‘blue’ and ‘yellow’ stimuli are equally effective in driving direct light-induced activity suppressions (‘masking’) in Opn1mwR mice, indicating the sensory control of the entrainment mechanism is separable from general light-induced changes in behaviour. In summary, our data challenge the simple view that the circadian system is maximally sensitive to blue light and suggest a new way of adjusting artificial lighting to promote healthy circadian entrainment.

Disclosures: J.W. Mouland: None. **F. Martial:** None. **A. Watson:** None. **R. Lucas:** None. **T. Brown:** None.

Poster

140. Vision: Subcortical Visual Pathways

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 140.03/K5

Topic: D.07. Vision

Support: NIH grant 1DP2EY022584
Sloan Research Fellowship in Neuroscience
Klingenstein-Simons Fellowship in the Neurosciences

Title: Central projections of molecularly distinct M1 intrinsically photosensitive retinal ganglion cells

Authors: *S. LEE, E. G. MIN, T. M. SCHMIDT;
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Abstract: Melanopsin-expressing, intrinsically photosensitive retinal ganglion cells (ipRGCs) are a class of atypical, ganglion cell photoreceptor. The M1 subtype of ipRGCs serve ambient irradiance detectors mediating non-image forming visual behaviors such as circadian photoentrainment and the pupillary light reflex. Despite initial reports of homogeneity within the M1 population, recent reports have suggested that M1 ipRGCs can be molecularly subdivided based on whether they express the transcription factor Brn3b (Brn3b+ M1 and Brn3b- M1). Although reports have shown axonal innervation of M1 ipRGCs in several non-image forming brain areas, whether those are Brn3b+ or Brn3b- has not been determined. We therefore performed virus injection (AAV2-FLEX-Chrimson-tdTomato) into eyes of $Brn3b^{Cre/+}$; $Opn4^{LacZ/+}$ mouse line to identify axons of Brn3b+ M1 ipRGCs in brain which will be co-labeled with Chrimson (Brn3b+) and beta-galactosidase (LacZ+) through immunohistochemistry. We confirmed that 20 % of LacZ+ M1 ipRGCs are co-labeled with Chrimson in retina, indicating that LacZ+ and Chrimson+ axons are of Brn3b+ M1 ipRGCs. The axons of Brn3b+ M1 ipRGCs are observed in multiple non-image forming targets. Interestingly, we also observed Brn3b+ axons arising from non-M1 cells, suggesting that either non-M1 ipRGCs or conventional RGCs also contribute projections to some non-image forming targets. Collectively, these results provide a high-resolution map of Brn3b+ M1 ipRGC projections in the mouse brain.

Disclosures: S. Lee: None. **E.G. Min:** None. **T.M. Schmidt:** None.

Poster

140. Vision: Subcortical Visual Pathways

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 140.04/K6

Topic: D.07. Vision

Title: Model of dynamical asymmetries in early vision parallel pathways

Authors: *A. R. R. CASTI;
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Abstract: The classic *parallel pathways hypothesis* for sub-cortical visual neurons posits that ON and OFF cells in the retina and lateral geniculate nucleus (LGN) form two non-interacting streams of visual information, with the dynamics of the streams essentially identical except for the expected effects of the sign inversion in their receptive fields. Evidence has emerged over the

past couple of decades that this hypothesis, while apparently valid for certain types of visual stimuli and response measures, is incorrect. For example, intracellular recordings of guinea pig brisk transient cells responding to transient changes in contrast revealed contrast-dependent spike rate rectification in OFF cells, whereas such effects were absent for ON cells (Zaghloul et al., 2003). Further, a pharmacological blockade of the ON pathway eliminates this contrast dependence in the OFF pathway responses, suggesting that the dynamical asymmetry owes to direct, non-reciprocal inhibition from ON cells to OFF cells.
The goal of this work was to incorporate asymmetric ON to OFF pathway inhibition in a model of retinal neurons, and to test its validity against extracellular recordings of ON and OFF ganglion cells in cats. Specifically, the aim was to quantify the importance of asymmetric feed-forward inhibition to observed differences in the spike responses of ON and OFF cells driven by spatially homogeneous spots with temporally noisy luminance modulation. The spot diameter also varied, allowing us to investigate potential size-dependent effects of the asymmetric inhibition. We optimized an integrate-and-fire model of a bipolar, amacrine, and retinal ganglion cell circuit of ON and OFF cells, which was uncoupled except for a cross-inhibitory conductance from the ON amacrine cell to the OFF ganglion cell. We found that the inclusion of asymmetric ON to OFF inhibition significantly improved the OFF cell model performance for large spot sizes (larger than the receptive field), while for smaller spot sizes the contribution of this cross-stream inhibition was negligible.

Disclosures: A.R.R. Casti: None.

Poster

140. Vision: Subcortical Visual Pathways

Location: Hall A

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Topic: D.07. Vision

Support: BrightFocus Foundation grant G2017027
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Title: Ocular hypertension alters retinal ganglion cell output to the visual thalamus

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Abstract: Ocular hypertension (OHT) is a major risk factor for glaucoma and is associated with changes to the structure and function of the optic nerve. It is unknown how and over what time course OHT affects retinal communication with visual nuclei in the brain. The goal of this study is to determine how OHT affects synapses made by retinal ganglion cells in the dorsal lateral geniculate nucleus (dLGN), a major visual relay nucleus in the thalamus. Intraocular pressure

was elevated by injection of 10 micron beads into the anterior chamber of the eye and experiments were performed 5-6 weeks following confirmation of modest (~30%) and sustained OHT. Control mice received saline injections and did not display OHT. In recordings from On-sustained alpha retinal ganglion cells, we found that OHT led to a reduction in dendritic complexity and suppression of spontaneous spiking. To assess retinogeniculate synaptic function, we generated a mouse line in which the light-gated channel Channelrhodopsin-2 is expressed in several subpopulations of melanopsin-expressing RGCs, including On-sustained alpha RGCs, allowing us to stimulate RGC axons in acute brain slices while recording post-synaptic responses in dLGN thalamocortical relay neurons (TC neurons). Synaptic currents evoked by ChR2 activation in the dLGN were mediated by AMPA-type glutamate receptors and were action potential dependent. Using pairs of stimuli (100 ms - 5 s intervals) and high-frequency stimulus trains (10 Hz), we found that synaptic transmission depressed more strongly in OHT brains, consistent with an increase in synaptic vesicle release probability. While there was no change in the amplitude of miniature synaptic currents, their frequency was slightly decreased. There was no change in the size or density of vGlut2-stained RGC synaptic terminals in the dLGN, although the dendritic complexity of dye-filled thalamocortical relay neurons, as assessed with a Sholl analysis, was slightly reduced, suggestive of a loss of synapses in dLGN from OHT mice. Finally, while OHT was associated with a decrease in the density of RBPMS-stained RGCs in the peripheral retina, there was no change in the density of ChR2-expressing RGCs suggesting that changes to synaptic function largely precede RGC loss in the ChR2-expressing subpopulation. These findings indicate that OHT leads to early-stages changes in RGC output to subcortical visual areas of the brain.

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Poster

140. Vision: Subcortical Visual Pathways

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Topic: D.07. Vision

Support: NIH Grant EY013588
NIH Grant T32 EY015387
NIH Grant P30 EY012576

Title: Extraretinal contributions to thalamic filtering of retinal spike trains

Authors: *P. C. ALEXANDER[1,2], H. J. ALITTO[2,3], T. G. FISHER[2,4], D. L. RATHBUN[2,5], W. M. USREY[2,3,1];
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Stanford, CA; [5]Inst. for Ophthalmology and Ctr. for Integrative Neurosci., Univ. of Tuebingen, Tuebingen, Germany

Abstract: Before visual information from the retina reaches primary visual cortex it is dynamically filtered by the lateral geniculate nucleus (LGN). While the structure of the incoming retinal spike train is a major factor in determining which retinal spikes are relayed by the LGN, extraretinal sources including intrathalamic inhibition and cortical feedback also play a primary role. To investigate the contribution of extraretinal signals to thalamic filtering we used data from monosynpatically connected pairs of retinal ganglion cells (RGCs) and LGN relay cells that were stimulated with binary white noise to train generalized linear models to predict which RGC spikes were relayed. Models that considered only the recent history of RGC spikes (feedforward model) were compared with models that also included the recent spiking activity of the connected LGN relay cell as a proxy for extraretinal influences (recurrent model). When tested using cross-validation, the recurrent models were significantly better at predicting which retinal spikes were relayed. Next, models were fit to data in which RGC-LGN pairs were stimulated with sine wave gratings that varied in either contrast or size. In this context, recurrent models, but not feed forward models, captured the presence of thalamic gain control in response to high contrast or large diameter stimuli. Thus, stimulus dependent recurrent suppression is sufficient to explain several known properties of retinogeniculate communication, including increased contrast sensitivity and increased contrast-dependent firing rate rectification within the LGN. Likewise, the increase in recurrent suppression in response to large diameter stimuli is consistent with previous reports of increased surround suppression in the LGN compared to the retina. Overall, these models are consistent with our previous experiment findings and provide deeper insight into how extraretinal mechanisms transform retinogeniculate communication.

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Poster

140. Vision: Subcortical Visual Pathways

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Program #/Poster #: 140.07/K9

Topic: D.07. Vision

Support: CRC870

Title: Functional binocular convergence in the retinogeniculate pathway

Authors: *S. WEILER[1], M. H. P. FERNHOLZ[1], J. BAUER[1], J. JÄPEL-SCHAEL[2], V. SCHEUSS[3], M. HUBENER[1], T. BONHOEFFER[1], T. ROSE[1];

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Abstract: The dorsal lateral geniculate nucleus (dLGN) is believed to forward the activity of specific retinal ganglion cell (RGC) classes via parallel, eye-specific, and largely unmodified visual processing streams to primary visual cortex (V1). This is supported by experiments showing that only a small number of RGCs contribute to the firing of a thalamocortical (TC) neuron (Litvina & Chen Neuron 2017). In addition, *in vitro* evidence suggests that functional binocular convergence is largely eliminated during the first postnatal weeks (Ziburkus & Guido J. Neurophysiol. 2006). In mice, this view has been challenged by recent trans-synaptic tracing experiments showing frequent binocular convergence of RGCs onto individual TC neurons (Rompani et al Neuron 2017). Similarly, we recently demonstrated that a fraction of TC neurons responds to visual stimulation of both eyes *in vivo* and that eye-specific responses of TC neurons can undergo prominent experience-dependent plasticity (Jaepel et al Nat. Neurosci. 2017). Here we address this apparent mismatch between structural tracing data, functional *in vitro* results, and recent *in vivo* recordings by directly mapping cellular and subcellular functional binocular convergence onto individual TC neurons in dLGN brain slices of adult mice. We established a novel optogenetic approach for quantification of eye-specific TC neuron responses by dual-color channelrhodopsin-assisted circuit mapping (2CRACM) using the red- and blue-light excitable opsins ChrimsonR and Chronos after eye-specific viral transduction of RGCs. We find, in contrast to previous *in vitro* approaches, that roughly half of the neurons in the binocular segment of dLGN receive monosynaptic functional input from both eyes. Binocular neurons are strongly dominated by input from one eye, and inputs of the non-dominant eye show a larger contribution of NMDA-receptor-mediated currents than inputs of the dominant eye. Patterned dual-color stimulation of short RGC axon stretches (subcellular 2CRACM) revealed that binocular TC neurons in the contralateral eye projection zone receive ipsilateral eye input if their dendrites extend into the ipsilateral termination patch in the dLGN, and vice versa. In addition, we find recently contested purely ipsilateral responses in the ipsilateral RGC projection patch. We conclude that previous recordings have underestimated the degree of functional binocular convergence onto mouse TC neurons. Our data provide the groundwork to address if functional retinogeniculate binocularity is modified after monocular deprivation, a question which we are currently addressing.

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Poster

140. Vision: Subcortical Visual Pathways

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Topic: D.07. Vision

Support: NIH Grant EY09593

Title: Receptive field structure of local interneurons in the murine dorsal lateral geniculate nucleus

Authors: *A. S. GORIN[1], S. AHN[1], U. CIFTCIOGLU[2], F. T. SOMMER[3], J. A. HIRSCH[2]; [1]Neurosci. Grad. Program, [2]Neurobio., USC, Los Angeles, CA; [3]Helen Wills Neurosci. Inst., Univ. of California Berkeley, Berkeley, CA

Abstract: Local inhibitory interneurons within the lateral geniculate nucleus (LGN) of the thalamus influence every spike that relay cells send to cortex. Visual processing in the LGN has been studied intensively in cat, where almost all relay cells have receptive fields made of two concentric subregions, a center and a surround, that have the opposite preference for stimulus contrast. Further, there are push-pull responses within each subregion—e.g. bright stimuli shone in an On subregion excite and dark ones inhibit. Push is supplied by retinal ganglion cells that have the same center sign as the target cell. Because local interneurons in cat also have center-surround receptive fields, we hypothesized that they synapse with relay cells of the opposite center sign to provide pull. It is difficult to target interneurons *in vivo*, however, and study their responses in depth. Thus, with the dual goals of learning about inhibition in thalamus and how this compares across species, we turned to mouse and used optogenetics to identify and target interneurons. Our previous studies of the mouse LGN focused on relay cells. We found that the largest single class of relay cells had receptive fields with a center-surround structure and push-pull responses. As in cat, the pull was stronger in the center than in the surround. Most other murine relay cells had various types of On-Off receptive fields. This diversity suggested that circuits for push-pull and other forms of inhibition differ between species. Our results show that the distribution of receptive-field sizes for interneurons and relay cells was similar. Our sample, however, includes interneurons with On, Off, or On-Off, but not center-surround, receptive fields. How, then, might murine interneurons contribute to spatially opponent push-pull responses in relay cells? One simplistic possibility involves convergent input from interneurons with small and large receptive fields aligned with that of the target relay cell and are maximally excited by stimuli that fill their entire receptive field. Thus, stimuli confined to the relay cell's center strongly excite interneurons with small receptive fields of the opposite sign to generate robust pull. Interneurons with large receptive fields that are opposite in sign from the relay cell's surround provide the pull observed in that subregion. Small stimuli evoke only weak output from these interneurons whereas large stimuli that cover the whole receptive field elicit strong responses that generate pronounced postsynaptic inhibition. We will test this and other potential circuits involving On-Off interneurons using a combination of multielectrode recording and computational modeling.

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Poster

140. Vision: Subcortical Visual Pathways

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Topic: D.07. Vision

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R01-EY023591
Eyesight Foundation of Alabama

Title: Photoreceptor-independent rapid adaptation in macaque LGN neurons

Authors: *P. TELLERS, L. C. SINCICH;
Dept. of Optometry and Vision Sci., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: During everyday vision, the light intensity photoreceptors are exposed to can change rapidly. Previously, we analyzed the rate of adaptation in LGN neurons *in vivo* during continuously presented stochastic stimuli (Tellers et al. 2018). Under these conditions LGN neurons exhibited substantial adaptation within the first 34 msec following presentation of the preferred stimulus valence (e.g. a brighter than mean stimulus value for an ON cell). Since neural adaptation can occur at many stages of the visual system, we wondered if the rapid adaptation observed in LGN neurons originates in the photoreceptors themselves. To test this idea, we targeted individual cones within the receptive field with adapting stimuli to determine if LGN spike probability was dependent on a specific cone within the receptive field center. We used a multiwavelength adaptive optics scanning laser ophthalmoscope to simultaneously image the cone mosaic and present movie stimuli in anesthetized macaques undergoing neuromuscular blockade. Cones were imaged with 840 nm light, while red and green stimulus channels were independently modulated to drive L and M cones equally (543 nm) or L cones preferentially (710 nm). The stimulus consisted of binarized white noise movies shown at 30 Hz, with spatial resolution of either 100 or 150 pixels/deg and with real-time retinally-stabilized delivery. Mean movie luminance was 195 cd/m^2. Receptive fields were located 0.4°-4° from the fovea, and mapped by spike triggered averaging. After selecting a pair of cones inside a neuron's receptive field center, we used one cone to adapt the neuron's response for 67 msec, while a subsequent probe stimulus, landing on either the same or a different cone, revealed the cone dependence of adaptation. We note that these stimulus conditions were always drawn from selected frames within ongoing random noise being presented to the surrounding non-targeted cones. In 4 ON parvocellular LGN neurons, we found that spike probability for the probed cone was always lower when the LGN neurons were adapted by a given cone, regardless of which cone was

shown the probe stimulus. When the adapted and probed cone differed, neural responses decreased by 64-95% (mean: 80%) in individual neurons after adaptation compared to a decrease of 54-96% (mean: 79%) when the adapted and probed cone were identical. None of the 4 neurons exhibited any significant difference in the adaptation rate between the two conditions. Our results suggest that most of the rapid adaptation in LGN neurons observed during ongoing stimulation does not originate in the photoreceptors, but is more likely a function of changes in downstream synaptic efficacy.

Disclosures: P. Tellers: None. **L.C. Sincich:** None.

Poster

140. Vision: Subcortical Visual Pathways

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Title: Possible parallel visual pathways between the lateral pulvinar and V2 thick/thin stripes in macaques

Authors: *Y. LIU, J. HU, S. YAO, T. TAKAHATA;
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Abstract: Recent studies have shown that there is another major visual stream besides traditionally well-studied LGN-V1 stream, which is “the pulvinar stream” driving through the midbrain, the pulvinar and extrastriate visual cortices. This stream appears to be evolved especially in the primate lineage, since most of other mammals are lack of it or their pulvinar is relatively very small. For example, “blind sight”patients can still feel an existence of visual objects even after they suffer severe damage in the striate cortex (V1), and that is likely because the pulvinar stream compensates the huge loss of the LGN-V1 stream. It is also known that there are at least three parallel visual pathways in the LGN stream: the parvocellular(P) pathway for shape/luminance/orientation/color coding, the magnocellular (M) pathway for movement direction and disparity coding, and the koniocellular (K) pathway for blue color coding. In the LGN and V1, they are segregated into different layers or sub-compartments. When they are relayed into V2, they are projected into three specific sub-compartments, and the P pathway specifically projects into “thin stripes”, and the M and K pathways projects into “thick” and “pale stripes”. However, existence of this type of parallel pathway has not been systematically tested in the pulvinar pathway. Previous electrophysiological studies have discovered the existence of two retinotopic organization through the pulvinar complex. In addition, the

connection between the pulvinar and V2 thick/thin stripes, but not pale stripes, has also been testified by a wide range of bidirectional tracer injection into the pulvinar. Here, we address possibility that there exist novel parallel visual streams through the pulvinar to V2 thin and thick stripe projections. Guided by conventional intrinsic signal optical imaging method, we identified the map of V2 sub-compartments, and three types of retrograde tracers (CTB-488, CTB-555 and BDA) were differentially injected in different stripes of V2. When we examined retrograde labeling within the pulvinar, we found 3-4 clusters of labeling. All clusters included all types of tracers next to each other, however, there was little overlap between tracers. These data showed that there are multiple sub-compartments that project to both stripes with corresponding receptive field of vision within the pulvinar, and there are P/M pathway-like segregation within each sub-compartment. Further studies are needed to clarify functional differences among sub-compartments of the pulvinar identified in this study.

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Poster

140. Vision: Subcortical Visual Pathways

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Topic: D.07. Vision

Support: National Eye Institute R01EY024890
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Title: Comparison of single neuron responses between two rostral subdivisions in the rat pulvinar / lateral posterior thalamic nucleus

Authors: *A. T. FOIK[1], L. R. SCHOLL[2], G. LEAN[2], A. HOANG[2], D. C. LYON[3];
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Abstract: The extra-geniculate visual pathway carrying visual information from the retina through the superior colliculus and the pulvinar/lateral-posterior nucleus (LP), is not well understood. While several studies have implicated this pathway, in particular, the pulvinar, in higher level cognitive tasks, we still know little about the basic functional neural properties. In rodents, such as squirrels and rats, (LP), while the nucleus as a whole is interconnected with visual cortex it can be divided into three main subdivisions, caudal (LPc), rostrolateral (LPrl), rostromedial (LPrm), based on whether there is specific, diffuse or no input from the superior colliculus, respectively. A similar pattern of SC input is found in other species as well, including primates. Here we recorded responses of single LPrl and rm neurons to drifting sine gratings to determine whether the regions could be distinguished functionally. Electrodes were targeted to

LPrl and LPrm using stereotaxic coordinates and confirmed through histology. Additional recordings were made in primary visual cortex (V1) for comparison. Experiments were performed in Long-Evans rats under light anesthesia (isoflurane/N_2O/O_2). We found that 53% (n = 125/241) of LPrm cells and 43% (n = 99/229) of LPrl cells responded to drifting gratings, compared to 79% of cells in V1 (n = 80/101). Orientation tuning in LP, as measured by the half-width at half-height, was similar between the two rostral subdivisions (LPrm: 46º; LPrl: 48º); both significantly broader than the V1 cell average (37º; both P = 0.003). In contrast, LP cells showed slightly higher direction selectivity (LPrm: 0.25; LPrl: 0.23) than in V1 (0.18). In addition, the optimal stimulus size for LP neurons was quite large (≥70º) compared to V1 (26º). The average response latency was also similar between rostral LP subdivisions (89 ± 3.9 ms in LPrm; 93 ± 3.7 ms in LPrl) and longer than in the V1 (76 ± 2.7 ms). Differences between the two rostral LP subdivisions were found for background activity and optimal TF. LPrm (12.35 ± 1.14 Hz) had significantly higher background activity than both the LPrl (9.12 ± 0.8 Hz; P = 0.036) and V1 (4.93 ± 0.8 Hz; P < 0.001; LPrl vs. V1 P < 0.001). The optimal TF was significantly higher in the LPrl (4.22 ± 0.3 cycle/s) than in LPrm (2.91 ± 0.22 cycle/s) and V1 (3.01 ± 0.3 cycle/s). Based on these preliminary results we suggest that LPrm and LPrl subdivisions represent separate information processing units with regard to temporal frequency, with LPrl being more representative of the superior colliculus from which it receives diffuse inputs and LPrm more representative of V1.

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Poster

140. Vision: Subcortical Visual Pathways

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Support: DFG Emmy-Noether (KR 4062/4-1)
EMBL Interdisciplinary Postdoc fellowship (EI3POD)
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Title: Fine-scale functional organization of the mouse superior colliculus

Authors: J. SIBILLE[1,2,3,4], L. FERRARESE[5], C. GEHR[1,2,3,4], H. ASARI[5], ***J. KREMKOW**[1,2,3,4];
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Abstract: The superior colliculus (SC) is a layered midbrain structure that plays a central role in visual processing in the mouse. About 90% of the retinal ganglion cells (RGC) project to the SC (Kremkow & Alonso 2018) and show cell-type specific axonal projection patterns within and across different SC layers (e.g. Huberman et al. 2008). This suggests that the parallel streams of visual information, originating in the retina (Baden et al. 2016), are processed and integrated within functionally specific neuronal circuits in the SC. Recent studies have identified the functional organization of the mouse SC, such as orientation columns (Feinberg & Meister 2015, Ahmadlou & Heimel 2015) and regionally specific motion processing (de Malmazet et al. 2018). However, the fine-scale functional architecture of the SC, in particular within the deeper visual layers (lower stratum griseum superficial "lSGS"), still remains unclarified. Here we targeted the visual layers of the SC by introducing a Neuropixels probe (Jun et al. 2017) tangentially to its surface along the anterior-posterior axis. The high-density recording sites on this probe - 384 recording channels over about 4mm - gave us measurements of visually evoked signals over unprecedentedly large parts of the SC, covering response areas up to 1 to 2 mm long. To visually stimulate the full retinotopy covered by our recording sites, we presented visual stimuli in a dome (Denman et al. 2017), and to characterize the functional response properties, we presented a series of stimuli, e.g. sparse noise, moving bars and gratings, looming, and chirp stimuli. In addition to these electrophysiological recordings, we started to investigate the SC circuits using two-photon calcium imaging of SC cells and RGC axons at different depths of the SC (100-300µm below the surface). Here we will present our preliminary data suggesting that the fine-scale functional organization of the mouse superior colliculus is more elaborate than previously thought.
References:
Kremkow & Alonso (2018) Annu Rev Vis Sci; Huberman, et al. (2008) Neuron; Baden et al. (2016) Nature; Feinberg & Meister (2015) Nature; Ahmadlou & Heimel (2015) Nat Commun; de Malmazet et al. (2018) Curr Biol; Jun et al. (2017) Nature; Denman et al. (2017) J Neurosci.

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Poster

140. Vision: Subcortical Visual Pathways

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Title: Locomotion independent encoding of visual motion in the mouse superior colliculus

Authors: *E. L. SAVIER, H. CHEN, M. LIU, J. CANG;
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Abstract: Animals rely on the rapid detection of visual information in their environment in order to avoid potential threats, like a colliding object or a predator. One way to detect such threats is through recognition of abrupt changes such as motion. Within the visual system, the superior colliculus (SC) plays a predominant role in the detection of motion and generates visually evoked defensive responses. Previous studies in mice have shown that the most superficial lamina of the visual layers of the SC (sSGS) contains neurons highly sensitive to movement direction. In other early visual areas, like the thalamus and the primary visual cortex, locomotion increases the response magnitude of individual neurons. These results suggest that the behavioral state of the animal can influence the encoding of the visual information and potentially lead to different behavioral outcome.
To test if such a state-dependent modulation existed in the SC, we conducted 2-photon calcium imaging at cellular resolution to monitor the responses of sSGS neurons to visual stimuli. Head-fixed mice were visually stimulated with drifting gratings and flashing spots displayed on a computer monitor while free to run on a treadmill. These stimuli were used to determine the receptive fields and direction selectivity of collicular neurons. First, we confirmed that the majority of neurons in the sSGS are visually responsive in awake condition and display high specificity for motion direction. Next, we characterized neuronal responses by separating trials according to whether the mice were stationary or running. Contrary to what has been reported in other visual areas, we found that locomotion does not change the response amplitude nor the direction tuning of neurons in the sSGS. In addition, we assessed the stability of tuning in these neurons through chronic imaging, and revealed that direction selective neurons maintain their response specificity across months. Together, our results indicate that collicular neurons maintain their tuning across time and behavioral states. This robust and stable encoding of visual information confirms the sSGS's role as a faithful feature detector in the early visual system.

Disclosures: E.L. Savier: None. **H. Chen:** None. **M. Liu:** None. **J. Cang:** None.

Poster

140. Vision: Subcortical Visual Pathways

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 140.14/K16

Topic: D.07. Vision

Support: National Eye Institute Intramural Research Program

Title: Superior colliculus in mice is necessary for detecting behaviorally relevant visual changes

Authors: *L. WANG, S. GOLDSTEIN, K. MCALONAN, R. J. KRAUZLIS;
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Abstract: One of the most important visual regions in mice is the superior colliculus (SC), an evolutionarily conserved midbrain structure best known for its role in guiding orienting movements. In mice, the SC is perhaps best recognized for its role in processing visual threats, such as looming stimuli that denote the approach of a predator. In several other species, the SC plays a pivotal role in detecting behaviorally relevant visual events in general, such as those used to guide perceptual choices. Here we tested whether mouse SC plays a similar role in visual event detection by transiently inhibiting SC activity during a visual orientation-change detection task.

Mice were trained to report visual orientation changes that could occur in either a left or right visual display by licking a central spout to obtain fluid rewards. During this yes/no task, we briefly and unilaterally activated SC intermediate layer GABAergic neurons expressing Channelrhodopsin (ChR2) in vGat-cre mice.

The current study has three primary findings: 1) SC inhibition induced spatially specific deficits in detection. Optogenetic stimulation caused a significant reduction (22 ± 2.4%, n=8 mice) in hit rates, as well as marked increases (83.7 ± 11.1ms) in mean reaction times for near-threshold orientation changes contralateral to the inhibited side. Neither hit rates nor reaction times for orientation changes ipsilateral to the inhibited side were affected. 2) Behavioral effects caused by SC inhibition were specific to a temporal epoch coincident with known visual responses in the SC. Activating SC GABAergic neurons for 100-ms (starting from 50ms after the orientation change onset) caused a contralateral detection deficit, whereas earlier or later SC inhibition did not. 3) SC inhibition reduced visual detection sensitivity. Psychometric curves revealed that SC inhibition during the visual epoch significantly increased Just-Noticeable-Differences (JND, 1.03 ± 0.39°) and detection thresholds (2.36 ± 0.36°) for contralateral orientation changes. Effects on detection thresholds (1.64 ± 0.64°) and lapse rates (0.063 ± 0.024) caused by SC inhibition were larger in the presence of a competing visual stimulus, indicating a role for stimulus competition or target selection for the mouse SC.

Together, our results demonstrate that the mouse SC plays a crucial role in detecting behaviorally relevant visual events that guide appetitive perceptual choices.

Disclosures: L. Wang: None. **S. Goldstein:** None. **K. McAlonan:** None. **R.J. Krauzlis:** None.

Poster

140. Vision: Subcortical Visual Pathways

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 140.15/K17

Topic: D.07. Vision

Support: Simons Foundation Award 543015SPI
NIH Grant 1K99EY028640-01A1
Helen Hay Whitney Foundation fellowship

Title: Functional architecture of motion direction in the mouse superior colliculus

Authors: *Y.-T. LI, Z. TURAN, M. MEISTER;
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Abstract: In the mammalian retina, ~30 distinct types of retinal ganglion cells (RGCs) send different visual features to the brain. The superior colliculus (SC), an evolutionarily conserved structure, receives direct retinal inputs from more than 90% of RGCs. How the circuits of the SC operate on these retinal inputs and create new visual representations remains unclear. To address this question, we investigated the population coding of basic visual features in the SC of awake mice. Here we focus on the direction of motion, an important feature for guiding animal behavior. We imaged the calcium responses to moving dots and moving bars of different directions in superficial SC neurons of C57BL6/J mice using two-photon microscopy. We found that direction-selective collicular neurons are organized in maps where neurons with a distance up to ~500 μm (~30° of visual angle) preferred the same direction. This is distinct from the salt-and-pepper pattern in the mouse primary visual cortex, where the similarity of preferred direction was reported only for pairs with a distance of ~20 μm (~1°). Furthermore, these direction maps in the SC span the vertical thickness to a depth of at least 300 μm. Throughout the map the preferred direction is largely orthogonal to the preferred orientation. To better understand the functional architecture at a larger scale, we used mutant mice that lack the dorsal cortex, thus revealing both hemispheres of the SC for wide-field optical imaging. Within this global map, the upward direction occupied a large portion on both sides, but the overall organization of preferred direction in the two hemispheres was not symmetric. The global map of direction preference appears aligned with certain patterns of optic flow but not others. We also observed that the functional architecture varies across individuals. To sum up, the unique functional organization of the SC, which is distinct from the organization of the cortex, indicates it may play a distinct role in coding visual features and guiding behavior.

Disclosures: Y. Li: None. **Z. Turan:** None. **M. Meister:** None.

Poster

140. Vision: Subcortical Visual Pathways

Location: Hall A

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Program #/Poster #: 140.16/K18

Topic: D.07. Vision

Support: NSF Grant IOS-1656838

DARPA Grant HR0011-18-2-0019, TA2

Title: Visual responses in the superior colliculus of a diurnal, precocial hystricognath rodent: Octodon degus

Authors: N. I. MARQUEZ[1], A. R. DEICHLER[1], I. PERALES[1], J.-C. LETELIER[1], G. J. MARÍN[2], J. MPODOZIS[1], ***S. L. PALLAS**[3];
[1]Biol., [2]Univ. of Chile, Santiago, Chile; [3]Biol., Univ. of Massachusetts, Amherst, MA

Abstract: The superior colliculus (SC) is a multisensory structure located on the dorsal surface of the midbrain, controlling spatial attention and orienting behaviors towards or away from salient locations in visual space. In commonly studied rodents, the SC is especially developed, and receives input from about 90% of retinal ganglion cells. The SC possesses a topographic map of the retina in its superficial layers, and most neurons in these layers respond briskly to transient visual stimulation, especially visual motion. The receptive fields (RFs) increase in size from superficial to deeper locations and exhibit a suppressive surround that inhibits the response at the center and restricts excitatory receptive field size. Most studies of the SC in rodents have used species belonging to the Sciuridae or Muridae families, which are mostly nocturnal and altricial. Interestingly, developmental studies in hamsters and mice (Muridae) have shown that RF organization is well established in newborns and that visual experience does not significantly refine RF structure. However, prolonged dark rearing (beyond P60) leads to enlargement of RF size, indicating that visual experience is critical for maintenance of refined RFs. Here we introduce a new rodent model for studying the development of the visual pathway, *Octodon degus* (Hystricognathi, Octodontidae). Unlike most Muridae and Sciuridae, degus are diurnal and precocial, opening their eyes and starting to walk from postnatal day one. These facts suggest that the visual organization of the degus' SC is well established before they are born. Therefore, in the degus, visual experience may not be as crucial in the maintenance of RFs as in altricial species. Alternatively, visual experience may be more important in refinement and maintenance of RFs in degus than in Muridae, given that they experience visual stimulation immediately after birth. As a first approach to resolution of this issue, we mapped the visual field topography in the SC by systematically recording along its rostro-caudal and latero-medial axes, at 500 µm intervals, determining the position of the RF (center, elevation, and azimuth). As shown in other species, RFs corresponding to the nasal visual field were smaller (5-6º, at 200 µm depth) than those corresponding to temporal visual fields (16-18º, at 200 µm depth). Likewise, RFs increased in size in deeper layers, reaching 18-20º at 1000 µm depth. Experiments in progress will test the effect of visual experience on development and maintenance of RF structure in degus by comparing these results with those obtained from degus raised in darkness.

Disclosures: **N.I. Marquez:** None. **A.R. Deichler:** None. **I. Perales:** None. **J. Letelier:** None. **G.J. Marín:** None. **J. Mpodozis:** None. **S.L. Pallas:** None.

Poster

140. Vision: Subcortical Visual Pathways

Location: Hall A

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Program #/Poster #: 140.17/K19

Topic: D.07. Vision

Support: Fondecyt 1170027

Title: The tectofugal system of marsupials: A tract tracing and histochemical study in the chilean opossum (Thylamys elegans)

Authors: A. DEICHLER, *G. J. MARÍN, M. RUIZ, T. VEGA-ZUNIGA, J. MPODOZIS; Biologia, Facultad de Ciencias, Univ. de Chile, Facultad de Ciencias, Universidad de Chile, Chile

Abstract: A defining feature of the tectofugal pathway (TFP) is a massive bilateral projection from the retino-recipient optic tectum to the sensory thalamus, a trait reported in members of all major groups of tetrapods: birds, non-avian reptiles, amphibians and mammals. Thus from an evolutionary perspective the bilateral TFP can be viewed as the ancestral tetrapod condition. However the prevalence of this trait in mammals is uncertain. Bilateral tecto-pulvinar projections are consistently found in rodents; however controversy comes when considering primates and carnivores, in which most tracing studies report only ipsilateral projections. This scenario poses an intriguing question on the evolutionary history of the mammalian TFP. Vision in primates and carnivores is highly binocular, a character normally associated to a predominant thalamofugal system, in detriment of collicular pathways. Following the nocturnal bottleneck hypothesis, a reduction in collicular pathways may have occurred at the origin of the mammalian lineage, perhaps leading to multiple events of TFP loss and regain during subsequent evolution. To generate a clearer picture on the mammalian TFP evolution, we performed a tract tracing and neurochemical characterization of the collicular ascending projections in a south american nocturnal marsupial, the yaca, *Thylamis elegans,* comparing it with our previous results in the diurnal rodent *Octodon degus*. Yacas are in a key phylogenetic position for this comparison as they belong to Didelphidae, a basal family of american marsupials. Reminiscent of the primate condition, yacas display a wide binocular field with prominent ipsi- and contralateral retinofugal projections to a laminated DLG. Our results show that in yacas the caudal pulvinar (PulC) receives a bilateral diffuse tectal projection, while the rostral pulvinar receives an ipsilateral topographic projection, just as found in degus and most mammalian groups. In both species, yacas and degus, intense expression of calretinin and VGluT2 are defining features of PulC neurons. Notably, compared to degus the contralateral pathway is significantly reduced in the yaca. Our results highlight the conservative character of the TFP in mammals and suggest possible historic scenarios that may lead to a decreased TFP in some members of this clade.

Disclosures: **G.J. Marín:** None. **A. Deichler:** None. **M. Ruiz:** None. **J. Mpodozis:** None. **T. Vega-Zuniga:** None.

Poster

140. Vision: Subcortical Visual Pathways

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 140.18/K20

Topic: D.07. Vision

Support: NIH Grant R01EY027718

Title: Construction of classical and competitive surrounds in the owl isthmi pars magnocellularis

Authors: *H. M. SCHRYVER[1], J. X. LIM[2], M. STRAKA[4], S. P. MYSORE[3]; [1]Psychological and Brain Sci., [2]Neurosci. & HHMI Janelia Res. Campus, [3]Psychological and Brain Sci. & Neurosci., Johns Hopkins Univ., Baltimore, MD; [4]Paradromics Inc, Austin, TX

Abstract: Selection for spatial attention involves identifying the location of the highest priority stimulus in the environment to guide behavior. This function relies on competitive interactions in the midbrain superior colliculus (in mammals, or optic tectum in birds), which, in turn, have been shown, in birds, to be mediated by competitive response suppression provided by a nearby inhibitory nucleus, the Imc (isthmi pars magnocellularis). Imc neurons have been demonstrated to exhibit classical (local) inhibitory surrounds as well as global competitive surrounds, but the sources of inhibition necessary to construct these surrounds are not well understood. Here, using microiontophoretic injections of GABA antagonists in the barn owl (Tyto alba) Imc, we investigate how these inhibitory surrounds are constructed. Results can provide valuable mechanistic insight into the function of a nucleus critically involved in implementing stimulus competition and selection in the optic tectum.

Disclosures: **H.M. Schryver:** None. **J.X. Lim:** None. **M. Straka:** None. **S.P. Mysore:** None.

Poster

140. Vision: Subcortical Visual Pathways

Location: Hall A

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Topic: D.07. Vision

Support: NIH grants EY026916
NIH grants NS073553

Title: Deactivating association cortex blocks reversal of hemianopia by multisensory training

Authors: *H. JIANG, E. M. WALKER, B. A. ROWLAND, B. E. STEIN;
Neurobio. and Anat., Wake Forest Sch. of Med., Winston-Salem, NC

Abstract: Previous studies in cat (Jiang et al., Nature Communications 2015) showed that hemianopia induced by unilateral visual cortex damage can be reversed by multisensory (auditory-visual) rehabilitation training. The training reinstates visual responsiveness in multisensory neurons of the ipsilesional SC, which renders them capable of supporting SC-mediated behavior in the previously blind hemifield. The training-induced reversal of hemianopia is effective within 4-5 weeks even if the animal is anesthetized during the training sessions (Jiang et al., SfN 2018). Presumably, the training effect is mediated by changes in the remaining visual pathway that involves projections via posterior thalamus to association cortex (i.e., the anterior ectosylvian sulcus, AES), and from there to the multisensory SC. The present study sought to test this possibility by blocking the AES cortico-collicular relay during the training sessions. Two cats were trained in a visual orientation task, after which hemianopia was induced by unilateral ablation of all contiguous areas of visual cortex. After 3 months, weekly multisensory rehabilitative training sessions were begun while the animals were anesthetized and AES activity was cryogenically blocked via implanted cooling coils. After 8 weeks of training there was no evidence of amelioration of visual deficit. Although animals responded with near perfect accuracy to a visual stimulus at every 15 degrees interval in the ipsilesional hemifield, they failed to respond to these same stimuli in the contralesional hemifield. They were also unreactive to threatening gestures in that hemifield and would not track visual stimuli that moved into it. To ensure that the animals were still capable of rehabilitation, they were then trained in the same way without AES deactivation. Hemianopia was reversed in one animal after 4 training sessions (weeks), and in the other after 5 training sessions. These data confirm the speculation that influences relayed from AES play a critical role in the reversal of hemianopia via multisensory training. Supported by NIH grants R01EY026916.

Disclosures: H. Jiang: None. **E.M. Walker:** None. **B.A. Rowland:** None. **B.E. Stein:** None.

Poster

140. Vision: Subcortical Visual Pathways

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 140.20/K22

Topic: D.07. Vision

Support: NIH Grant EY026916

Tab Williams Family Foundation

Title: The temporal principle of multisensory integration in localization

Authors: *N. L. BEAN, B. E. STEIN, B. A. ROWLAND;
Wake Forest Sch. of Med., Winston Salem, NC

Abstract: The responses of individual neurons in the superior colliculus (SC) to spatiotemporally congruent pairs of visual-auditory stimuli are typically greater than those elicited by the individual component stimuli ("multisensory enhancement"). Pairs of visual-auditory stimulus presented at significant temporal disparities elicit less robust responses than stimuli delivered within relative temporal proximity. The dependence of multisensory enhancement on relative temporal proximity has been summarized as the "temporal principle of multisensory integration", which is consistent with the idea that cross-modal stimuli derived from the same event are typically proximate in time. Prior work suggests that a visual-auditory stimulus-onset asynchrony (SOA) of 25 ms (visual before auditory, V25A) maximizes the average multisensory enhancement in a large population of SC neurons (Miller et al., 2016). Here we determined whether this heuristic also predicted enhancement in the ability of animals to localize visual-auditory stimulus pairs presented at different temporal disparities. Adult cats (n=2) were trained to detect and approach visual and auditory stimuli at randomly-selected locations. Stimulus intensities were titrated to two levels of effectiveness: weakly-effective (30-40% accuracy) and strongly-effective (50-60% accuracy). Animals were tested with brief (50 ms) visual and auditory stimuli presented individually, or together at the same spatial location but at a variety of different SOAs, from A200V to V200A, at each intensity level. Multisensory enhancement in overt behavior was highly consistent with the observed heuristic. Enhancement was maximized at both effectiveness levels around simultaneity and the V25A temporal disparity, and rapidly degraded outside of that. While the accuracy of responses to visual-auditory pairs at other temporal disparities almost always exceeded the accuracy of responses to either individual stimulus, they did not consistently exceed the predictions of a separate activation model of localization. This trend was evident for pairs of weakly-effective and strongly-effective stimuli. These results describe a temporal principle in multisensory detection and localization behavior that parallels the temporal principle evident in individual SC multisensory neurons. Supported by NIH grant EY026916 and the Tab Williams Family Foundation.

Disclosures: N.L. Bean: None. **B.E. Stein:** None. **B.A. Rowland:** None.

Poster

140. Vision: Subcortical Visual Pathways

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 140.21/K23

Topic: D.07. Vision

Support: NIH Grant EY024458

Title: Dark-rearing precludes the auditory enhancement of visual localization

Authors: *S. A. SMYRE, Z. WANG, B. E. STEIN, B. A. ROWLAND;
Neurobio. and Anat., Wake Forest Sch. of Med., Winston Salem, NC

Abstract: The brain is richly endowed with multisensory neurons that can integrate cues from different senses to enhance perception and behavior. However, this neural capability for 'multisensory integration' requires extensive experience to develop. For example, in the absence of visual-auditory experience, multisensory neurons in the midbrain and cortex remain robustly responsive to visual and auditory cues, but are unable to synthesize that multisensory information to enhance their physiological responses. Presumably, these observations are reflective of a general brain requirement, and the absence of visual-auditory experience would compromise perception and overt behavior in similar ways. Here, we tested this assumption by examining whether animals reared in such conditions would show the typical multisensory benefits when trained to detect and localize external events. Cats (n=2) were raised to adulthood (1-2 years) in a dark room to preclude visual-auditory experience. They were then trained in the dark to detect, localize, and orient towards briefly-activated (50 ms) visual or auditory cues. The cues appeared at randomly-selected locations in a perimetry apparatus within the central 90° of space. They also learned to remain still (no-go) on trials containing no stimulus ("catch trials"). After 1-3 months of this unisensory localization training, stimulus intensities were lowered so that unisensory response accuracy at each location was no greater than 25-30%, thereby ensuring high sensitivity to any benefits that might be realized when those cues were combined. The animals were then tested with these visual and auditory cues alone or in combination at each location. In contrast to normal animals, the multisensory response accuracies of the dark-reared animals were never better than the predictions of separate activation by each cue and in some cases were significantly lower. These findings are consistent with the responses of individual multisensory neurons and suggest that the brain uses multisensory experience to develop the capacity to integrate these inputs to facilitate overt behavior. Supported by NIH/REI RO1 EY024458.

Disclosures: S.A. Smyre: None. **Z. Wang:** None. **B.E. Stein:** None. **B.A. Rowland:** None.

Poster

140. Vision: Subcortical Visual Pathways

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Program #/Poster #: 140.22/K24

Topic: D.07. Vision

Support: NIH Grant EY026916
NIH Grant EY027686
NIH Grant NS073553
Tab Williams Family Foundation

Title: Can the hemianopic hemisphere compete with its intact counterpart after rehabilitation?

Authors: *A. S. DAKOS, E. M. WALKER, H. JIANG, B. E. STEIN, B. A. ROWLAND; Neurobio. and Anat., Wake Forest Sch. of Med., Winston-Salem, NC

Abstract: Loss of visual cortex on one side of the brain causes profound blindness in the contralesional visual hemifield (hemianopia) despite the presence of visual circuits far from the site of physical damage (e.g., the midbrain superior colliculus, SC). Previous studies in cat have shown, however, that secondary (excitotoxic) damage induced by the lesion silences the visual responses of neurons in the multisensory layers of the ipsilesional SC (see Jiang et al., 2009; 2015). The visual activity of neurons in these layers can be restored by multisensory (auditory-visual) training, so that they can once again support contralesional visuomotor orientation behaviors to individual visual events. However, these restored capabilities are presumed to be at a competitive disadvantage with those for ipsilesional space due to the vast interhemispheric asymmetry in visual processing circuits (ipsilesional SC vs. contralesional SC + visual cortex, see Sprague, 1966). Thus, when visual events appear in the two hemifields simultaneously, events in ipsilesional space are likely to have a greater impact on the brain's visuomotor circuitry. To evaluate this possibility, and the magnitude of any such disadvantage, the present study employed a visual choice paradigm. Hemianopic cats, rehabilitated by multisensory training, were provided with identical visual stimuli in each hemifield, and allowed to choose the target for an orientation response in order to obtain a food reward. Responses to either stimulus were rewarded equally. Surprisingly, the previously blind hemifield was not disadvantaged in this task. 2/3 tested animals strongly preferred the target in the previously blind hemifield, and did so at multiple stimulus intensity levels. Indeed, the relative intensity of the visual stimulus in that hemifield had to be reduced by 40% in order to mitigate this preference. In addition, the ability to synthesize visual and auditory inputs to facilitate orientation responses to minimally effective cues was equally robust in the two hemispheres and consistent with the principle of inverse effectiveness. These results not only underscore the effectiveness of the training paradigm in restoring vision in the previously blind hemifield, but reveal an unexpected potency in the visual processing capability of the remaining visual circuit. Supported by NIH grants R01EY026916, F31EY027686, T32NS073553, and the Tab Williams Family Foundation.

Disclosures: A.S. Dakos: None. **E.M. Walker:** None. **H. Jiang:** None. **B.E. Stein:** None. **B.A. Rowland:** None.

Poster

141. Visual Cortex: Functional Architecture and Circuits I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 141.01/K25

Topic: D.07. Vision

Support: KAKENHI19K12743
KAKENHI17K07050

Title: A theoretical study of differences in the contrast and length tuning of visual cortical neurons between cats and rodents

Authors: *M. MIYASHITA[1], S. TANAKA[2];
[1]Natl. Inst. of Technol., Shizuoka, Japan; [2]The Univ. of Electro-Communications, Tokyo, Japan

Abstract: In the cat primary visual cortex, most of neurons selectively respond to specific orientations of local contours presented in the visual field, and are regularly arranged according to neurons' preferred orientations, forming orderly orientation maps. Numerous experimental studies have been reported on the dependence of neuronal activity upon the size or luminance contrast of visual stimuli. On the other hand, little is known about the response properties of neurons in the rodent visual cortex, where orientation-selective neurons are sparsely and randomly distributed in the salt-and-pepper-like fashion. Recently we have reproduced qualitatively different orientation representations based on the same self-organization model only by changing the value of one parameter: the probability of excitatory intracortical connections, p. The salt-and-pepper-like orientation representation emerged at $p < p_c$, whereas orderly orientation maps appeared at $p > p_c$. In this study, in order to elucidate how the structure of orientation representation affects neuronal response properties, we performed computer simulations of the dynamics of neural networks composed of leaky integrate-and-fire units that receive the self-organized thalamo-cortical inputs. In the simulations, we employed sinusoidal grating patches as visual stimuli, and changed the luminance contrast and length of the grating patches along the stimulus orientations. In the model visual cortex showing either orderly map or salt-and-pepper-like representation, the neuronal firing rate in response to longer grating patches was saturated or suppressed, although firing rate in the salt-and-pepper-like representation was generally much lower than that in the orderly maps. Particularly at higher stimulus contrasts, neurons in the salt-and-pepper-like representation elicited maximum responses when the size of grating patches was similar to the size of classical receptive fields (CRFs). When only CRFs were stimulated by grating patches, the firing rate of neurons in orderly maps monotonically increased as luminance contrast increased up to 100%, whereas that in the salt-and-pepper-like representation showed plateau at contrasts higher than 50% and no responses were observed at contrasts lower than 10%. The simulation results suggest that the regularity of orientation

representation contributes to the emergence of orientation-selective neurons with a wide dynamic range of luminance contrast.

Disclosures: M. Miyashita: None. **S. Tanaka:** None.

Poster

141. Visual Cortex: Functional Architecture and Circuits I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 141.02/K26

Topic: D.07. Vision

Support: Macquarie University Postgraduate Research Fund

Title: Beyond the retinotopic framework of the primary visual cortex: Category-dependent feedback information of non-foveal visual stimuli coded by the foveal cortex

Authors: *A. I. COSTANTINO, M. COLTHEART, P. SOWMAN, M. WILLIAMS;
Dept. of Cognitive Sci., Macquarie Univ., Sydney, Australia

Abstract: Introduction Feedback connections from higher to lower visual areas are ubiquitous in the human brain, suggesting that they might play a crucial role in visual perception. How feedback mechanisms modulate visual perception is still debated, but the majority of models including both attentional and predictive coding models, predict that feedback activations are retinotopically mapped in primary visual cortex. However, recent studies have suggested the existence of a foveal cortex activation after the presentation of peripheral (non-foveal) stimuli in a time-course consistent with feedback timings. They have demonstrated that this foveal feedback is position-invariant; that this activation coded information about object's shapes; and that the magnitude of the activation correlates with performances. Here we extend these findings, investigating: (1) whether this is a general mechanism, or it is feature-dependent (i.e., present only during a shape discrimination task); and (2) which is the source of the feedback. **Methods** We tested 25 subjects in a block-designed functional magnetic resonance imaging (fMRI) study. Participants performed a same/different task on peripherally presented pairs of stimuli belonging to the same category (i.e., male faces, female faces, cars and bikes). We recorded reaction times and accuracy, and also explored the multi-voxel pattern (MVP) activation of four regions of interest (ROIs): the foveal region of primary visual cortex, the peripheral region of primary visual cortex, the fusiform face area (FFA) and the lateral occipital complex (LOC). **Results** Our preliminary results show category-dependent information both in the foveal cortex and in the FFA/LOC but not in other ROIs, confirming the previous findings that the foveal cortex codes information about peripheral objects. **Conclusions** Our results suggest that this feedback to the foveal region of primary visual cortex to peripherally presented objects is a task-dependent, general mechanism. As no category information was found in the peripheral primary visual

cortex ROI, we argue that the information cannot be simply due to spreading across the cortex but may be generated in FFA or LOC. This highlights the FFA and LOC as possible candidates of the foveal feedback for faces and objects.

Disclosures: A.I. Costantino: None. **M. Coltheart:** None. **P. Sowman:** None. **M. Williams:** None.

Poster

141. Visual Cortex: Functional Architecture and Circuits I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 141.03/K27

Topic: D.07. Vision

Support: NIH Grant RO1EY027018

Title: Bilateral changes to white matter structure following unilateral resection in pediatric patients

Authors: *A. M. S. MAALLO[1,2], E. FREUD[3,4], C. PATTERSON[5], M. BEHRMANN[1,2]; [1]Carnegie Mellon Univ., Pittsburgh, PA; [2]Ctr. for the Neural Basis of Cognition, Pittsburgh, PA; [3]Dept. of Psychology, York Univ., Toronto, ON, Canada; [4]Ctr. for Vision Res., Toronto, ON, Canada; [5]Dept. of Pediatrics, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Children with unilateral resections of ventral occipito-temporal cortex (VOTC) do not necessarily evince a perceptual impairment, even when specialized category-selective regions, such as the fusiform face area, are resected. We aim to elucidate whether unilateral resections modulate the integrity and organization of the contralesional white matter pathways. To this end, we analyzed diffusion MRI data acquired from 6 children with cortical resections to the VOTC. We used a deterministic fiber tracking algorithm to define the two major white matter pathways connecting the occipital lobe and the temporal lobe: the inferior longitudinal and inferior fronto-occipital fasciculi. We then compared in the patients and the controls the microstructural indices including fractional anisotropy, and axial and radial diffusivity of the tracts, and found that the changes were influenced by the presurgical conditions of the patients. In subgroup 1 consisting of 3 patients presenting with neurological comorbidities, we found changes to white matter microstructure in both the ipsilesional and contralesional tracts. However, in subgroup 2 with 3 patients without neurological disorders other than the etiology of the surgery, we found changes only to the ipsilesional white matter microstructural indices that were restricted to the proximate regions of the resection. Next, we characterized the network properties of the entire contralesional hemisphere using graph theoretic measures including efficiency, and mean node degree and clustering coefficient. We compared these in the patients and the controls using 34 cortical regions defined by gyri-based parcellation as nodes. In subgroup 1, we found only

increased clustering coefficient. Interestingly, in subgroup 2, despite the evidence of localized white matter damage due to the resection, we found attenuated graph theoretic measures in the supposedly healthy hemisphere. These results suggest first, that the damage to white matter microstructure is specific to the site of the resection without anterograde degeneration of the tracts in the absence of confounding neurological comorbidities, and second, that there is reorganization in the contralesional hemisphere that may serve as the foundation for the (re)organization of function concomitant with unilateral cortical resections.

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

Poster

141. Visual Cortex: Functional Architecture and Circuits I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 141.04/K28

Topic: D.07. Vision

Support: NIMH IRP

Title: Affective processing of face stimuli in human primary visual cortex

Authors: *T. T. LIU, J. Z. FU, S. JAPEE, L. G. UNGERLEIDER, E. P. MERRIAM;
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Abstract: Fearful faces evoke stronger neural responses than neutral faces in multiple brain regions in both monkeys and humans, including the amygdala, the fusiform face area, and the primary visual cortex. We hypothesized that processing of face valence in visual cortex is accomplished through feedback from the amygdala. If so, then fMRI activity in visual cortex should reflect the conjunction of both feedforward and feedback inputs, and these two sources of input should have distinct laminar profiles. The purpose of this experiment was to isolate and separately measure these two sources of input to visual cortex using ultra high-field, high-resolution fMRI. Participants viewed a series of face stimuli that were closely-cropped and balanced for low-level visual properties. Face stimuli were updated every 1000 ms with an interstimulus interval of 100 ms, and were blocked by emotional expression (fearful, happy, neutral). Participants performed a gender judgement task unrelated to facial expression. We measured fMRI activity using gradient-echo BOLD at both 3T and 7T, and at three different spatial resolutions. We observed a robust and reliable valence effect (greater fMRI responses to fearful faces) in visual cortex, replicating previous studies. We also measured a pronounced bias with cortical depth: fMRI responses were larger and noisier in and around larger draining veins, which dominated the measurements in superficial cortical layers. Our results highlight the need

for alternate pulse sequences and analysis strategies for studying layer-specific activity with fMRI.

Disclosures: T.T. Liu: None. **J.Z. Fu:** None. **S. Japee:** None. **L.G. Ungerleider:** None. **E.P. Merriam:** None.

Poster

141. Visual Cortex: Functional Architecture and Circuits I

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Priority Programs (SPP1665, JA 945/4-1, HE 2471/12-1 and SPP1926, HE 2471/18-1)
SFB874 (DFG project number 122679504) (to S.H. and D.J.)
German-Israeli Project Cooperation (DIP, JA 945/3-1, SL 185/1-1)

Title: Subtraction and division fo visual cortical responses by the serotonergic system

Authors: *Z. AZIMI[1,2], K. SPOIDA[3], R. BARZAN[1,2], T. SURDIN[3], M. D. MARK[3], S. HERLITZE[3], D. JANCKE[1];
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Abstract: Subtraction and division are fundamental operations throughout cortical sensory systems to achieve normalization of input and hence, adjust dynamic range. In the visual system, polysynaptic local, horizontal distal, and top-down cortical circuits have been identified providing the substrate for such arithmetic functions. However, it remains unclear, how these origins are orchestrated and whether the known list of origins is complete. Here, we suggest the serotonergic (5-HT) system as another player involved in controlling response normalization in the primary visual cortex (V1). In general, except for studies in the somatosensory and olfactory cortex, which used optogenetic approaches to target 5-HT neurons specifically, little is known about the involvement of the serotonergic system and its cortical receptors in the dynamics of sensory processes. In the present study, we examined the effect of increased activity of 5-HT neurons in the dorsal raphe nucleus (DRN) on spontaneous and visually evoked activity in V1 of the mouse (ePetCre, n=14). To this end, we combined *in vivo* optogenetic stimulation of 5-HT neurons in the DRN with wide-field calcium imaging and multiunit recordings in V1. We show that spontaneous and visually evoked activity in V1 decreases following optogenetic stimulation of the DRN. This reduction in responses is mediated through different mechanisms: Using

selective receptor blockers we demonstrate that inhibitory (5-HT1A) receptors account for subtractive suppression of baseline spontaneous activity, while depolarizing (5-HT2A) receptors promote divisive suppression of the gain of evoked V1 responses. These components led to normalization of response amplitude over a range of stimulus contrasts in both awake and anesthetized state. Our data suggest that serotonergic input from the DRN recruits established inhibitory and excitatory polysynaptic cortical circuits involved in visual contrast normalization, unfolding an immediate regularization of sensory information via a distinct pathway.

Disclosures: Z. Azimi: None. **K. Spoida:** None. **R. Barzan:** None. **T. Surdin:** None. **M.D. Mark:** None. **S. Herlitze:** None. **D. Jancke:** None.

Poster

141. Visual Cortex: Functional Architecture and Circuits I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 141.06/K30

Topic: D.07. Vision

Title: A model for the origin of motion sensitivity in primary visual cortex

Authors: *A. W. FREEMAN, Y. ZHENG;
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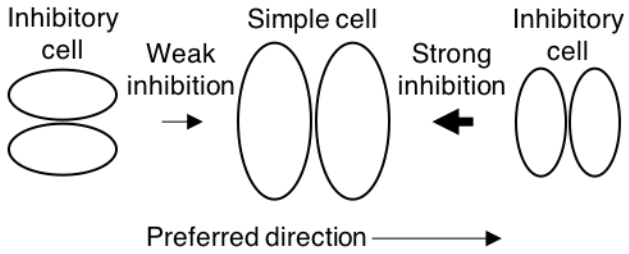
Abstract: Introduction. Motion-sensitive neurons are prevalent in primate and carnivore primary visual cortex: these cells respond well to stimulus motion in one direction and poorly to motion in the opposite direction. Direction-selective neurons were discovered sixty years ago but the mechanisms underlying this selectivity are still obscure. One possible contributor is intracortical inhibition: inhibitory neurons are orientation-selective (Cardin, Palmer, Contreras, 2007) and their preferred orientation on one side of an excitatory cell may differ from that on the opposite side. This heterogeneity could, in turn, result in asymmetries of the excitatory response for a grating moving in one or the other direction. My aim was to test computationally whether heterogeneous inhibition could lead to direction selectivity. **Methods.** Each simple cell in the model received converging input from on- and off-centre subcortical neurons, producing orientation selectivity. The preferred orientation varied across the cortical surface because each cell received a unique pattern of inputs. Each inhibitory cortical neuron received the same inputs as its nearest simple cell and therefore had the same orientation preference. **Results.** Simple cells displayed varying degrees of direction selectivity, from none to maximal (no response in the antipreferred direction). To test the hypothesis, I computed the difference in orientation preference between a simple cell and inhibitory cells in its neighbourhood. In a significant majority of cases a grating moving in the antipreferred direction passed through a region in which the preferred orientation was similar to that of the simple cell, evoking strong inhibition. Conversely, as shown in the figure, responses were strong in the preferred direction because the

grating passed through a region containing inhibitory neurons with differing orientation preference. **Conclusion.** The model predicts that the preferred motion direction of a simple cell is from heterogeneous areas in the orientation preference map (such as pinwheels) to iso-orientation domains.



Disclosures: A.W. Freeman: None.

Poster

141. Visual Cortex: Functional Architecture and Circuits I

Location: Hall A

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Program #/Poster #: 141.07/K31

Topic: D.07. Vision

Support: NSF-IIS-1718991
NSF-DGE-1106400
NIH/NEI T32 EY007043
NIH 1R01EB026955

Title: A geometric theory for complex cells

Authors: Y. CHEN[1], D. M. PAITON[2], *B. A. OLSHAUSEN[3], F. T. SOMMER[4];
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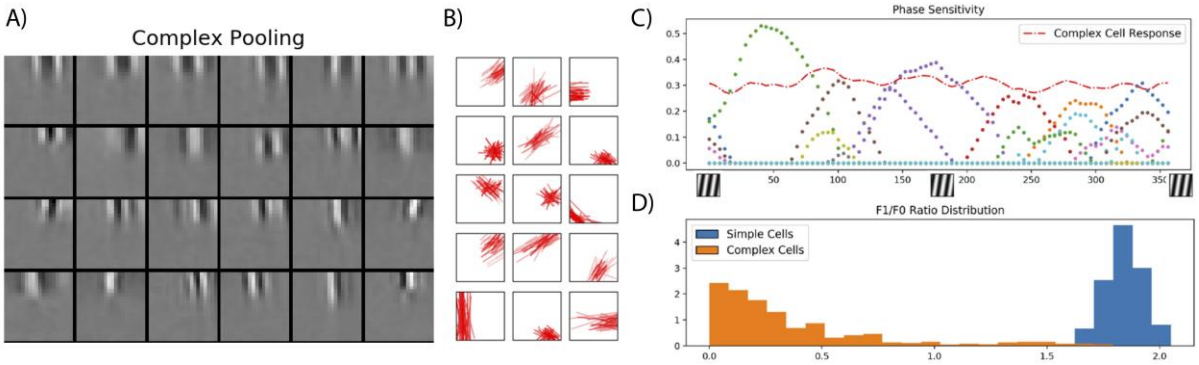
Abstract: A fundamental role of the visual cortex is to represent structure in natural scenes, including local features and their smooth transformations. It is believed that simple cells learn and recognize these local features, which can be modeled using sparse coding. Previous models of complex cells have been motivated either by the objective to build invariant representations or to reflect the multivariate higher-order statistics of natural images. Here we provide a unifying geometric perspective for understanding simple and complex cells based on the recently proposed Sparse Manifold Transform (SMT) model. The SMT is a hierarchical model combining sparse coding (neurons in the first layer) and manifold smoothing (neurons in the second layer) to create temporally smooth representations that reflect transformations in the sensory input. We

have formulated a variant of SMT that constrains the second layer to have minimum-wiring length. Learning in this model leads to cells in layer 1 and 2 which resemble simple and complex cells, respectively. Panel A below shows that orientations and locations of receptive fields (RFs) of layer 1 cells pooled by the same layer 2 cell are similar. Panel B summarizes this result for 21 randomly selected complex cells, visualized as a needle plot showing positions and orientations of simple cell subunits. During a phase sweep of the optimal grating stimuli for one layer 2 cell, the response of cells in layer 1 is phase sensitive (Panel C, dotted lines) and phase invariant for the layer 2 cell (Panel C, red line). The F1/F0 ratio distribution of all cells in the model is bimodal, separating cells in the two layers (Panel D). Our model provides concise functional explanations for both cell types: Simple cells represent a discretized sampling of the smooth data manifold in the sensor space, while complex cells represent localized smooth functions on the manifold. Thus, the hierarchical network enables complex cells to span the manifold and build an untangled population representation which tends to preserve the identity of the signal while straightening transformations.



Disclosures: **Y. Chen:** None. **D.M. Paiton:** None. **B.A. Olshausen:** None. **F.T. Sommer:** None.

Poster

141. Visual Cortex: Functional Architecture and Circuits I

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Topic: D.07. Vision

Support: NIH Grant EY022090
NIH Grant EY027383

Title: Distinct subnetworks for spatial processing in mouse V1

Authors: *A. M. MEIER, E. B. HAN, A. BURKHALTER;
Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Mouse primary visual cortex (V1) exhibits a binary modular pattern of connectivity, M2 muscarinic acetylcholine receptor expression, and visual response tuning in upper cortical layers, termed ‘patches’ and ‘interpatches’ [Ji et al. 2015]. This anatomical pattern presents the opportunity to use the tools of mouse genetics in studying cortical modularity. While the single-cell response properties and connectivity of cortical modules (such blobs and stripes in primate) have been extensively investigated, the interactions between modularity and spatial processing has yet to be determined. In this study, we addressed this question by using calcium imaging in awake mouse V1 to determine the correlation structure of patch and interpatch responses and their possible dependence on stimulus properties. Specifically, we examined response correlations when exposed to large vs. small stimuli, hypothesizing that differences in lateral connectivity between module types could give rise to different circuit-level responses depending on stimulus size. We recorded visual responses via GCaMP 2-photon imaging from awake mice locomoting on a treadmill while square wave circular gratings were presented to the eye. Transgenic mice expressing GCaMP6f in Emx1-positive (excitatory) cells, crossed with Chrm2-tdTomato mice for labeling patches and interpatches, were used. Noise response correlations were determined between each pair of recorded neurons by comparing correlated variability of responses to repeated exposures to identical stimuli. After imaging, mice were sacrificed and cortex was flatmounted, sectioned, and imaged with epifluorescence microscopy to determine the patch-interpatch pattern of recorded regions. We compared noise correlations in pairs containing two patch cells, two interpatch cells, and one cell of each type. We found that in response to full-field stimuli, interpatch-interpatch pairs showed the greatest degree of synchrony, while small-field stimuli (30 degree diameter) elicited the greatest correlations between patch-patch pairs. Correlations were reduced as distance between cell pairs increased, however, significant differences between patch-patch and interpatch-interpatch pairs were found independent of cell distances. These results suggest that patch cells coordinate their activity to process small stimuli and fine details, in agreement with their higher average spatial frequency preferences [Ji et al. 2015]. Interpatch cells coordinate to process wide-field stimuli, possibly making use of their long-range lateral connections to detect optic flow or looming stimuli.

Disclosures: **A.M. Meier:** None. **E.B. Han:** None. **A. Burkhalter:** None.

Poster

141. Visual Cortex: Functional Architecture and Circuits I

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Brain/MINDS-AMED
JSPS KAKENHI (25221001, 25117004 and 19H01006)

Takeda Science Foundation
01GQ1413 (BMBF)

Title: Dynamic functional changes of neurons in visual cortex after eye opening

Authors: *F. KISHINO[1], M. UEMURA[1], S. TRÄGENAP[2,3], M. KASCHUBE[2], K. OHKI[1,4]; [1]Dept. of Physiol., Grad. Sch. of Med., Univ. of Tokyo, Tokyo, Japan; [2]Frankfurt Inst. for Advanced Studies, Frankfurt, Germany; [3]The Intl. Max Planck Res. Sch. for Neural Circuits, Frankfurt, Germany; [4]Intl. Res. Ctr. for Neurointelligence, Univ. of Tokyo, Tokyo, Japan

Abstract: Neuronal function matures during the early stage of life. In mouse primary visual cortex (V1), the distribution of preferred orientation changes after eye-opening. At eye-opening, neurons responsive to cardinal (i.e. vertical and horizontal) orientations outnumber those responsive to oblique ones, and this cardinal bias becomes small in adults. Currently, it is unclear whether this decrease in cardinal bias is due to a shift in the distribution of responsive cells, or due to a change of tuning in individual cells. In order to address this issue, we first conducted acute two-photon calcium imaging at multiple time points and found that the cardinal bias decreases mainly during the first week of eye-opening (P14-21). Next, to examine how this functional change occurs in this period, we pursued the orientation tuning of identical neurons in V1 during the first week of eye-opening every other day using chronic two-photon calcium imaging. We found three types of changes contributing to the decrease in cardinal bias: 1) neurons shifting their preferred orientation from cardinal toward oblique, 2) neurons not responsive to grating stimuli at eye-opening acquiring response to oblique orientations, and 3) neurons responsive to cardinal orientations at eye-opening becoming unresponsive. Also, some neurons showed changes that do not decrease the cardinal bias and some remained stably selective to the same orientation across all days. We conclude that individual neurons show changes in both their response amplitude and their selectivity during the first week of eye-opening and that part of these changes contribute to a decrease in cardinal bias.

Disclosures: F. Kishino: None. **M. Uemura:** None. **S. Trägenap:** None. **M. Kaschube:** None. **K. Ohki:** None.

Poster

141. Visual Cortex: Functional Architecture and Circuits I

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Program #/Poster #: 141.10/K34

Topic: D.07. Vision

Support: NSERC Grant RGPIN-2015-06215

Title: Changes in GABAergic and glutamatergic proteins in the young-old and old-old monkey visual cortex

Authors: D. AHUJA, E. JEYANESAN, K. ARBABI, *K. M. MURPHY;
McMaster Univ., Hamilton, ON, Canada

Abstract: The discovery that application of GABA sharpened orientation tuning in the aging monkey visual cortex highlighted inhibitory mechanisms as key players regulating functional degradation during aging. In the aging human visual cortex, pre- and post-synaptic GABAergic protein do not merely decrease. Instead, an intricate pattern of changes is observed (Pinto et al. 2010). Only GAD65 and Gephyrin showed age-related declines in human V1, but other proteins such as NR2A and myelin basic protein also decline (Siu et al. 2015; 2017). In monkey V1, GAD65 protein declines in animals aged 20-25 years (Liao et al. 2016) which corresponds to the "young-old" category used to describe human aging, but there is little information about age-related changes for other GABAergic or glutamatergic proteins. Furthermore, recent studies are finding that visual and neural declines continue from young-old to old-old humans. Here we asked how the expression of a large collection of GABAergic and glutamatergic proteins change in the aging monkey visual cortex by examining tissue from both young-old and old-old monkeys.We quantified the expression of 15 GABAergic and glutamatergic proteins in V1 of macaque monkeys that were young adults (<20 years, n=2), young-old adults (20-25 years, n=3) or old-old adults (>25 years, n=3). Synaptoneurosomes samples and immunoblotting was used to quantify the expression of GAD65, GAD67, Gephyrin, GABAaa1, GABAaa2, GABAaa3 VGAT, CB1, VGLUT1, PSD-95, NR1, NR2A, NR2B, GluA2, Ube3A. Following incubation, in primary and secondary antibodies, the bands were quantified using densitometry. Each sample was run 4 times for a total of 480 observations. These data are being analyzed using a high-dimensional analysis pipeline to determine if there are age-related clusters and to construct phenotypes that describe the changes in young-old and old-old monkey visual cortex. PCA is used for dimension reduction, robust sparse k-means for clustering, and our new process for identifying high-dimensional features that are combined to construct the aging phenotypes. PCA showed that all of the proteins except NR2B contribute to age-related changes and Dim1 was dominated by GAD65, GABAaa3, GABAaa1, VGAT, PSD-95, NR1, CB1, GAD67; Dim2 by Gephyrin, GluA2, NR1, NR2A; and Dim3 by VGlut1, GABAaa2. These results show that the visual cortex continues to change from young-old to old-old monkeys in the expression of GABAergic and glutamatergic proteins and those changes will contribute to age-related declines in neural functions and visual processing.

Disclosures: D. Ahuja: None. **E. Jeyanesan:** None. **K. Arbabi:** None. **K.M. Murphy:** None.

Poster

141. Visual Cortex: Functional Architecture and Circuits I

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Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 141.11/K35

Topic: D.07. Vision

Support: NSERC Grant RGPIN-2014-06503

Title: Positive impact of an enriched environment during development of the mouse visual system

Authors: *O. BIBOLLET-BAHENA[1], S. HO-TRAN[1], A. ROJEWSKI[1], S. BELANGER[2], C. F. CASANOVA[1];
[1]Univ. Montreal, Montreal, QC, Canada; [2]Labeo Technologies, Montreal, QC, Canada

Abstract: Environmental factors such as climate, food availability, predator presence and social interactions influence the development and behavior of living creatures. Studies have demonstrated that exposure to an enriched environment (EE) promotes neuronal plasticity and function recovery. In this study, we examined the impact of housing environmental conditions during the development of the visual system. Control mice and mice that were exposed to an EE from birth were compared during adulthood (> P60). The EE consisted in group housing in larger cages containing several toys, hiding places, nesting material and a spinning wheel that were moved or replaced at regular intervals. Control mice were individually housed in standard cages (with only nesting material), following weaning. Network connectivity, retinotopic maps, responses to contrast, to spatial frequencies and temporal frequencies were assessed by acquiring functional visual maps by brain optical imaging of intrinsic signals. Retinotopic maps, and responses to contrast, spatial and temporal frequencies allowed delineating the primary visual cortex and the extrastriate areas. Our retinotopic maps show that visual cortical areas are larger in mice reared in an EE compared to control animals ($p<0.05$). There were no significant differences between females and males within each group. In addition, neurons in mice raised in EE show optimal responses to higher spatial frequencies. We are presently evaluating the implications of these differences on network connectivity of the visual system. The latter is being compared between the two mouse populations by seed-based correlation and clustering analyses. In order to establish when in development the differences start appearing, we are acquiring cortical maps, evaluating optokinetic reflexes and performing immunohistochemistry on mice weekly from P17 to adulthood. In conclusion, this study indicates that enriching the environment during development favorably affects the structure-function of the visual cortex. Supp: NSERC to CC.

Disclosures: O. Bibollet-Bahena: None. **S. Ho-Tran:** None. **A. Rojewski:** None. **S. Belanger:** None. **C.F. Casanova:** None.

Poster

141. Visual Cortex: Functional Architecture and Circuits I

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Program #/Poster #: 141.12/K36

Topic: D.07. Vision

Support: NIH Grant P51 OD011092
NIH Grant EY029753-01

Title: Quantitative analysis of ocular dominance columns in foveal and non-foveal eccentricities in macaque monkeys

Authors: A. EDATHODATHIL, *R. M. FRIEDMAN, A. W. ROE;
Div. of Neurosci., Oregon Hlth. & Sci. Univ. - ONPRC, Beaverton, OR

Abstract: In primates, ocular dominance columns (ODCs) appear as alternating left and right eye pattern of stripes that typically lie perpendicular to the border between V1 (primary) and V2 (second) visual cortical areas. We have been investigating the functional architecture of foveal visual cortex, which receives a high density of cone photoreceptors inputs from the retina. Previous studies have reported that, compared with non-foveal locations, the widths of foveal ODCs are larger and foveal patterns appear more complex (disordered) (LeVay et al. 1985, Rosa et al. 1988, Horton and Hocking 1996). We have now examined this quantitatively and with a larger database. We applied a semi-automated method to measure ODC width and complexity to a large set of ODC intrinsic optical imaging maps (n=56 macaque monkey; rhesus 33, cynomologous 23). Imaged visual cortical locations were divided into 4 regions based on eccentricity (roughly foveal 1° and 1-2°, and non-foveal 2-5° and 5-10°). ODC widths were determined by measuring widths of the columns at every point along ODC centerlines. ODC complexity was determined by measuring the angles between the ODC border tangents (at each point along left/right eye borders of ODC columns) and the V1/V2 border; the larger the distribution of angles, the greater the complexity. We found significant range of ODC widths across the population (means ranging from ~300 µm to 500 µm). Contrary to some reports, OD widths were narrower in foveal (~350 µm) compared to non-foveal (~400 µm) locations. Consistent with previous observations, ODC complexity was greater in the central foveal than non-foveal eccentricities. These methods applied to previously published ODC maps for comparison revealed similar results. We found no difference in the population with respect to species, age, sex and weight; except ODC complexity was greater for cynomolgus than rhesus monkeys. Our results suggest that there may indeed be differences in foveal ODC organization that reflect specialized processing of foveal inputs in macaque monkeys. These normative measures will be useful for evaluating other cortical organizations, as well as for evaluating the

establishment of cortical organizations during development and in animals with abnormal visual experience.

Disclosures: **A. Edathodathil:** None. **R.M. Friedman:** None. **A.W. Roe:** None.

Poster

141. Visual Cortex: Functional Architecture and Circuits I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 141.13/K37

Topic: D.07. Vision

Support: NSF Grant CRCNS 1822650

Title: Neural encoding manifolds relate (sparse) responses in mouse visual system to circuit motifs

Authors: ***L. DYBALLA**[1], M. S. HOSEINI[3], G. D. FIELD[4], M. P. STRYKER[3], S. W. ZUCKER[1,2];
[1]Dept. of Computer Sci., [2]Dept. of Biomed. Engin., Yale Univ., New Haven, CT; [3]Ctr. for Integrative Neurosci., Univ. of California, San Francisco, San Francisco, CA; [4]Dept. of Neurobio., Duke Univ., Durham, NC

Abstract: Inferring circuit structure and dynamics at the cellular level in the mouse visual system is confronted with two major challenges. The first challenge is the diversity of stimuli needed to exercise the circuits at a natural activity level. Spatial frequency gratings are too impoverished a stimulus set, but unconstrained 'natural' images defy analysis. We have begun to meet this challenge through the use of a stimulus ensemble that includes flow patterns, a class of naturalistic visual stimuli that span spatial frequency, contrast, orientation and directionality, as well as conventional grating stimuli (Dyballa et al, PNAS, 2018). A greater challenge is the sparsity of data, especially when recorded simultaneously from multi-site electrodes, because many different circuits can 'explain' the existing data. We propose to meet this challenge by using machine learning techniques to infer a manifold of neurons that is informative of the general type of circuit as well as the function of different neurons. It differs from the standard approach in which trials are embedded in 'neural coordinates.' Our manifold, by contrast, embeds neurons in functional coordinates derived from the stimuli. It is defined so that those neurons that are close to one another on the manifold respond similarly in both activity and time to similar features within the stimulus ensemble. A key advantage of the manifold approach is that it constrains the underlying class of circuit models rather than the details. For example, a densely interconnected group of neurons yields one type of manifold, for a range of sizes and densities. Other classes include neurons sampled from disconnected circuits, or those organized into dense sub-circuits with connections between them. This last example yields a smooth, but non-linear

manifold, whose coordinates span relevant stimulus dimensions, and suggests a second advantage of our approach: nearby neurons on the manifold can be mapped back to anatomy, thereby completing the loop to actual circuit possibilities. Preliminary experiments with mouse retina and visual cortex data reveal examples of these different manifold structures and provide a foundation for understanding the computational transition between retina and cortex in awake, behaving mice.

Disclosures: L. Dyballa: None. **M.S. Hoseini:** None. **G.D. Field:** None. **M.P. Stryker:** None. **S.W. Zucker:** None.

Poster

141. Visual Cortex: Functional Architecture and Circuits I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 141.14/K38

Topic: D.07. Vision

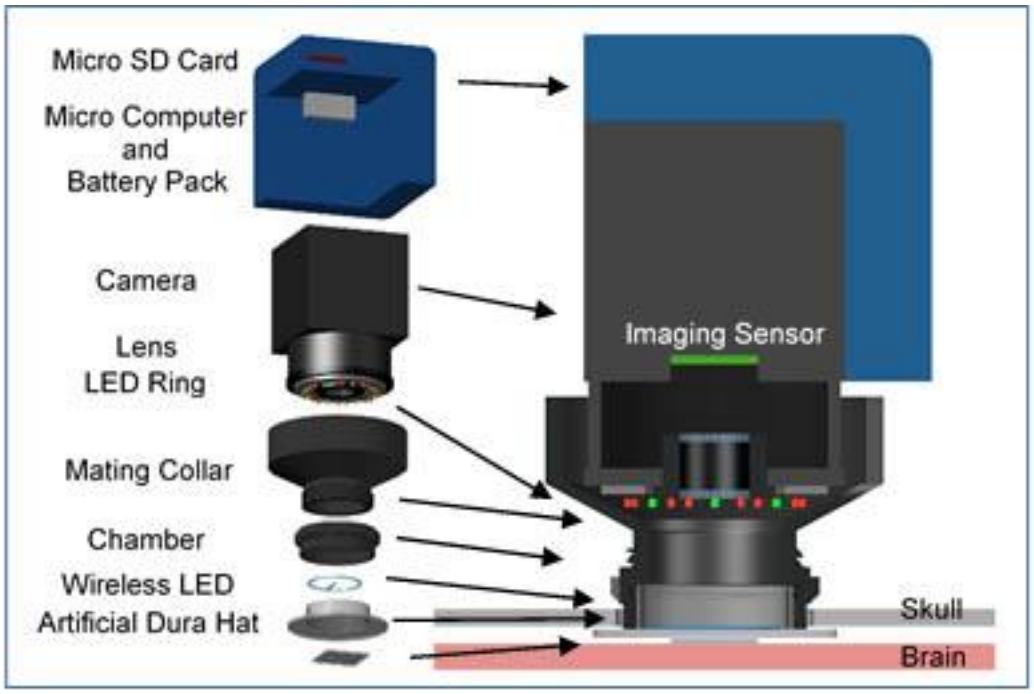
Support: NIH: 5R01NS093998-03
ONPRC: PPQ 1003383-021 YR 59 Pilot
NIH: P51 OD011092

Title: Development of multimodal wireless brain interfaces in nonhuman primates

Authors: *M. M. CHERNOV[1], D. ZARAZA[1], R. M. FRIEDMAN[1], Y. YANG[3], J. A. ROGERS[4], A. W. ROE[2];
[1]Neurosci., [2]Oregon Hlth. and Sci. University- ONPRC, Beaverton, OR; [3]Mechanical Engin., [4]Materials Sci. and Engin., Northwestern Univ., Evanston, IL

Abstract: Our goal is to enable study of neural brain circuits during visual behavior in more naturalistic and social contexts. To study the role of functional domains in visual perception and attention, we have previously developed combined optical, electrical, and optogenetic approaches conducted through optical windows over areas V1, V2, and V4 in the nonhuman primates (e.g. Ruiz et al J Neurophys 2013, Tanigawa et al Front Neural Circ 2016, Chen et al PNAS 2017, Chernov et al PNAS 2018). These studies were performed in either anesthetized or head-fixed subjects using optical imaging to identify functional domains and electrical, near infrared, or optogenetic stimulation to modulate them. In this project, we develop a lightweight camera system combined with wireless optogenetic stimulator (Neurolux) that eliminates the need for head-fixing the animals and opens up an opportunity to study and modulate functional domains while the subjects are performing behavioral tasks while sitting in a chair and, ultimately, in a home cage. Our system uses a lightweight 12-bit camera that is able to collect HD-resolution images at 30 frames per second, with interchangeable lens and integrated green/red LED illumination with digitally controlled adjustment. We show that imaging of

functional domains in the primate visual cortex with our system produces imaging of comparable quality to a benchtop system, while relieving the need for head fixation and significantly reducing motion artifacts. In addition, we show the ability to optically image changes in cortical activity following wireless optogenetic stimulation using an implantable RF-powered micro-LED device. In the future, we plan to expand our work to enable stimulation of multiple sites and the use of multiple wavelengths, allowing for a versatile platform for studying the cortical basis of primate behavior.



Disclosures: **M.M. Chernov:** None. **D. Zaraza:** None. **R.M. Friedman:** None. **Y. Yang:** None. **J.A. Rogers:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurolux. **A.W. Roe:** None.

Poster

141. Visual Cortex: Functional Architecture and Circuits I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 141.15/K39

Topic: D.07. Vision

Support: NIH Grant R01EY011488

Title: Functional synaptic organization of ocular dominance within the dendritic field of layer 2/3 neurons in ferret visual cortex

Authors: *C. TEPOHL, B. SCHOLL, D. FITZPATRICK;
Max Planck Florida Inst., Jupiter, FL

Abstract: Neurons in primary visual cortex (V1) exhibit binocular responses that differ in ocular dominance – the relative strength of the inputs from the two eyes. How the ocular dominance properties of individual neurons are generated from the pattern of excitatory inputs that synapse within their dendritic field remains unknown. To address this question, we used *in vivo* calcium imaging to examine the functional properties of dendritic spines and the soma of individual layer 2/3 neurons of ferret V1. To characterize synaptic inputs, we assessed dendritic spine responses to drifting gratings presented independently to each eye. We extracted the orientation preference, direction preference, and ocular dominance of individual dendritic spines for comparison with the properties of the somatic response.
Each neuron received synaptic inputs that exhibited a broad range of ocular dominance values and there was no obvious relationship to the ocular dominance of the soma. This led us to explore whether the spatial arrangement of synaptic inputs within the dendritic field could be a factor that contributes to somatic ocular dominance. We found no differences in the distribution of ocular dominance values between apical and basal dendrites. However, by comparing the functional distribution of synaptic inputs on individual dendritic branches to a shuffled distribution of all inputs, we found that branches could exhibit non-random organization. On several branches, synaptic inputs were more similar or dissimilar to each other in terms of ocular dominance and orientation preference than would be expected by chance, consistent with a branch-level organization of these functional properties. In addition, we observed a non-random fine-scale spatial organization of ocular dominance within individual dendritic branches. Shuffling the spatial position of synaptic inputs on the same dendritic segment revealed above chance level local clustering of ocular inputs for both the dominant and non-dominant eyes. Taken together, these results suggest that multi-scale spatial arrangements of synaptic inputs within the dendritic field may contribute to the functional integration of eye-specific synaptic inputs.

Disclosures: C. Tepohl: None. **B. Scholl:** None. **D. Fitzpatrick:** None.

Poster

141. Visual Cortex: Functional Architecture and Circuits I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 141.16/K40

Topic: D.07. Vision

Support: Allen Institute For Brain Science

Title: Activation of neuromodulatory axon projections in mouse visual cortex during periods of locomotion and pupil dilation

Authors: R. S. LARSEN, ***E. E. TURSCHAK**, T. L. DAIGLE, D. R. OLLERENSHAW, H. ZENG, J. ZHUANG, J. WATERS;
Allen Inst. For Brain Sci., Seattle, WA

Abstract: Cortical neuron network activity is altered during periods of arousal, locomotion, exploration, and attention. These brain states are also accompanied by physiological changes such as pupil dilation and motor movements, including running. The mouse neocortex receives axonal projections from cholinergic, noradrenergic, and serotonergic neurons, and these projections are thought to drive the changes in cortical activity that accompany a shift in behavioral state. We therefore sought to examine the roles of neuromodulators during periods of arousal, locomotion, and exploration by imaging the activity of neuromodulatory axons in the mouse primary visual cortex. To accomplish this, we expressed GCaMP6s in cortical-projecting axons of ChAT-Cre, Dβh-Cre, and Slc6a4-Cre mice and performed *in vivo* 2-photon microscopy. We examined fluorescence changes during periods of locomotion and pupillary changes. We observed that the fluorescence of both cholinergic and noradrenergic axons increased during periods of locomotion and pupil dilation, with the fluorescence of the axons rising <1s before pupil dilation. Locomotor activity was accompanied by pupil dilation and was preceded by a rise in axonal fluorescence with timing and amplitude that matched the subsequent pupil dilation. However, axon fluorescence was more sustained than expected from the pupil dilation, suggesting that there are additional factors that affect cholinergic and noradrenergic axon activity in primary visual cortex during locomotion. While additional studies are needed to establish a causal relationship, our results provide evidence that activities of the axons of multiple neuromodulatory systems are correlated with physiological state changes.

Disclosures: R.S. Larsen: None. **E.E. Turschak:** None. **T.L. Daigle:** None. **D.R. Ollerenshaw:** None. **H. Zeng:** None. **J. Zhuang:** None. **J. Waters:** None.

Poster

141. Visual Cortex: Functional Architecture and Circuits I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 141.17/L1

Topic: D.07. Vision

Support: 1R01EY027402-02
5T32 EY007135-23
Whitehall Foundation
Knights Templar Eye Foundation
Alfred P. Sloan Foundation

Title: Spatiotemporal evolution of V1 laminar activation to dioptic and dichoptic stimulation

Authors: *B. M. CARLSON, K. DOUGHERTY, M. A. COX, A. MAIER;
Psychology, Vanderbilt Univ., Nashville, TN

Abstract: Each of our two eyes provides a separate signal to our brain. These signals combine to create the singular perspective that characterizes visual perception. This binocular combination is achieved by fusing congruent views across both eyes and by resolving any interocular differences. Where and how this binocular combination and resolution occurs is not entirely clear. The first major meeting point of the two eyes' signals in the primate primary visual pathway is granular layer 4C of primary visual cortex (V1). To gain a better understanding of how binocular signals are combined once they meet in visual cortex, we recorded neuronal responses across all layers of V1 of three fixating macaques using a linear multielectrode array. Previous work has shown that the synaptic inputs to V1 layer 4C can be estimated via current source density (CSD), a quantitative measurement of localized net depolarization. We computed CSD from our recordings. Then, we examined the temporal dynamics of CSD within V1 layer 4C as well as their propagation across neighboring layers in response to monocular, binocular congruent (dioptic), and binocular incongruent (dichoptic) stimulation using static grating stimuli. Both dioptic and dichoptic stimuli evoked a larger synaptic response than monocular stimulation. We found that binocular incongruent stimuli evoked a significantly different CSD response, including in the initial volley of layer 4C activation, compared to binocular stimuli that match between the eyes. We also tried adapting one eye for several hundred milliseconds before adding a second stimulus to the other eye, as this type of stimulation has been shown to aid the visual system in resolving conflict between the eyes. Monocular adaptation resulted in an almost two-fold reduction of layer 4C CSD, independent of whether the resulting binocular stimulation was congruent between the eyes or not. However, following this initial transient, layer 4C CSD responses significantly discriminated between congruent and conflicting binocular stimulation. These results suggest that the initial volley of V1 synaptic activity is significantly affected by the congruency of binocular stimulation, suggesting that the resolution of interocular conflict involves the earliest stages of cortical visual processing.

Disclosures: B.M. Carlson: None. **K. Dougherty:** None. **M.A. Cox:** None. **A. Maier:** None.

Poster

141. Visual Cortex: Functional Architecture and Circuits I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 141.18/L2

Topic: D.07. Vision

Support: Paul G. Allen Family Foundation
NSFC 31871055
Guangdong Science and Technology Department Program 2017B030314026

Guangdong Science and Technology Department Program 2018B030334001

Title: Cell subtype specific modulation of cortical synchrony by arousal states

Authors: *L. LI[1], L. HUANG[2];
[1]Sun Yat-sen Univ., Guangzhou, China; [2]Allen Inst. for Brain Sci., Seattle, WA

Abstract: Arousal states such as locomotion profoundly impact both spontaneous oscillation pattern of the membrane potential (Vm) as well as the sensory evoked responsiveness of cortical neurons. Synchronized or coordinated neuronal activities underlie cognitive, emotional and social behaviors, yet how arousal states modulate the synchrony between individual neurons remains less understood. Employing in vivo two-photon Calcium (Ca) imaging, we investigated this issue by examining the pair-wise cross-correlation distributions between layer (L) 2/3 neurons in primary visual cortex (V1) using transgenic mouse lines in which Ca indicators GCaMP6f and GCaMP6s are cell-type specifically expressed in genetically defined neuron populations (i.e. the within-type correlation). We found that locomotion modulated the within-type correlation profiles of V1 neurons in a cell-type dependent manner. More interestingly, Somatostatin-expressing inhibitory interneurons exhibited two physiologically distinct subtypes, whose synchrony was modulated differently by locomotion. It has been reported that visual stimulation in general had a desynchronizing effect, our data further suggested the desynchronization by sensory input might also depend on neuron types or subtypes.

Disclosures: L. Li: None. **L. Huang:** None.

Poster

141. Visual Cortex: Functional Architecture and Circuits I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 141.19/L3

Topic: D.07. Vision

Support: Max Planck Society, and the University of Tuebingen

Title: Central versus peripheral visual location prevalence as diagnostic for feedforward and feedback mechanisms across visual cortical hierarchy for visual recognition

Authors: *L. ZHAOPING[1,2];
[1]Max Planck Inst. for Biol. Cybernetics, Tuebingen, Germany; [2]Ctr. for Integrative Neurosci., Univ. of Tuebingen, Tuebingen, Germany

Abstract: Some phenomena, e.g., visual crowding (the reduced capability to recognize an object in visual clutter), are stronger in the peripheral visual field. Given the attentional bottleneck, which massively reduces visual input information flow starting from the primary visual cortex

(V1) to higher visual cortical areas (Zhaoping, Current Opinion in Neurobiology, 2019), I recently proposed (Zhaoping, Vision Research, 2017) that the top-down feedback from higher to lower visual cortical areas for recognition is weaker or absent in the peripheral visual field, since attended objects are typically brought to the central visual field. This feedback facilitates analysis-by-synthesis-based object recognition, it involves assessing the agreement between the brain's internal model of the visual inputs for a visual object and the actual visual input in lower visual areas such as V1, where the relevant sensory input has yet to be filtered out by the attentional bottleneck, so that a good or poor agreement makes the object more or less likely perceived. This feedback analysis is particularly helpful for recognition when visual inputs are noisy or uncertain.

This proposal successfully predicted (Zhaoping and Ackermann, Perception, 2018) that, unlike vision in the central visual field, vision in the peripheral visual field can perceive reversed depth in a binocularly anticorrelated random-dot stereogram (aRDS), in which a bright dot in one eye corresponds to a dark dot in the other eye and vice versa, and which excites V1 neurons tuned to the binocular spatial disparity opposite to the disparity between the corresponding dots (Cumming and Parker, Nature, 1997).

I propose that the prevalent visual field location, i.e., central or peripheral, for any visual phenomenon is diagnostic for the feedforward or feedback neural mechanisms responsible. Accordingly, the reduction of feedforward visual information from V1 causes crowding unless the feedback analysis compensates sufficiently; the reversed depth in an aRDS is invisible in the central visual field since the feedback vetoes the feedforward sensory inputs which violate the brain's internal model that the inputs should be binocularly correlated. Further, in visual backward masking, perception of a briefly presented target is disrupted by a mask presented shortly after, but not before, the target. If this is caused by a conflict between the top-down feedback for the target and the feedforward input from the mask, then I predict that the masking effect is weaker in peripheral visual field. We discuss the central-peripheral diagnostic in relation to other visual phenomena, including visual illusions.

Disclosures: L. Zhaoping: None.

Poster

141. Visual Cortex: Functional Architecture and Circuits I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 141.20/L4

Topic: D.07. Vision

Support: NIH U01NS094368-03

Title: Heterogeneous responses in V1 laminar circuits after focal suppression of lateral network

Authors: *A. R. ANDREI, S. R. DEBES, I. M. CHELARU, R. JANZ, V. DRAGOI;
McGovern Med. School, Univ. of Texas, Houston, TX

Abstract: The cortex has a distinct architecture, with neurons stacked in columns and connected horizontally in certain layers. Horizontal connections have been implicated in a wide variety of neural response properties (i.e., contrast gain control, surround suppression) and are hypothesized to be altered in disease states (i.e., schizophrenia). However, the nature of this modulation remains poorly understood, primarily due to the technical difficulty of isolating the contribution of horizontal projections from other inputs. Here we employed a novel approach using optogenetics in non-human primates. We take advantage of the limited spatial spread of blue light within the relatively large NHP brain. Here we asked whether inactivating a portion of the horizontal network would have uniform or heterogeneous effects on neural populations within a cortical column. We suppressed the activity of glutamatergic neurons in upper layers using GtACR2, a chloride-conducting channelrhodopsin, and recorded the activity of neighboring, laminar neural populations (n=236 cells, 2 monkeys, 24-ch Plexon U-probes located ~300 μm away from light source) while NHPs performed a contrast detection task. In the absence of a visual stimulus, the optogenetic suppression had no effect on the firing rate of the adjacent neurons. However, in the presence of a visual stimulus, we observed 4 distinct, contrast-dependent, firing rate patterns within the laminar population. The 4 response motifs were present in distinct proportions across layers, and found in stable proportions across recording sessions and monkeys. These response patterns are well captured by the standard normalization model. However, our results demonstrate that the normalizing drive provided by the horizontal network differentially affects individual neurons within the same column. Using a computational model, we demonstrate that these 4 motifs can be attributed to variations in stimulus sensitivity of the horizontal network input. Surprisingly, our most common response motifs are described by either very weakly sensitive horizontal input (most common, n=91) or very strongly sensitive horizontal input (2nd most common, n=61). To summarize, our study provides the first causal evidence that the horizontal network provides a source of heterogeneous, normalizing drive to neurons within a column. Further, we propose that the 4 response motifs are indicative of distinct classes of connection patterns between neurons in nearby columns. These distinct connection motifs provide a source for the previously unexplained heterogeneity of stimulus responses found across V1 neurons.

Disclosures: A.R. Andrei: None. **S.R. Debes:** None. **I.M. Chelaru:** None. **R. Janz:** None. **V. Dragoi:** None.

Poster

141. Visual Cortex: Functional Architecture and Circuits I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 141.21/L5

Topic: D.07. Vision

Support: NIH EY002682
NIH EY11744
P30-EY008126

Title: Connections of functionally distinct regions within and adjacent to visual area V3 in a prosimian primate

Authors: *M. K. BALDWIN[1], R. FAN[2], A. W. ROE[3], J. H. KAAS[4];
[1]Psychology, Univ. of California At Davis, Davis, CA; [2]Stanley Manne Res. Inst., Chicago, IL; [3]Zhejiang Univ., Zhejiang, China; [4]Psychology Dept., Vanderbilt Univ., Nashville, TN

Abstract: Several schemes have been proposed for the organization of cortical fields rostral to dorsal V2 in primates. In a previous study in which dorsal V3 in prosimian galagos was mapped using intrinsic signal optical imaging (Fan et al., 2010), an unresponsive zone or "gap" was often present within the territory of dorsal V3. This unresponsive zone failed to respond to the same stimuli that consistently elicited responses medially and laterally in V3 regardless of whether the stimuli were presented in the upper or lower visual quadrant. In the current study we further examined this unresponsive cortical region by placing anatomical tracer injections guided by optical imaging into cortex rostral to V2, both within responsive dorsal V3 as well as in the unresponsive gap. Thalamocortical and corticocortical connections were then analyzed. Our results indicate that the unresponsive gap within V3 has different visual connections than adjoining parts of dorsal V3. While injections into V3 result in strong connections with the lateral pulvinar, injections into the unresponsive zone result in strong connections with an architectonically distinct division of the pulvinar located at its most lateral extent. This division is similar in appearance and architectonic staining patterns to the pulvinar shell division described in anthropoid primates. Cortical connections of V3 and the unresponsive zone were also different. In contrast to responsive dorsal V3, injections involving the unresponsive zone included connections with the upper field visual representation of other visual areas. Together, our results provide evidence that dorsal V3 is divided by part of another visual area that represents the upper visual quadrant and has visual response properties that are distinct from V3.

Disclosures: M.K. Baldwin: None. **R. Fan:** None. **A.W. Roe:** None. **J.H. Kaas:** None.

Poster

142. Visual Processing Beyond V1

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 142.01/L6

Topic: D.07. Vision

Title: Distributed and retinotopically asymmetric processing of coherent motion in mouse visual cortex

Authors: *K. SIT[1], M. J. GOARD[1,2,3];
[1]Psychological and Brain Sci., [2]Molecular, Cellular, and Developmental Biol., [3]Neurosci. Res. Inst., Univ. of California: Santa Barbara, Santa Barbara, CA

Abstract: Perception of visual motion is important for animal behaviors such as visually guided navigation, pursuit of prey, and avoidance of threats. Cortical contributions to global motion processing have been extensively studied in primate visual cortex, where single unit recordings have revealed that specific higher visual areas (HVAs) are specialized for processing global coherent motion. Supplementing prior research, recently developed transgenic mice with pan-neuronal calcium indicator expression allow for comprehensive and unbiased measurement of motion processing at multiple novel spatial scales. Here, we used widefield and 2-photon calcium imaging to probe the mesoscale organization of coherent motion processing in mouse visual cortex. Widefield fluorescence imaging revealed differences across HVAs in response to coherent motion, providing support for HVA modularity in mouse visual cortex, as in primates. Interestingly, we also discovered a prominent spatial distribution of coherent motion processing that was tightly coupled to retinotopy, with neurons representing the inferior visual field exhibiting increased sensitivity to the coherent motion present in visual stimuli. Although the retinotopic asymmetry was independently present across multiple visual cortical regions, the effect was particularly strong in primary visual cortex (V1). The underlying distribution of motion responsiveness persisted over a broad range of visual stimuli, including a set of natural movies and artificial random dot kinematograms. Two-photon calcium imaging confirmed that coherent motion responsiveness was related to vertical retinotopy preference in individual neurons. These results suggest that, beyond differential processing by specialized HVAs, there are additional organizational principles underlying distributed processing of coherent motion in the mammalian visual cortex.

Disclosures: K. Sit: None. **M.J. Goard:** None.

Poster

142. Visual Processing Beyond V1

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 142.02/L7

Topic: D.07. Vision

Support: T32NS099578

Title: Characterizing the spatiotemporal tuning properties of mouse visual cortex

Authors: *N. MESA[1], E. E. TURSCHAK[2], J. ZHUANG[3], S. E. DEVRIES[2], J. WATERS[2]; [1]Univ. of Washington, Seattle, WA; [2]Allen Inst. For Brain Sci., Seattle, WA; [3]Neural Coding Dept., Allen Inst. for Brain Sci., Seattle, WA

Abstract: Mice can perform complex visual behaviors, including prey capture and association between visual cues and reward. Mice have roughly ten visual areas, including various higher visual areas, which may be specialized to perform these behaviors. Various studies have found evidence of functional specialization of different mouse visual areas. and neurons in different visual areas have preferences for different temporal frequencies (TFs), spatial frequencies (SFs), stimulus orientation, and direction. In primates, these properties change systematically across visual cortex. In mice, however, little is known about whether or how these properties change differ across individual visual areas. We used a combination of widefield calcium imaging and 2-photon imaging to map the temporal frequency and spatial frequency tuning preferences of neurons across visual cortex in mice that transgenically express GCaMP6f. We used a variety of Cre reporter lines to investigate how SF and TF change across layers as well as across cortex. We found that there are gradients of temporal frequency tuning properties across mouse visual cortex, meaning that certain parts of the visual field have higher TF tuning than others. We also found that the relationship between temporal frequency and retinotopic location is not consistent across visual areas.

Disclosures: N. Mesa: None. **E.E. Turschak:** None. **J. Waters:** None. **J. Zhuang:** None. **S.E. DeVries:** None.

Poster

142. Visual Processing Beyond V1

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 142.03/L8

Topic: D.07. Vision

Support: NIH Grant R01 EY022090
NIH Grant R01 EY027383
McDonnell Center for Systems Neuroscience

Title: Canonical and non-canonical features of the mouse visual cortical hierarchy

Authors: *R. D'SOUZA[1], Q. WANG[2], A. MEIER[1], W. JI[1], H. KENNEDY[3], K. KNOBLAUCH[3], A. H. BURKHALTER[1];
[1]Neurosci., Washington Univ. Sch. of Med., St. Louis, MO; [2]Allen Inst. for Brain Sci., Seattle, WA; [3]Stem Cell and Brain Res. Inst., Univ. Lyon, Univ. Claude Bernard Lyon 1, Inserm, Bron, France

Abstract: Visual perception relies on generative intracortical mechanisms that shape sensory representation depending on contextual cues. While the mouse has emerged as a popular animal model to investigate how visual signals are contextually influenced, the cortical network in mouse is much more densely interconnected than in primate, with almost all possible connections between visual areas shown to exist. It is unclear, therefore, whether such an ultra-dense system is organized as a sequential ranking of areas, as suggested by hierarchical models of cognition, or whether it involves interareal pathways that do not conform to conventional feedforward and feedback relationships. To identify the presence of an areal hierarchy, we examined the laminar patterns of axonal projections between ten previously identified mouse visual areas. The anterograde tracer biotinylated dextran amide (BDA) was injected into each area in adult C57BL/6 mice of both sexes, and termination patterns were analyzed in the nine target visual areas in serial coronal sections for each injection. BDA labelled projections from primary visual cortex (V1) terminated most densely in layers 2 to 4 (L2-4) in extrastriate areas, with relatively weaker terminations in L1. Conversely, projections from each extrastriate area to V1 strongly targeted L1 with sparser terminations in L2-4. We therefore measured the density ratio (DR), defined as the ratio of the optical density of BDA labelled axons in L2-4 to that in L1, for each pathway with the reasoning that the DR would provide a quantitative metric for identifying hierarchical relationships between areas. A beta regression analysis was used to determine optimal hierarchical level values for each area such that DRs for any areal pair predicted the hierarchical distance between them. The analyses showed that the network is organized as three non-overlapping levels. V1 and the lateromedial area (LM) form the first two processing stages, whereas the remaining areas form an interconnected network that often violate feedforward/feedback relationships, but with the anteromedial (AM) and postrhinal (POR) areas at the top of the hierarchy. Single unit recordings from each area showed that receptive field sizes typically increase with higher hierarchical positioning, but is dependent on the processing stream to which the area belongs. Altogether, the analyses indicate that unlike early visual areas, association cortical areas exhibit features that are distinct from sensory-motor hierarchies, and suggest that top-down influence on early visual areas is more strongly weighted towards control by hierarchically higher areas in mouse than in primate.

Disclosures: **R. D'Souza:** None. **Q. Wang:** None. **A. Meier:** None. **W. Ji:** None. **H. Kennedy:** None. **K. Knoblauch:** None. **A.H. Burkhalter:** None.

Poster

142. Visual Processing Beyond V1

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 142.04/L9

Topic: D.07. Vision

Support: Max Planck Society

Boehringer Ingelheim Fonds

Title: Area-specific mapping of binocular disparity across mouse visual cortex

Authors: A. LA CHIOMA, T. BONHOEFFER, ***M. HUBENER**;
Max Planck Inst. of Neurobio., Martinsried, Germany

Abstract: Binocular disparity, the difference between left and right eye images, is a powerful cue for depth perception. In primates and cats, individual neurons sensitive to binocular disparities are found in almost all regions of the visual cortex, with distinct disparity tuning properties across primary and higher visual areas. Mouse primary visual cortex (V1) has been shown to contain disparity-tuned neurons, but it is unknown how these signals are processed beyond V1. Furthermore, comparison of disparity tuning across different mouse visual areas might help delineating their functional roles in visual processing, which are still largely unclear. We therefore used two-photon calcium imaging to characterize binocular disparity in V1 and in two higher visual areas, LM and RL, which contain substantial visuotopic representations of the binocular field of view. We find that disparity signals are prominent in all three areas of mouse visual cortex. Preferred disparities markedly differ among visual areas, with area RL encoding visual stimuli very close to the mouse. Tuning for near disparities in RL was evident using both phase-shifted, oriented gratings and random dot stereograms for stimulation, and was also seen in awake animals.
Moreover, disparity preference is systematically related to visual field elevation, such that neurons with receptive fields in the lower visual field are overall tuned to near disparities, likely reflecting an adaptation to natural image statistics. Our results reveal ecologically relevant areal specializations for binocular disparity processing across mouse visual cortex. Since it was recently shown that RL also contains many neurons responsive to whisker stimulation, we speculate that this area might contain a multimodal representation of the space immediately in front of the mouse.

Disclosures: **M. Hubener:** None. **A. La Chioma:** None. **T. Bonhoeffer:** None.

Poster

142. Visual Processing Beyond V1

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 142.05/DP07/L10

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: D.07. Vision

Title: Neural mechanisms for border-induced brightness perception in early human visual cortex: An intracranial electrophysiology study

Authors: *L. LUO[1], Q. WANG[2], F. FANG[1];
[1]Peking Univ., Beijing, China; [2]Beijing Key Lab. of Epilepsy, Epilepsy Center, Dept. of Functional Neurosurgery, Sanbo Brain Hosp., Beijing, China

Abstract: The brightness of surfaces is a critical attribute for visual scene processing and is influenced not only by the physical luminance of the surface but also by the contrasts of surrounding borders. This perceptual phenomenon is vividly demonstrated by the classic Cornsweet illusion, where two surfaces with equal illuminances appear to be different in brightness due to the change of illuminance on the border between them. However, the neural mechanisms behind, especially the role of early human visual cortex, are still under debate. To examine the neural substrate of Cornsweet illusion, we used stereo-encephalography (SEEG) in epilepsy patients with intracranial electrodes implanted for their presurgical evaluation to record neural population activities directly from primary visual cortex (V1). First, spatial receptive fields (RFs) of each recording sites were measured with checkerboard mapping stimuli. Stimuli sets consisted of 3 conditions (Cornsweet, Real and Control) were designed on an individual basis to make sure that a uniform luminance region (i.e., the surface) of each stimulus was positioned over the RFs of the majority of targeted V1 sites. The Cornsweet stimuli were embedded in a black background. The perceived luminance changes induced by counterphase flipping of the border contrasts were measured using a two-interval forced-choice task, where patients were asked to compare the Corsweet stimuli with the Real stimuli, whose surface luminance were modulated homogeneously. The same measurements were repeated for the Control stimuli, which were identical to the Cornsweet stimuli except for the grey background, leading to weaker illusion effects as confirmed by the behavioral results. In Real condition, the contrasts between surface regions were set as perceptually equivalent to the matching Cornsweet stimulus. All stimuli were presented in 1-Hz counterphase flicker while the patients were performing a fixation task. We found that changes of the border contrasts of Cornsweet stimuli elicited significant evoked potentials in most V1 sites with RFs locating within the physically constant surfaces. The Cornsweet responses show longer latencies and reduced high-gamma (70 – 150 Hz) power compared with the Real responses. More importantly, a subset of V1 sites show differences between the Cornsweet and Control conditions as well, with the former being more similar to the Real responses according to the cluster-based, sliding-window ANOVAs. Our results provided human electrophysiological evidence that the neural mechanisms of border-induced brightness perception of surfaces arise at early stages of visual processing.

Disclosures: L. Luo: None. **Q. Wang:** None. **F. Fang:** None.

Poster

142. Visual Processing Beyond V1

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Program #/Poster #: 142.06/L11

Topic: D.07. Vision

Support: KAKENHI 17H01754, 16K01962, 17J40112, JP15H05921
Mitsubishi-zaidan 30317

Title: Responses to figure-ground organization in natural image patches by monkey V4 neurons

Authors: *K. SAKAI[1], K. KIMURA[1], M. SHISHIKURA[1], Y. YAMANE[2,3], H. TAMURA[2,4]; [1]Dept. of Computer Sci., Univ. Tsukuba, Tsukuba, Japan; [2]Osaka Univ., Osaka, Japan; [3]JSPS, Tokyo, Japan; [4]CiNet, NICT, Osaka, Japan

Abstract: Segregation of natural images into objects and background is a crucial step for understanding scenes and recognizing objects. As a step towards understanding the formation of surfaces based on figure-ground (FG) segregation, we investigated the responses of V4 neurons to signal figures and ground, with a focus on local image information contained in natural images. We recorded spiking activities of a population of macaque V4 neurons in response to a variety of natural image patches and their variants (e.g., mirrored images, filled images, and reversed contrast) that extended approximately three times larger than the extent of the classical receptive field (CRF) of the neurons. Approximately one third of the visually responsive neurons showed the response modulation depending on the positional relation between the CRF of the neuron and the figural region of the stimulus. However, these neurons with FG modulation showed low consistency in FG determination across the stimuli (approximately 60%). We examined the optimal stimuli for the neurons with FG modulation to estimate the spatial structure of the receptive field that represents the responses to figure and ground. Because the individual neurons showed the low consistency, we expected that their optimal FG configuration differs across neurons while they exhibit a facilitatory sub-region in response to figure on and around their CRF. To determine the spatial structure of the optimal FG configuration, we tagged luminance intensity to figure and performed spike triggered stimulus average (STA). Specifically, we separated the stimulus patches into two groups---one with brighter figures compared to ground, and the other with darker figures. Then, we generated STAs for each group and the difference was taken between the two to cancel out luminance contrast. We compensated nonuniformity of luminance in the natural images by subtracting the simple ensemble average of the stimuli from the STA. The estimated STAs exhibited a wide variety of structure but the most showed an antagonistic arrangement of a facilitative region on and around the CRF center and a suppressive region off the center. The linear STAs showed significant contribution to the reconstruction of the neural response. The antagonistic structure might be suitable in the detection of FG from local structures. A wide variety of sub-regions in shape and spatial extent suggests the limitation of individual neurons in the detection of FG from a variety of natural images and the necessity of population coding by integrating the responses of multiple neurons.

Disclosures: K. Sakai: None. **K. Kimura:** None. **M. Shishikura:** None. **Y. Yamane:** None. **H. Tamura:** None.

Poster

142. Visual Processing Beyond V1

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 142.07/L12

Topic: D.07. Vision

Support: ANR-16-CE37-0002-01

Title: Processing of rotationnal symmetry in the non-human primate brain

Authors: P. AUDURIER[1], Y. HÉJJA-BRICHARD[1], P. J. KOHLER[2], A. M. NORCIA[2], J.-B. DURAND[1], ***B. R. COTTEREAU**[1];
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Abstract: Symmetry is a highly salient feature of the natural world that is specifically processed by numerous species of the animal kingdom and notably by primates. Previous neuroimaging measurements have identified several visual areas (in particular along the ventral pathway) that have selective responses to reflection symmetry in humans and to a lesser extent in macaques. Here, we wanted to determine whether another form of symmetry - rotational symmetry - is also processed by both primate species. We adapted the experimental protocol from a previous human fMRI study to characterize BOLD responses to rotational symmetry in rhesus macaque. Recordings were performed at 3T in two awake monkeys using a dedicated 8-channel coil positioned above the animal's head. We used a block design where the stimuli alternated between a baseline (i.e. fixation point only), a sequence of textures that contained rotational symmetry and a sequence of control textures that shared the same low-level features but had no rotation symmetry. The rotation symmetry textures were exemplars from one of four distinct classes, interleaved between the runs. These classes differed on the maximum order of rotation symmetry (i.e. the number of rotations that leave the pattern unchanged) they contained: 2, 3, 4 or 6. Eye movements were monitored while the monkey performed a fixation task. We only analyzed runs where fixation was confirmed for more than 85% of the time. Sixteen and twenty valid runs were collected for each symmetry order in the first and second macaques, respectively. From a general linear model computed using SPM 12 and that included saccadic eye movements and motion related noise signals as regressors of non-interest, we found that all the symmetry conditions led to significantly stronger responses than their respective control in areas V3 and V4, as well as in more anterior regions along the lower bank of the superior temporal sulcus (STS). Responses in all these regions depended parametrically on the maximum order of rotation symmetry in the stimuli, with higher symmetry orders leading to stronger activations. These properties are strikingly similar to those observed in humans using the same protocol.

Altogether, our results suggest that the cortical networks that process rotational symmetry in human and macaque are at least partially homologous.

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Poster

142. Visual Processing Beyond V1

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MOST(Taiwan) 107-2420-H-002-029-DR

Title: Disparity dependent luminance contrast response functions in human dorsal visual areas

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Abstract: The perceived depth from disparity in random dot stereograms depends on luminance contrast in the image. Here, we investigated the neural mechanisms underlying such effect by using a block-design fMRI experiment. We measured the BOLD activation in retinotopically defined visual areas as a function of luminance contrast and disparity. The test stimuli were square random dot stereograms (20.16 x 20.26 degree) that gave the percept of either a flat surface (zero disparity) or a sinewave modulated in depth (raised cosine surfaces). The luminance contrast ranged from 5% to 80%. The task of the observer was to indicate whether the peak of the depth modulated surface was to the left or the right of the fixation. The accuracy of depth judgement increased with luminance contrast. In all visual areas, BOLD signals increased monotonically with luminance contrast. In the early visual areas, including V1, V2, V3and hV4, such contrast response functions were independent from disparity modulation. In areas V3A, V3B and KO (defined by a separate localizer that contrasts activations to moving edges from uniform motion), the contrast response functions saturated at relatively low contrast compared at the zero disparity condition. However, the BOLD response in the raised cosine surface condition showed no sign of saturation even at the highest contrast and reached an activation level up to 40% greater than that in the no disparity condition. Such disparity modulated contrast response function was highly correlated with the change of perceived depth under different luminance contrasts. Our results suggested that disparity information can reduce the contrast gain control signal in V3A, V3B and KO and thus not eliminates the saturation in the response function but also increases the contrast dependent activation to a greater level.

Disclosures: **C. Chen:** None. **P. Chen:** None.

Poster

142. Visual Processing Beyond V1

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Support: NIH Grant R00EY025768
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Title: Complex feature sets constrained by deep generative image models drive visual evoked potentials in macaque monkeys

Authors: H. SCOTT[1], I. FRUEND[4,5,6], ***A. C. SNYDER**[1,2,3];
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Abstract: The visual world contains more information than the brain can process. One effective method for compressing visual information uses a statistical model in which important and probable feature sets are summarized by a relatively small number of independent latent variables. Such models form the basis of recently popularized artificial neural networks for tasks like visual object classification. The generative form of these networks, called Generative Adversarial Networks (GANs), are trained to create images maximally similar to rich natural images using complex, yet low dimensional, feature sets. Since both artificial and natural neural networks face similar image-compression needs, we asked if the primate visual system might have evolved to be sensitive to latent feature sets similar to those learned by a GAN. To test this, we presented passively-fixating rhesus macaque monkeys images sequences generated by a GAN while we recorded EEG with 32 scalp electrodes. We hypothesized that if primate brain structures are sensitive to GAN latent variables, then those brain areas would respond quasi-linearly to modulations of image features within the manifold of images generated by the GAN. We rhythmically modulated the latent features of the stimuli and measured the elicited steady-state visual evoked potential (SSVEP). Importantly, the SSVEP to latent feature modulation differed from SSVEPs elicited by other kinds of image modulation, such as by luminance, contrast, hue, Fourier noise or pixel noise. This suggests parts of the primate visual system are particularly tuned to complex latent feature sets useful for compactly summarizing natural images.

Disclosures: **A.C. Snyder:** None. **H. Scott:** None. **I. Fruend:** None.

Poster

142. Visual Processing Beyond V1

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Title: Organizing principles in the transformation of representations in ventral visual pathway

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Abstract: Understanding specific transformations that underlie our ability to recognize objects is one of difficult open problem in sensory neuroscience. Recent work has shown that artificial convolutional networks are capable of simulating the responses of neurons in the early to middle visual cortex. However, interpreting the transformations performed by these networks remains a challenge. We have structured a two-layer quadratic convolutional model to include major known elements of neural computations in the brain, including contrast normalization, quadratic nonlinearities, and contextual modulation, including cross-orientation suppression. We applied this model to neurons from cat V1, macaque V2, and macaque V4. In all three areas, the models outperformed or matched the leading machine learning methods, that included gradient boosted tree models. Neurons in all areas varied in the strength of the contribution from their linear or the quadratic terms. Therefore we divided neurons into four classes based on which term dominated within which layer. V1 neurons were more likely to use the linear term than neurons from V2 and V4 in either layer. For neurons that were quadratic in the first layer, we curved Gabors to characterize their feature selectivity and found that adding curvature to the Gabors improved the fit. Using the curved Gabors, we saw that the excitatory and suppressive components tended to be locally orthogonal in V2 and V4 as well as an increase in relative curvature from V2 to V4. We also compared the dominant excitatory orientations between the first layer and second layer and found that they tended to be parallel with each other in V1 and V4. Finally, we noted that excitation and suppression in neurons that were quadratic in the second layer tended to be aligned.

Disclosures: R. Rowekamp: None. **T. Sharpee:** None.

Poster

142. Visual Processing Beyond V1

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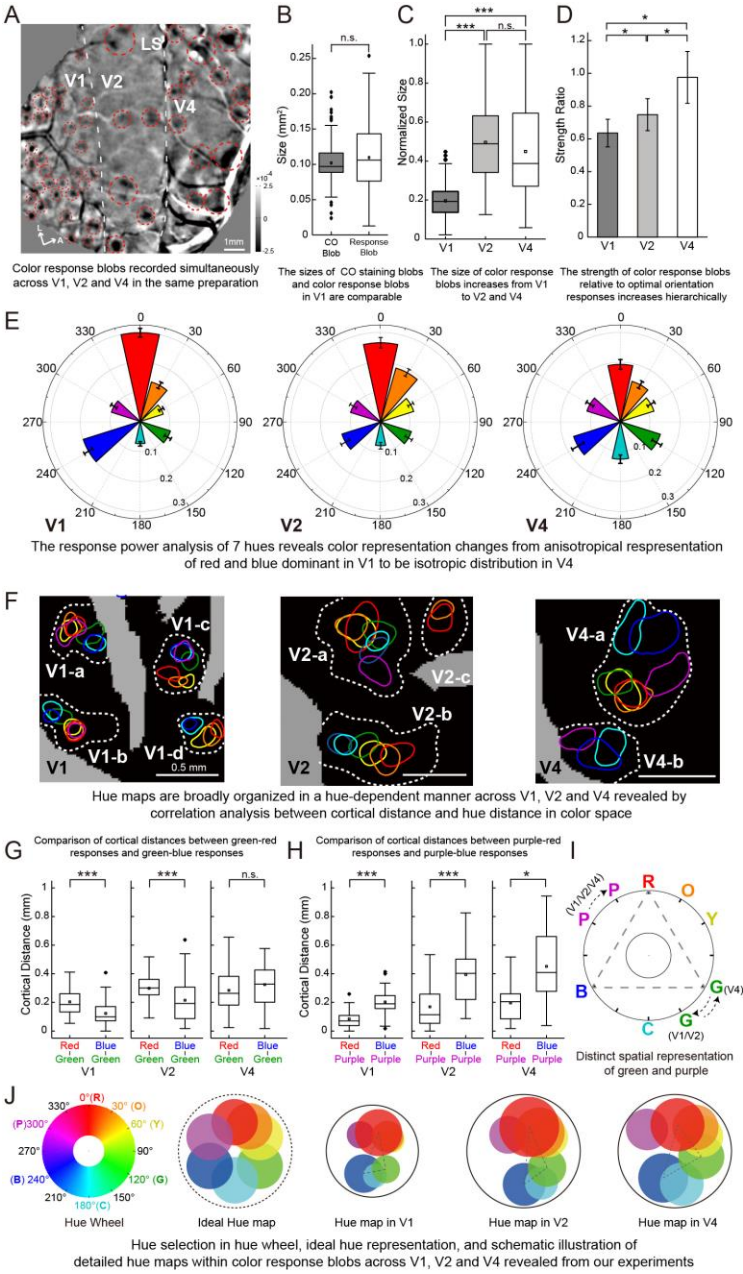
Title: Detailed hierarchical functional architectures of chromatic responses across macaque V1, V2 and V4 cortices

Authors: *Y. LIU[1,2], X. ZHANG[1], Y. LU[1], H. GONG[1,2], J. YIN[1], Z. CHEN[1], L. QIAN[1], S. SHIPP[1], I. M. ANDOLINA[1], N. MCLOUGHLIN[3], W. WANG[1,2];
[1]Inst. of Neuroscience, Chinese Acad. of Sci., Shanghai, China; [2]Univ. of Chinese Acad. of Sci., Beijing, China; [3]Univ. of Manchester, Manchester, United Kingdom

Abstract: Color perception depends on transforming spectral signals from the retina into neural representations in visual cortex. Visualizing the functional architecture of spectral information processing in V1 color-response blobs, V2 thin stripes and V4 globs in trichromatic primates instructs our understanding of this process. How, precisely, does the representation of hue change across V1, V2 and V4? To address this question, we used intrinsic signal optical imaging to simultaneously record V1, V2 and V4 cortical population responses to colored stimuli in macaques. We use both chromatic gratings and 8 isoluminant hues to delineate the functional organization of color responses in V1, V2 and V4. We found consistently shaped domains of color response blobs whose overall response size and strength increased from V1 to V2 and V4. Response power analysis revealed that “endspectral” hues (reddish and blueish) are anisotropically dominant in V1 and V2 but become more isotropically distributed in V4. Furthermore, we found hue maps within these color-response blobs exhibiting a general hue-dependent organization, consistent with learlier resports as adjacent hues activated neighboring cortical sub-domains. However, red hue retains a significantly greater representation across the hierarchy. In addition, the locus of extraspectral purple is variable, but consistently biased toward red in all three areas, whereas, green is represented towards blue in V1 and V2 but “back” to the middle of red and blue maps in V4. Together, these results delineate how detail the cortical functional organizations of hue maps within retained color-response blobs change hierarchically across V1, V2, and V4.



Disclosures: **Y. Liu:** None. **X. Zhang:** None. **Y. Lu:** None. **H. Gong:** None. **J. Yin:** None. **Z. Chen:** None. **L. Qian:** None. **S. Shipp:** None. **I.M. Andolina:** None. **N. Mcloughlin:** None. **W. Wang:** None.

Poster

142. Visual Processing Beyond V1

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Topic: D.07. Vision

Support: NIH Grant EY005864-29
NIH Grant 5F31EY026791-03

Title: Population-level deficits in contour representation in V4 of amblyopic macaques

Authors: *J. PAI, B. N. BUSHNELL, N. MAJAJ, J. A. MOVSHON, L. KIORPES;
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Amblyopia is a disorder of form vision, typically in one eye, associated with abnormal visual input during a critical period of development. Most studies of the neuronal correlates of amblyopia have used simple stimuli (e.g. gratings) while recording from early visual cortex in anesthetized animals. Visual responses to amblyopic eye stimulation are in some cases impaired, but to a lesser degree than behaviorally measured visual losses, implying that there are additional deficits downstream of V1 and V2. Recent experiments suggest that integration of contour information across space involves interactions between V1 and V4, an extrastriate area thought to encode form and shape information. Since behavioral studies show losses in contour integration ability, we were interested in whether this processing is disrupted in amblyopia. We recorded simultaneously from neural populations in V1, V2, and V4 in 3 awake, fixating, macaques, 2 strabismic amblyopes and a visually normal control. We recorded multiunit activity from 2 96-channel Utah arrays in each animal, one along the V1/V2 border and one in V4, in response to monocular stimulation of the fellow (FE) and amblyopic eye (AE). Stimuli were arrays of randomly oriented Gabor patches containing collinear sets of 3, 5, or 7 Gabors, centered over the multi-unit receptive field. V4 neurons were much more responsive to Gabor contours than V1 neurons. Sites responding to the FE were more numerous than those responding to the AE. The interocular difference grew from V1 (31% FE sites, 18% AE sites) to V4 (69% FE sites, 22% AE sites). We used linear discriminant analysis to assess the ability of neural populations to discriminate collinear form during FE or AE viewing. In V4, populations driven by the FE performed markedly better than those driven by the AE, and performance increased as a function of increasing contour length. V1 performance was lower than in V4, with little difference between the eyes and little effect of contour length. These results suggest that 1) contour information is carried by population-level representations in V4 but not V1, and 2) deficits in these representations at the level of V4 or onwards may explain the behavioral deficits of amblyopia.

Disclosures: **J. Pai:** None. **B.N. Bushnell:** None. **N. Majaj:** None. **J.A. Movshon:** None. **L. Kiorpes:** None.

Poster

142. Visual Processing Beyond V1

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Topic: D.07. Vision

Support: NIH Grant 5F31EY026791-03
NIH Grant EY005864-29

Title: Population-level deficits in global form representation in V4 of amblyopic macaques

Authors: *B. N. BUSHNELL, N. J. MAJAJ, J. A. MOVSHON, L. KIORPES;
New York Univ., New York, NY

Abstract: Amblyopia is a disorder of form vision, typically in one eye, associated with abnormal visual input during a critical period of development. Most studies of the neuronal correlates of amblyopia have used simple stimuli (e.g. gratings) while recording from early visual cortex in anesthetized animals. Visual responses to amblyopic eye stimulation are in some cases impaired, but to a lesser degree than behaviorally measured visual losses, implying that there are additional deficits downstream of V1 and V2. To study correlates of form discrimination, we recorded responses from neural populations in V4 of 3 awake, fixating macaques (2 strabismic amblyopes and 1 visually normal control) to Glass pattern stimuli. Glass patterns are fields of dot pairs arranged according to geometric rules that create a global percept of radial or concentric structure. We titrated the strength of the percept by altering the density and spacing of the dot pairs, and the fraction of pairs that conform to the geometric rules ("coherence", with 0 creating random dipoles or "noise"). Prior studies in V1 and V2 have demonstrated selectivity for local orientation but not for global form. We recorded multiunit activity from 96-channel Utah arrays in V4, in response to monocular stimulation of the fellow (FE) and amblyopic eye (AE). We briefly presented Glass pattern stimuli over the multiunit receptive field. FE sites were generally more responsive to visual stimulation. Activity at many sites was binocular, but dominated by the FE; most monocular sites were driven by the FE. For responsive sites driven by either eye, all Glass patterns were usually effective, and global forms were not consistently preferred. However, we used linear discriminant analysis to assess the ability of neural populations to discriminate radial or concentric Glass patterns from noise – population responses reliably discriminated both global patterns; performance typically improved monotonically with coherence. Performance was better for the FE than the AE at all densities and dot spacings. These results show that V4 neurons can differentiate Glass patterns from noise. Consistent with behavioral measures, performance is impaired for AE sites relative to the FE in V4 on this global form discrimination.

Disclosures: **B.N. Bushnell:** None. **N.J. Majaj:** None. **J.A. Movshon:** None. **L. Kiorpes:** None.

Poster

142. Visual Processing Beyond V1

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Topic: D.07. Vision

Support: NEI RO1EY018839
T32NS099578

Title: Long-range apparent motion tuning in ventral visual area V4

Authors: ***A. W. BIGELOW**, T. KIM, W. BAIR, A. PASUPATHY;
Biol. Structure, Univ. of Washington, Seattle, WA

Abstract: Apparent motion (AM) stimuli, created by displacing an object intermittently across the visual field, have been invaluable for probing the neural and cognitive substrates of motion perception. Psychophysical studies have demonstrated robust motion percepts for large spatial and temporal displacements of up to 10 deg of visual angle and 300 ms interstimulus intervals. While neurons in primary visual cortex (V1) and along the dorsal pathway (e.g. MT, MST, LIP) show direction selectivity for short stimulus displacements (<0.25 deg), for long-range displacements ($\geq$ 0.5 deg), they fail to exhibit direction selectivity even in area MT where RFs are many times the step size. Thus, no correlate exists for the percept of long-range apparent motion (LAM) at the level of individual neurons. Here we investigate whether neurons in area V4 might provide the neural basis for this illusory percept. Area V4 is an intermediate visual area in the ventral pathway implicated in the processing of form, color and texture. However, there are strong reciprocal connections between V4 and dorsal stream areas. Many V4 neurons also exhibit direction selectivity, suggesting that V4 may play an important role in integrating information across the visual streams into a unified visual percept. We recorded individual neural responses to AM stimuli with spatial displacement of 0.6-2.1 deg in an awake, fixating macaque. Stimuli were either scintillating Julesz patches (changing with every screen jump) or Gabor patches similar to the design of Hedges et al. (2011). We recorded from 85 well isolated neurons in one animal and, strikingly, 35/85 showed tuning for the direction of LAM, something not found in area MT. Because stimuli in both directions are presented at the exact same spatial locations, our results cannot be explained on the basis of spatial inhomogeneities in the RF. To our knowledge, these results identify the first neural correlate for long-range apparent motion. Furthermore, these are also novel results as our stimuli are non-Fourier: both the Julesz patches and the grating stimuli are drift/micro-balanced. Many neurons along the dorsal stream, e.g., V1, MT, are not driven by second-order stimuli; thus, our study highlights a cortical area that may

participate in the perception of higher order motion. These results are complimentary to earlier studies showing that the ventral stream is activated during viewing of LAM and that LAM persists despite plastic deformations in shape if objects are topographically similar. Moreover, our study suggests that ventral visual areas, particularly higher cortical areas such as V4, likely play an important role in the perception of dynamic stimuli.

Disclosures: A.W. Bigelow: None. **T. Kim:** None. **W. Bair:** None. **A. Pasupathy:** None.

Poster

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Title: Clustering of motion signals in ferret PSS

Authors: D. C. KHAMISS, A. A. LEMPEL, *K. J. NIELSEN;
Neurosci., Johns Hopkins Univ., Baltimore, MD

Abstract: One of the crucial functions of the visual motion pathway is to integrate multiple moving elements into global motion signals. Motion integration has classically been studied using plaid patterns, which are composed of two component gratings drifting in separate directions. The resulting plaid then appears to move in a third, intermediate direction. Using these stimuli, neurons in primary visual cortex (V1) of non-human primates have been shown to represent the motion of the individual components (‘component’ neurons). In contrast, a population of neurons in higher-order area MT represent the movement of the plaid (‘pattern’ neurons), indicative of motion integration. We have recently demonstrated that the ferret visual system contains a motion pathway similar to that of the primate. As in the primate, ferret V1 extracts component motion signals, while a higher-order area - area PSS - contains a population of pattern neurons. Here, we further investigated processing of motion signals in PSS to elucidate possible mechanisms underlying the observed motion integration. In particular, we focused on the functional organization of tuning properties in PSS. To this end, we used multi-site silicon probes to record from multiple PSS neurons simultaneously. By recording neural responses to gratings and plaids, we determined basic direction selectivity and the degree of motion integration for every neuron. We then quantified similarities in tuning properties between neurons as a function of the distance between them. Our data reveal that direction preferences tended to be similar across PSS neurons as long as distances between neurons were small (between 50 and 250 um). Yet, clustering of direction preferences in PSS appears to be weaker than that reported for primate MT. To refine the analysis, we classified cells as component or

pattern cells based on their degree of motion integration. We then analyzed differences in direction preferences for pairs of nearby component cells, as well as pairs of nearby pattern cells. While the pairs of component cells tended to exhibit disparate direction preferences, we found that nearby pattern cells typically preferred very similar motion directions. This clustering might be explained by the fact that motion integration across a diverse range of component directions is required to generate consistent pattern responses. Furthermore, we are currently making use of the ferret's unique suitability for developmental studies to investigate how this functional architecture emerges during the maturation of the motion pathway.

Disclosures: D.C. Khamiss: None. **A.A. Lempel:** None. **K.J. Nielsen:** None.

Poster

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Topic: D.07. Vision

Support: NIH grant R01EY022443

Title: Image segmentation and neural representation of multiple spatially-separated stimuli in cortical area MT

Authors: *S. WIESNER, X. HUANG;
Dept. of Neurosci., Univ. of Wisconsin Madison, Madison, WI

Abstract: Segmenting objects in natural scenes is a fundamental function of vision. To examine the neural mechanism underlying image segmentation, we previously investigated how neurons in the middle-temporal (MT) cortex of macaque monkeys represent overlapping visual stimuli moving transparently in different directions. We have found that a group of neurons in MT show a directional bias and prefer the component direction at a specific side, either clockwise (C) or counter-clockwise (CC), of two stimulus directions (Xiao & Huang, 2015). In natural vision, it is common to encounter multiple moving stimuli that are spatially separated. However, the rules by which neurons in area MT represent multiple, spatially-separated stimuli are not well understood. To investigate this question, we recorded from neurons in area MT of fixating macaques. The stimuli were two random-dot squares placed side by side with the border centered on the RF. The two square patches had equal luminance and each square was 10° wide. The random dots moved coherently in a given direction within the aperture of each patch. The motion directions of the two patches were separated by 60°. We varied the vector-averaged direction of the two patches and characterized the tuning curves in response to the bi-directional stimuli. We varied the stimulus configurations to determine whether MT neurons exhibited a spatial bias toward the stimulus component at one side of the RF and/or a directional bias toward a component direction.

We observed that some MT neurons showed a directional bias to spatially separated stimuli as found using overlapping stimuli. We also found that MT neurons showed a significant spatial bias toward one side of the RF, characterized by the response weights to the two stimuli. The spatial bias occurred regardless of whether the patch at the preferred location moved in the C- or CC-side direction, or whether the two patches were arranged side-by-side horizontally or vertically. The spatial bias cannot be explained by the preference for a single stimulus at different spatial locations since the bias was often toward the stimulus component that elicited a weaker response when present alone. The spatial bias cannot be explained as a bias toward the fovea. By recording from multiple single neurons simultaneously, we found that different neurons showed spatial biases toward different patches in the same recording session, suggesting that the spatial bias was not due to selective attention. The spatial bias, together with the directional bias, may provide a heterogeneous neural code for individual stimulus components and may facilitate the segmentation of multiple objects in natural environments.

Disclosures: S. Wiesner: None. **X. Huang:** None.

Poster

142. Visual Processing Beyond V1

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Support: NEI EY013644

Title: Speed tuning in head coordinates as an alternative explanation of depth selectivity from motion parallax in area MT

Authors: *Z.-X. XU[1], G. C. DEANGELIS[1,2];
[1]Brain and Cognitive Sci., [2]Ctr. for Visual Sci., Univ. of Rochester, Rochester, NY

Abstract: The retinal image motion of an object generally reflects both self-motion and movement of the object in the world. When an object is stationary in the world and the observer is translating, retinal velocity depends on the object's distance from the point of fixation (motion parallax, MP). Depth from MP is known to depend on the ratio of retinal velocity to eye velocity (motion-pursuit law, Nawrot and Stroyan, 2009), and previous work has shown that neurons in macaque area MT are selective for the sign of depth by combining retinal image motion with smooth eye movement command signals (Nadler et al. 2008, 2009). A more recent study suggested that MT responses are gain modulated by the direction of eye movement to generate depth-sign selectivity (Kim et al. 2017).
It remains unclear exactly how eye velocity and retinal velocity interact to determine the responses of MT neurons to depth. We re-analyzed data from Nadler et al. (2008) to characterize

the joint tuning of MT neurons for retinal velocity and eye velocity. Consistent with previous findings, the effect of eye velocity on responses to retinal image motion is well-described by a gain modulation for some neurons. However, for other neurons with slow speed preferences, retinal speed tuning clearly shifts with eye velocity, causing a diagonal structure in the joint tuning profile. This observation leads to an alternative explanation for MP-based depth tuning in MT: MT neurons might be tuned to velocity in head-coordinates (the sum of retinal and eye velocities), instead of being tuned to the ratio of retinal and eye velocities.
To test this hypothesis, we simulated neurons with log-Gaussian speed tuning in a space ranging from retinal to head coordinates, and we generated depth tuning curves from model responses. We find that speed tuning which is shifted toward head coordinates can predict depth tuning from MP similar to that observed in MT. Thus, depth tuning in MT could arise from even a modest shift toward head-centered speed tuning. We fit PSTHs from MT neurons with both this head-coordinate speed tuning (HT) model and a gain modulation (GM) model. The HT model (median $R^2 = 0.48$) slightly outperformed the GM model (median $R^2 = 0.47$) in fitting PSTHs, but both models equally well predicted depth tuning curves.
Our results reveal that a more extensive sampling of the joint tuning for retinal and eye velocities will be necessary to distinguish the HT and GM models. Nevertheless, our findings suggest that depth tuning based on MP might be related to a shift toward coding velocity in head coordinates. We are exploring whether this type of joint tuning would be valuable for computing depth from MP when objects also move in the world.

Disclosures: Z. Xu: None. **G.C. DeAngelis:** None.

Poster

142. Visual Processing Beyond V1

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Title: Macaque area MT is specialized for 2D motion processing

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Abstract: The macaque middle temporal (MT) area is a key structure implicated in the processing of visual motion. Its role in the perception of two-dimensional (2D) motion is well established, and recent studies suggest that some MT neurons are also selective for three-dimensional (3D) motion signals. In this study, we measured the selectivity of MT neurons for 2D and 3D motion, and tested for functional correlations between neuronal activity and behavior during a motion-in-depth (MID) discrimination task. All stimuli subtended 3° and were presented within the classical receptive field. We first assessed 2D motion selectivity during passive fixation. We then assessed MID direction (toward or away) selectivity during the discrimination task using a motion coherence paradigm. The MID stimuli were defined by a volume of dark and bright dots which moved either towards or away from the animal's cyclopean eye. To evaluate the sensitivity of MT neurons to different 3D motion signals, we presented stimuli defined by: (1) combined stereoscopic and perspective cues, (2) only stereoscopic cues, or (3) only perspective cues (single eye views of the combined-cue stimuli). As controls, we presented leftward and rightward 2D motion stimuli (presented monocularly and binocularly). Four lines of evidence converge to suggest that MT is specialized for 2D motion processing. First, MID tuning curves measured with perspective cue stimuli tended to either lack direction selectivity or have opposite direction preferences for the two eyes, which is inconsistent with 3D motion selectivity. Instead, this finding is consistent with 2D motion selectivity, since MID produces 2D retinal patterns with opposite net directions in the two eyes. For many neurons, significant ocular dominance was also observed. Second, responses measured with stereoscopic and combined-cue MID stimuli were highly correlated with perspective cue responses measured when the dominant (but not the non-dominant) eye was stimulated. Third, a weighted linear combination of the two eyes' perspective cue responses predicted the combined-cue responses. An independent test indicated that the same weights applied to 2D motion responses predicted the responses to the stereoscopic cue MID stimuli. Fourth, few neurons showed significant choice probabilities during the MID discrimination task. These results suggest that macaque area MT is specialized for the processing of 2D motion signals, and that previous reports of 3D motion selectivity may reflect a combination of 2D selectivity and ocular dominance. Selectivity for 3D motion likely arises in subsequent stages of visual motion processing.

Disclosures: L. Thompson: None. **B. Kim:** None. **B. Rokers:** None. **A. Rosenberg:** None.

Poster

142. Visual Processing Beyond V1

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 142.19/L24

Topic: D.07. Vision

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Title: "Changing gears to see fast and slow" - Hierarchical computation of velocity across V1, MT, and MST in non-human primates

Authors: *J. LUO[1], K. HE[1,2], X. LI[1], Y. LU[1], I. M. ANDOLINA[1], S. SHIPP[1], W. WANG[1,2]; [1]Inst. of Neuroscience, Chinese Acad. of Sci., Shanghai, China; [2]Univ. of Chinese Acad. of Sci., Beijing, China

Abstract: Primates are capable of perceiving moving objects across a wide range of speeds. Translational motion signals are computed primarily by direction-selective neurons at different processing stages of the primate visual pathway (from V1 to MT and MST). Previous studies using dot stimuli in human psychophysics, monkey physiology and spatiotemporal filter model simulations demonstrate that V1 direction-selective neurons with unimodal direction tuning curves at low speeds are transformed into "orientation" neurons at high speeds with bimodal tuning curves encoding motion streak signals. Exactly how motion direction and streak signals are integrated across low and high speeds along the visual hierarchy remain unclear. By single-unit recordings in V1, MT and MSTd in behaving macaques, we studied the neuronal responses across the visual hierarchy to a large range of velocities (from 0 to 150 °/s). We found that at different higher speeds, almost all V1 neurons over 8°/s and 15% of MT neurons over 30°/s exhibited clear bimodal tuning to motion streak signals, while 44% of MT neurons and most MSTs neurons maintained unimodal direction tuning curves at all tested speeds (remaining MT cells were unclassified). This transformation towards invariant representation of velocity indicates a hierarchical bottom-up nonlinear integration of direction and motion streak signals. We also tested the same neurons to classical plaid grating stimuli for component and pattern motion responses with respect to the unimodal/bimodal tuning properties. We found significant positive correlations between bimodal and component, and unimodal and pattern responses. Finally, we used a modified population cascade energy model to simulate how the responses of the bimodal neurons can become unimodal from V1 to MT and MST. These results demonstrate how linear and non-linear integrations of direction and motion-steak signals across the dorsal stream support computation of a large range of velocities from slow to fast.

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Poster

143. Spatial and Chromatic Vision

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Title: High-resolution artificial vision with shape perception via a chronically implantable 1024-channel neuroprosthesis in monkey visual cortex

Authors: *X. CHEN[1], F. WANG[1], B. LI[1], P. ROELFSEMA[1,2,3];
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Abstract: Globally, 40 million people across the world are blind, many of whom could potentially regain functional vision through a neuroprosthetic device that interfaces directly with the visual cortex. Electrical stimulation of the visual cortex is known to elicit the percept of a dot of light at a particular location in visual space, known as a 'phosphene.' However, the ability to create coherent artificial percepts, consisting of readily interpretable shapes and contours, has remained elusive and only sparsely documented in the literature (Dobelle 1974). Here, we present results obtained from a high-resolution, high-density, high-channel-count prosthesis for the visual cortex, consisting of 1024 electrodes (sixteen 8x8 Utah arrays) that were chronically implanted in V1 and V4 of the left hemisphere, in each of two monkeys. We developed customized cranial implants (Chen et al., 2017); new and improved surgical tools and techniques; and a large-scale microstimulation and recording system. Initially, the monkeys performed a simple task in which they made eye movements to the location of a phosphene that was elicited by V1 stimulation via individual electrodes. We found a significant correlation between saccade end points and the neurons' RFs. Next, the monkeys were tested on more complex tasks: a line orientation discrimination task, in which microstimulation was delivered on several electrodes simultaneously, creating the percept of either a horizontally or a vertically oriented line; a direction-of-motion task, in which microstimulation was delivered on a sequence of electrodes; and a letter discrimination task, in which microstimulation was delivered on 10-15 electrodes simultaneously, creating a percept in the form of a letter. They successfully reported the identity of these artificially generated percepts- performing above chance levels even with novel combinations of electrodes. Finally, over a period of several years, we monitored the current thresholds needed to elicit phosphene percepts, as well as electrode impedance levels and the quality of neuronal signals recorded on each of the channels. This proof-of-concept

demonstrates the potential of a visual cortical neuroprosthesis for restoration of functional, life-enhancing vision in the blind.

Disclosures: **X. Chen:** None. **F. Wang:** None. **B. Li:** None. **P. Roelfsema:** None.

Poster

143. Spatial and Chromatic Vision

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Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

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Topic: D.07. Vision

Support: Improvement of internationalization in the field of research and development: CZ.02.2.69/0.0/0.0/17_050/0008466

Title: Cortical visual prosthesis: A detailed large-scale simulation study

Authors: ***J. ANTOLIK**[1], Q. SABATIER[2], C. GALLE[3], Y. FREGNAC[4], R. BENOSMAN[2]; [1]Fac. of Mathematics and Physics, Charles Univ., Prague, Czech Republic; [2]Inst. de la Vision, Paris, France; [3]Gensight Biologics, Paris, France; [4]UNIC-CNRS, Gif-sur-Yvette, France

Abstract: Recent advances in applying optogenetics in primates initiated the development of light based prosthetic implants for sensory restoration. Thanks to being the most well explored cortical area that is readily accessible at the surface of the brain, vision restoration via direct optogenetic activation of primary visual cortex is one of the most promising early targets for a optogenetics based prosthetic program. However, two fundamental elements of the cortical optogenetic prosthesis remain unclear. First, the exact mechanisms of neural dynamics under direct cortical stimulation, especially in the context of living, active and functionally specific intra-cortical neural circuitry, is poorly understood. Second, we lack protocols for transformation of arbitrary visual stimulus to light activation pattern for optogenetic activation of cortical region that would induce a percept similar to that induced by the visual stimulus. In this study we address these issues using a large-scale spiking neural network modeling strategy of high biological fidelity. We examine the relationship between specific spatial configuration of light delivered to cortex and the resulting spatio-temporal pattern of activity evoked in the simulated cortical circuitry. Using such virtual experiments, we design a protocol for translation of a specific set of stimuli to activation pattern of a matrix of light emitting elements and provide a detailed assessment of the resulting cortical activations with respect to the natural vision control condition. In this study we restrict our focus to the grating stimulus class, which are an ideal starting point for exploration due to their thoroughly characterized representation in V1 and well-defined information content. However, we also provide an outline of a straight-forward road-map for transforming this grating centric stimulation protocol towards general strategy capable of transforming arbitrary spatio-temporal visual stimulus to a spatio-temporal optogenetically

induced intracortical activity pattern, thus enabling vision restoration via optogenetic V1 activation.

Disclosures: **J. Antolik:** None. **Q. Sabatier:** None. **C. Galle:** None. **Y. Fregnac:** None. **R. Benosman:** None.

Poster

143. Spatial and Chromatic Vision

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 143.03/L27

Topic: D.07. Vision

Support: NIH Grant EY07977

Title: Figure-ground separation depends on texture differences and texture composition

Authors: *J. D. VICTOR[1], M. M. CONTE[2];
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Abstract: Separating figure from ground is a prerequisite for object identification. Local cues, such as luminance and orientation, play an important role in this process. These cues may act by signaling a difference between figure and ground, resulting in thresholds that are determined solely by the figure-ground difference in image statistics. But thresholds may depend not just on the difference across the border, indicating that the compositions of figure and ground also play a role.
To distinguish these possibilities, we examined figure-ground thresholds in a series of psychophysical experiments in which image statistics within figure and ground were independently manipulated. Target stimuli were synthetic black-and-white images in which five randomly-positioned circular figures, constituting 25% of the total area and defined by one set of local image statistics, were superimposed on a background, which was defined by a different set of image statistics. The image statistics that defined figure and ground were drawn from a 10-parameter space that incorporated luminance, contrast, edge orientation, and corners (Vision Res. 2015), a domain in which we had previously shown that perceptual sensitivities corresponded to informative aspects of natural images (eLife 2014). In a 2-alternative forced-choice task, subjects (N=3) were asked to distinguish this target stimulus from a distractor whose statistics were spatially homogeneous and matched the area-average statistics of the target.
Consistent across subjects, figure-ground thresholds were determined primarily by the absolute value of the difference between their image statistics. Thresholds were approximately 0.2 for first-order statistics (luminance), 0.3-0.5 for second-order statistics (contrast and edge orientation), and 0.6-0.8 for third- and fourth-order statistics (corners and crossings). Thresholds for fourth-order statistics were lower than for third-order statistics; this was similar to our

previous findings for texture segmentation. While the main determinant of thresholds was the difference between figure and ground, the composition of figure and ground also played a role. The impact of figure and ground composition ranged from 5% of the unexplained variance for first-order statistics to over 40% for higher-order statistics. Overall, the kinds of image statistics in the figure that produced low thresholds were distinct from the kinds of image statistics in the ground that produced low thresholds. This suggests that figure-ground separation makes use not only of the texture difference across the border, but also of prior expectations for the composition of figure and ground.

Disclosures: J.D. Victor: None. **M.M. Conte:** None.

Poster

143. Spatial and Chromatic Vision

Location: Hall A

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Topic: D.07. Vision

Support: NIH NRSA Grant T90DA043219

Title: Temporal straightening capabilities of models for human vision

Authors: L. CASSARD, *E. P. SIMONCELLI;
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Abstract: Sensory systems make predictions about future observations given the recent past, and we have recently hypothesized that this is achieved by transforming them to an internal representation that follows straighter temporal trajectories (the “straightening hypothesis”, Hénaff *et al.*, 2018; 2019). This hypothesis is supported by both perceptual (Hénaff *et al.*, 2018; 2019) and neural (Bai *et al.*, 2018) evidence, which reveal straightening of natural image sequences, but increased curvature (“entangling”) of artificial sequences that fade between an initial and final frame.
Straightening of natural video trajectories, as well as entangling of artificial sequences, can also be observed in the responses of a two-stage model mimicking the nonlinear functional properties of the early visual system. Artificial neural networks trained for object recognition, on the other hand, exhibit an increase in curvature for all sequences. Thus, optimizing for object recognition does not provide these networks with the straightening capabilities found in the human visual system (Hénaff *et al.*, 2019). To further investigate the straightening performance of artificial neural networks, we examined the responses of a biologically-inspired network optimized for image compression (Balle, *et al.*, 2017). The network consists of three stages, each computing responses of a set of (learned) linear filters, and normalizing their responses by a weighted combination (also learned) of other rectified filter responses. We find that this image

compression network can achieve temporal straightening behaviors not seen in object recognition-trained networks, and more consistent with those of human observers. Specifically, we find that the image compression network exhibits straightening of natural sequences, as well as entangling of unnatural sequences. We conclude that the temporal straightening capabilities of primate visual systems are consistent with, and may depend on, nonlinear response properties found in biological neurons.

Disclosures: **L. Cassard:** A. Employment/Salary (full or part-time):; Howard Hughes Medical Institute. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH Training Program for Computational Neuroscience. **E.P. Simoncelli:** A. Employment/Salary (full or part-time):; Howard Hughes Medical Institute.

Poster

143. Spatial and Chromatic Vision

Location: Hall A

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Program #/Poster #: 143.05/L29

Topic: D.07. Vision

Support: NIH Grant EY021462

Title: Measuring predictability of natural movies

Authors: *C. M. ZIEMBA[1], O. J. HÉNAFF[2], R. L. T. GORIS[1];
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Abstract: Predicting future states of the environment is a central goal of brain function. The imperative to make accurate temporal predictions likely shapes sensory processing throughout evolution, development, and learning. Yet, we lack the basic tools to interrogate the mechanisms underlying natural temporal prediction. What kind of naturally occurring sensory sequences can be predicted well? Are some more predictable than others? Are some easier to learn than others? If so, why? Answers to even these basic questions are beyond the reach of current approaches to the study of predictive behavior. As a first step towards addressing these questions, we introduce a novel psychophysical paradigm to measure the predictability of short natural movies.
We operationalize predictability as the ability to discriminate a temporal violation from “natural” time for a five-frame image sequence, taken from a short movie. Each trial, observers judge which of two image halves follows a previously seen sequence of four images. One half is the correct choice; the other half violates the natural progression of time. The easier it is to detect a temporal violation, the more predictable we deem a sequence to be. We control task difficulty by

manipulating the magnitude of the temporal violation. To get reliable measurements of task performance, we present many unique four-frame sequences (each with a different starting point in the movie). We quantify perceptual predictability of the entire movie by pooling responses across all presented sequences and fitting a conventional psychometric function to the choice data as a function of the magnitude of the temporal violation ("predictability functions"). Preliminary results suggest that different natural movies exhibit very different levels of predictability: some movies yield very steep predictability functions, others yield very shallow ones. Importantly, a control experiment revealed that these performance differences cannot be attributed to differences in frame-discriminability. Together, these findings suggest that our task offers a much needed vehicle for measuring the predictability of natural movies, hence providing a testbed for connecting perceptual predictability to the structure of sensory representations.

Disclosures: C.M. Ziemba: None. **O.J. Hénaff:** None. **R.L.T. Goris:** None.

Poster

143. Spatial and Chromatic Vision

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Topic: D.07. Vision

Support: NIMH Grant 5R01MH112583-02

Title: A human behavioral task for manipulating visual cortical prediction error

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[1]Grad. Program in Neurosci., [2]Dept. of Radiology, [3]Dept. of Psychology, Univ. of Minnesota, Minneapolis, MN

Abstract: Predictive coding theory posits that external information corrects hypotheses about the world that are continuously generated in the brain. We developed a human behavioral task designed to model prediction error using a contrast surround suppression paradigm. In classic surround suppression, the perceived contrast of a central target texture is reduced by the presence of a surrounding texture that is similar to the center, but not by a surrounding texture that differs from the center. Our paradigm manipulates the effects of both texture regularity (i.e. gratings vs. naturalistic textures) and similarity (i.e. texture identity or orientation) on contrast surround suppression. To quantify how higher order image statistics impact the degree of suppression, we included a phase-scrambled (noise) condition in which the same texture image was used for both center and surround, with phase structure of the surround randomized.
Male and female participants completed a four-alternative forced choice contrast discrimination task that measured contrast discrimination thresholds for target textures with and without texture surrounds. All stimuli had normal pixel intensity distributions. Image luminance contrast was

calculated as the standard deviation of the pixel intensity distribution. Discrimination thresholds were measured at nine pedestal contrasts for each condition. By fitting Naka-Rushton function derivatives to these thresholds, we estimated the dependence of neuronal firing rates in early visual cortex on stimulus contrast.
Response estimates for gratings with parallel surrounds were consistently lower than for the orthogonal surrounds, replicating previous findings. Perceived contrast of broadband textures was also suppressed by the presence of surrounding texture; however, rotation of the surround did not produce a release from suppression, while noise surrounds did result in higher internal response. Our results indicate that phase structure modulates surround suppression and therefore the prediction signal. This paradigm will be incorporated into future high-field fMRI experiments to determine the cortical depths at which prediction and prediction error are implemented in the primary visual cortex. Furthermore, this paradigm will be used as the basis for a computational model of the neural circuits underlying predictive coding.

Disclosures: **E.S. Semaya:** None. **K.B. Weldon:** None. **C.A. Olman:** None.

Poster

143. Spatial and Chromatic Vision

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Topic: D.07. Vision

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Title: Double-opponent cells in macaque primary visual cortex: Receptive field structure and spatial integration

Authors: A. DE[1,2], ***G. D. HORWITZ**[1,2];
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Abstract: The primary visual cortex (V1) of macaque monkeys houses a variety of physiologically distinct cell types. Of those, simple and double-opponent (DO) cells respond to light edges and preserve information about spatial phase. Simple cells have receptive fields (RFs) that can be modeled as Gabor functions, and they integrate ON and OFF luminance signals roughly linearly across their RFs. Less is known about the RF properties of DO cells. To investigate the similarities and differences between these two cell types, we compared their spatial RF structures quantitatively and examined whether DO cells integrate visual signals linearly across their RFs.We recorded extracellular spiking activity from well-isolated V1 neurons in awake, fixating macaques (two male Macaca mulatta). Each neuron was visually

stimulated with colorful, dynamic white noise patterns. Spike-triggered averaging was used to reveal spatial weighting functions. The spatial weighting function of each cell was fit with an oriented Gabor model and an unoriented Difference of Gaussian (DoG) model. The Gabor model provided the better description for almost every simple and DO cell we studied. The superiority of the Gabor fits was slightly clearer for simple cells than DO cells. To determine whether DO cells integrate visual signals linearly across their RFs, we used an online closed-loop stimulus generation technique to identify chromatic edges that drove identical spike count responses but varied in color. Many DO cells responded to the spatial color differences similarly to how simple cells respond to spatial luminance differences. These observations are consistent with qualitative models of simple and DO cells receiving input from the same set of LGN afferents with a minor change to the wiring.We speculate that DO cells converge onto complex cells to eliminate information about the spatial phase on cone-opponent signals, just as simple cells are thought to converge onto complex cells to eliminate information about the spatial phase of cone non-opponent signals. Such a role is consistent with the color and orientation tuning of one class of complex cells.

Disclosures: **A. De:** None. **G.D. Horwitz:** None.

Poster

143. Spatial and Chromatic Vision

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Topic: D.07. Vision

Support: Whitehall Foundation
Univ. of Texas at Austin
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Title: Graded rod saturation and the correspondence with receptive field properties in mouse V1

Authors: *I. RHIM[1,2], G. COELLO-REYES[1,2], I. M. NAUHAUS[1,2,3];
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Abstract: The visual system's dynamic range to different light levels can mostly be attributed to the complementary sensitivity of rods and cones. Previous studies of mouse visual cortex are largely based on rod inputs, which can be gleaned from the fact that visible wavelengths were able to drive all regions of the visual field; 'S' (short) cone opsin dominates the cone mosaic in the ventral 2/3 of the retina and is only sensitive to UV wavelengths. In the mouse, cones are greatly outnumbered by rods throughout the entire retina (35:1), yet the average cone packing density (per mm) is similar to the primate periphery. More generally, rod inputs cannot

differentiate between colors and are routed through relatively sluggish and spatially broad circuitry in the retina. Here, we tested whether spatio-temporal tuning in mouse V1 is modulated by the level of rod saturation. To begin, we quantified rod saturation using V1 responses to S- and M-isolating stimuli. We previously showed that the mouse retina's dorsoventral M-to-S cone opsin gradient is recapitulated in V1 and higher visual areas to form a "cone opsin map" that is aligned with the cortex's vertical retinotopy (Rhim et al., J. Neurophysiol. 2017). Here, we show that the cone-opsin map gradually disappears as we reduce light levels, which enables us to perform the first quantification of rod vs. cone contributions to cortical processing, as a function of photon flux at the input of the intact mouse eye. Importantly, the monitor and preparation span the dynamic range of rod vs. cone processing so that our lowest and highest light levels yield close to 0% and 100% rod saturation, respectively. In turn, we are able to address questions about differential contributions of rods and cones to spatio-temporal processing in the cortex. We demonstrate that the cortex encodes slightly higher spatial frequencies under scotopic vision, and significantly higher temporal frequency under cone vision. These findings are verified using rod-deficient KO mice (Gnat1 -/-).

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Poster

143. Spatial and Chromatic Vision

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Program #/Poster #: 143.09/L33

Topic: D.07. Vision

Support: MOST 104-2320-B-002-065-MY3

Title: Response asymmetry of red and green in macaque primary visual cortex

Authors: *W.-M. HUANG[1], H.-Y. WU[1], Y.-C. PEI[2], C.-I. YEH[1];
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Abstract: Contrast between long-wavelength (L-cone) and middle-wavelength (M-cone) signals is one of the channels (the L-M channel) that processes chromatic information in early visual pathway. Recent studies have found that the weights of L-cone and M-cone inputs in the primary visual cortex (V1) are not equal - many V1 neurons tend to prefer red (L-cone) to green (M-cone) stimuli (Conway and Livingstone, 2006; Lafer-Sousa, et al., 2012; Shirhattia and Ray, 2018). However, the red-green imbalance has not been reported in the lateral geniculate nucleus (LGN, Derrington, Krauskopf and Lennie, 1984; Gegenfurtner, 2003). These results raise a question that whether the red-over-green preference is generated within V1. We addressed this question by measuring neuronal activities with Neuronexus neural probes (64 channels) in different layers of macaques V1. Color sparse noise were composed using equiluminance red and

green with constant short-wavelength cone weight, and were used to measured spatio-temporal receptive fields. Cortical layers and electrode depths were identified based on the reconstruction of the lesion sites in CO-staining slides and the current source density analysis. We analyzed both single-unit activities and local field potential (LFP). For single-unit activities, we calculated the signal-to-noise ratio (SNR) as the ratio of the variances of the receptive field at the peak time and 0-40ms before stimulus onset (Yeh et al., 2009). Response preference was quantified as the ratio of the SNRs of red and green maps. We found that a higher number of macaque V1 neurons preferred red to green (n=172 out of 203). The red-over-green preference was stronger for layers 2/3 neurons than for layer 4 neurons. Based on LFP signals induced by red or green stimuli, the red induced a broader visual spread (the visual spread was calculated as the variance of the fitted Gaussian function, Xing et al., 2009) and a larger gamma-band (20-60 Hz) power than the green. Overall, our results showed that the red/green asymmetry was likely originated within V1. The disparity between red and green signals was amplified in the superficial layers of V1, which could be the neural basis of the perception asymmetry of red and green colors (Sakurai and Mullen, 2006).

Disclosures: **W. Huang:** None. **H. Wu:** None. **Y. Pei:** None. **C. Yeh:** None.

Poster

143. Spatial and Chromatic Vision

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Program #/Poster #: 143.10/L34

Topic: D.07. Vision

Support: NRF-2017M3C7A1029659

Title: Chromatic sensitivity affected by depressive symptoms

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Abstract: Previous studies have suggested that depression is associated with impaired chromatic sensitivity using subjective self-report measures (Barrick et al., 2002; Goodwin & Jamison, 1990). However, it has not been addressed whether impaired chromatic sensitivity is directly related to perception of color. In the current study, we investigated whether depressive symptoms, inferred by Beck Depression Inventory-II (BDI-II) score (Beck et al., 1996), modulated chromatic sensitivity and perception of color. The stimuli were Gabor patches (3-deg visual angle) with cardinal chromatic contrast (L-M axis and S axis), which was systematically varied in cone contrast (7 levels low to high). In each trial, a standard patch (middle contrast level) was presented with a comparison patch (one of 7 contrast levels) left and right side of the

central fixation. Two Gabor patches were tilted 45° and -45° from vertical orientation. After 500ms of the stimulus presentation, Mondrian-like masks consisting of randomly arranged, multicolored squares were presented for 200ms to prevent color afterimage. Participants (N=41) were engaged in a two-alternative forced choice task, in which they reported the orientation of more colorful stimulus (left or right) by pressing designated keys on a computer keyboard. Participants were divided into three groups; low (0~13), middle (14~19), and high severity (20~63) of depressive symptoms depending on the BDI-II score. Our results showed that vividness discrimination function for L-M cone-contrast tended to be steeper in the high depressive symptom group compared to the low depressive symptom group. These results suggest that depressive symptoms may affect chromatic contrast sensitivity and color perception.

Disclosures: **J. Song:** None. **S. Hong:** None. **C. Kim:** None.

Poster

143. Spatial and Chromatic Vision

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Program #/Poster #: 143.11/L35

Topic: D.07. Vision

Support: R01MH116914

Title: Narrowband gamma oscillations in human visual cortex display color selectivity

Authors: *E. BARTOLI, W. H. BOSKING, Y. Y. CHEN, M. BEAUCHAMP, S. A. SHETH, D. YOSHOR, B. L. FOSTER;
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Abstract: Visual gamma oscillations are narrowband signals in the ~20-60 Hz range occurring in early visual cortex. The amplitude and frequency of these narrowband gamma (NBG) responses are dependent on stimulus attributes, such as size and contrast of grating or edge-like features falling within the receptive field. These stimulus dependencies appear consistent with the well-known orientation tuning of early visual cortices. However, it has recently been shown that visual NBG responses are enhanced by red/orange hues in the non-human primate. This observation challenges the view that image edges and contours are critical for NBG responses, as appropriate color stimuli without spatial structure robustly drive NBG responses.
In the present work, we examined whether NBG recorded intracranially from human visual cortex also displays color selectivity. Four subjects performed a visual color task, where they viewed full-screen colors (9 colors, equally spaced in CIELAB space, presented for 500 ms and with ISI between 1.5-2 s). Importantly, stimuli were static solid color images containing no spatial structure.
Spectral analysis revealed different time-frequency response profiles depending on the color hue.

A common feature was a transient broadband response at stimulus onset and offset, partially related to visual evoked potentials. Critically, NBG responses were tuned for red and orange colors preferentially, with features (spectral and temporal) similar to those classically observed for visual gratings. The average NBG amplitude enhancement varied according to the hue value from ~40% for red to ~3% for gray ($p<0.01$). To further test the impact of these findings for natural vision, we explored the influence of stimulus color on NBG responses to natural images. In one subject, we presented color and grayscale versions of the same image (e.g. red bell pepper). A sustained narrowband response was observed for the color image and not for the same image in grayscale, while the image features present in the receptive field were unchanged. Together, these findings suggest that visual NBG responses show a dependence not only on image structure (edges/contours) but also on color (red/orange hues), extending to the human brain prior evidence of such an effect in the monkey. The direct comparison between grayscale and color stimuli further underlines the importance of color profile in shaping the spectral response to natural images. Future models of NBG genesis need to incorporate color selectivity as an additional dimension of stimulus attributes modulating the occurrence of gamma range signals, further adding to the controversy regarding their functional role.

Disclosures: **E. Bartoli:** None. **W.H. Bosking:** None. **Y.Y. Chen:** None. **S.A. Sheth:** None. **D. Yoshor:** None. **B.L. Foster:** None. **M. Beauchamp:** None.

Poster

143. Spatial and Chromatic Vision

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 143.12/L36

Topic: D.07. Vision

Support: ERC project BrainBit (GA n. 692943)

Title: Whole brain map of neuronal responses triggered by colored visual stimuli in zebrafish larvae

Authors: *C. FORNETTO[1], N. TISO[2], F. S. PAVONE[1,3], F. VANZI[1,4];
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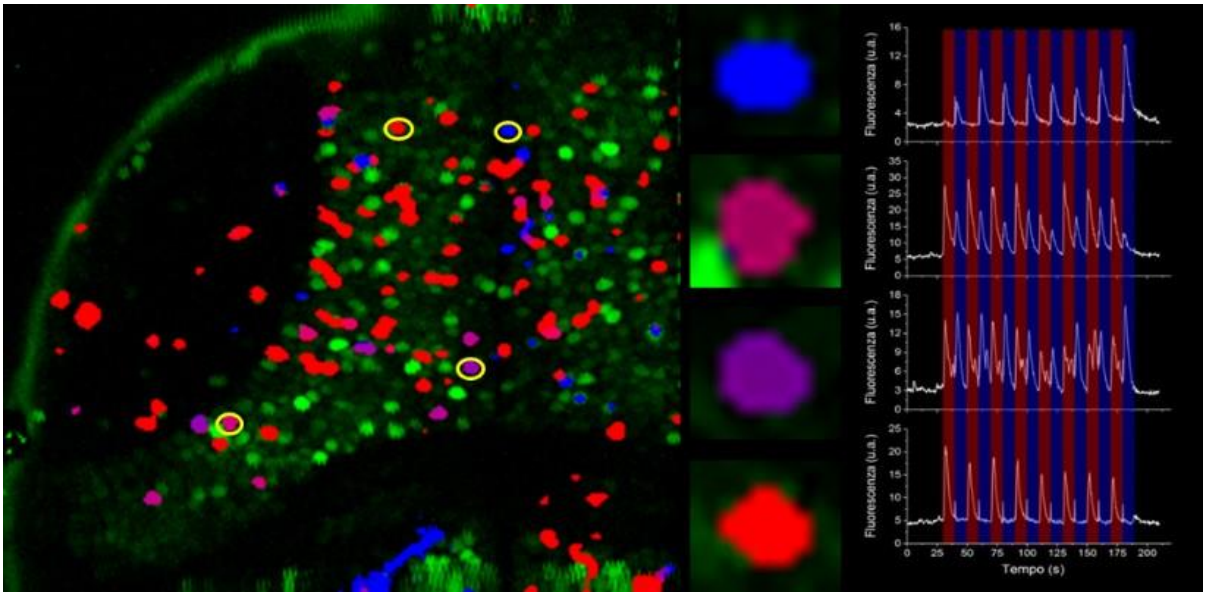
Abstract: Mapping neuronal circuits activated during the response to controlled stimuli requires imaging large volumes with enough spatial and temporal resolution to map cellular networks and their activity.
Zebrafish larvae represent an ideal model for these studies, both for their optical transparency and a simple central nervous system allowing single-cell recordings of activity in the whole

brain. In spite of this simplicity, zebrafish is capable of complex behaviors at very early stages of development (4-5 days post fertilization), in particular visually-guided behaviors such as Opto-Motor Response, Opto-Kinetic Response, phototaxis and pray capture. In this work we employ a nuclear-localized GCaMP6s transgenic line to study visual responses in larval zebrafish brain and the phototactic behaviors associated with color sensing and discrimination. While the different encephalic regions involved in processing visual information have been identified, the actual neuronal circuits activated during stimulation with visual colored stimuli have not yet been characterized. This study requires mapping neurons, in different areas of zebrafish brain, responsive to controlled stimuli at each of the four wavelengths relevant for color vision (362, 415, 480, 570 nm; Guggiana-Nilo and Engert, Front. Behav. Neurosci., 2016) while imaging neuronal activity with two-photon microscopy. The figure shows neurons of the optic tectum responding to 480 and 570 nm flashes. A mosaic two-photon fluorescence acquisition (covering a volume of 800x400x200 μm^3) has been implemented for full volumetric reconstruction of the whole encephalon. For the identification of responsive neurons, we developed a linear regression analysis and we obtained a whole brain map of color-responsive neurons with cellular resolution. This approach provides a comprehensive map of all neurons involved in color-driven responses in the zebrafish larva, opening the way to a detailed dissection of the circuits responsible for different phototactic behaviors.



Disclosures: **C. Fornetto:** None. **N. Tiso:** None. **F.S. Pavone:** None. **F. Vanzi:** None.

Poster

143. Spatial and Chromatic Vision

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

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Topic: D.07. Vision

Support: NIH Grant EY18363
NSF Grant 1457238
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Title: Eye movements enhance visual acuity

Authors: J. INTOY, ***M. RUCCI**;
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Abstract: Tests of visual acuity are commonly taken to measure the limits in visual resolution imposed by the optics of the eye and neural sampling of the retina. However, previous studies have shown that fine spatial vision also depends on eye movements. Humans continually move their eyes during fixation, when small rapid gaze shifts (microsaccades) interrupt an otherwise incessant jitter of the eye (ocular drift). Previous studies have shown that these fixational eye movements (FEM) enhance discrimination of fine patterns both by precisely centering the stimulus within the foveola and by converting spatial information in the form of temporal modulations on the retina.
Here we investigated the overall contribution of FEM to visual acuity in humans. We recorded eye movements during execution of the most common test of visual acuity, the Snellen eye chart, and eliminated their consequences on the retinal input via high-resolution gaze-contingent control. Observers reported the orientations of six optotypes on either the 20/20 line of an eye chart for monocular viewing (n=7) or the 20/16 line for binocular viewing (n=6). Eye movements were recorded by means of a Dual Purkinje image eye-tracker. Custom gaze-contingent calibration procedures enabled precise localization of the lines of sight. We found that both components of FEM, microsaccades and drift, are finely tuned to the task. Conjugate microsaccades precisely shifted gaze from one optotype to the next. Drift movements were considerably slower and more curved in the Snellen test than during fixation on a marker, resulting in a luminance flow that amplified the power of the optotypes on the retina by ~50%. We used retinal stabilization, a procedure that immobilizes the stimulus on the retina, to counteract the consequences of FEM on the visual input flow. Acuity fell by more than 0.15 logMAR under retinal stabilization, corresponding to more of 2 lines in the Snellen chart. These findings reveal that visual acuity relies on a surprising degree of oculomotor control. Humans finely tune their FEM, both microsaccade and ocular drift, in a way that greatly contributes to normal visual acuity.

Disclosures: **M. Rucci:** None. **J. Intoy:** None.

Poster

143. Spatial and Chromatic Vision

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 143.14/L38

Topic: D.07. Vision

Support: University of Oregon

Title: Visual response properties and functional organization of the octopus optic lobe

Authors: *J. PUNGOR[1], C. M. NIELL[2];
[2]Inst. of Neurosci., [1]Univ. of Oregon, Eugene, OR

Abstract: Cephalopods have remarkable eyes that instruct a rich repertoire of visually guided behaviors, from hunting prey and avoiding predation, to finding mates and communicating with conspecifics. Although superficially similar, the camera-type eyes of cephalopods and vertebrates emerged independently, resulting in one of the most stunning examples of convergent evolution. Cephalopods therefore have a vertebrate-like sensor (eye) connected to an invertebrate-like processor (brain). Despite the enticing complexity and tremendous capability of this visual system, relatively little is known about visual coding and neural computations in cephalopods, as there has been no direct measurement of the visual response properties of neurons in the central visual system. In this study, we aimed to identify the visual features extracted by the octopus visual system using two-photon calcium imaging of the primary visual processing area of their central brain, the optic lobe. By loading the fluorescent calcium indicator Cal-520 AM-ester into an ex-vivo preparation of the central brain and eyes of Octopus bimaculoides, we were able to visualize the activity of hundreds of cells across multiple layers of the optic lobe simultaneously. We presented an array of visual stimuli on a projection screen on the side of the imaging chamber, and recorded the response dynamics of cell populations throughout the lobe. We found that cells have spatially localized receptive fields, and many are selective for distinct features of the stimuli, including luminance polarity (On/Off). Furthermore, we found a retinotopic organization of response to stimuli across the optic lobe, and patchy organization for On/Off preference. These data are the first measurements of neural coding and functional circuit organization in the central visual system of cephalopods, and provide a foundation to begin studying how the octopus brain performs the computations necessary to guide visual behavior.

Disclosures: J. Pungor: None. **C.M. Niell:** None.

Poster

144. Eye Movements: Saccades in Nonhuman Primates

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 144.01/L39

Topic: E.01. Eye Movements

Support: Fondation pour la Recherche Médiacale (FRM)
CNRS

Title: Tracking a moving visual target in the monkey: Influence of the path frequency

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Abstract: Multiple behavioral studies report that the ability to smoothly track (without saccades) a moving target depends upon the "predictability" of its path; trackings are saltatory, i.e., composed of several saccades when the path is "unpredictable". When the set of possible paths that the moving target can take is limited, the predictability of one path depends upon the frequency of its occurrence. Thus, a target that moves seldom along a particular path is less predictable than a target moving always along the same path. In this study, we tested in two monkeys how the frequency of occurrence (the probability) of a path influenced the triggering and accuracy of tracking eye movements.
After the monkey fixated a static target located straight ahead for a variable interval (750-1500 ms) and a short blank interval (300 ms), the central target re-appeared and moved along one out of four possible oblique paths. The monkey was rewarded for tracking the moving target until it disappeared in the peripheral visual field. Considering each target path among four possible ones, five frequencies of occurrence were tested during separate blocks of trials (recorded during different days): 10% (rare), 25% (equiprobable), 50% (likely), 70% (very likely) and 100% (certain). In the complementary fraction of trials (90%, 75%, 50%, 30% and 0%), the target path was randomly selected among the three remaining paths. Also, two target velocities were used (20 and 40°/s, one for each block).
After target motion onset, before the interceptive saccade onset, the eye drifted further toward the quadrant where the target moved during the "certain" and "very likely" conditions than during the other ones. The latency of interceptive saccades was not influenced by the frequency of the target path. Their accuracy and precision were not influenced either, neither by the frequency of the target path nor by the amount of pre-saccadic drift. After the interceptive saccade, the eye followed the moving target, with no noticeable difference between the different path frequencies. Regardless of the probability, no consistent difference was observed between the Position/Time landing ratios (horizontal or vertical landing position divided by landing time) of interceptive saccades and the post-saccadic pursuit velocities. Finally, the accuracy and

precision of catch-up saccades did not depend on the frequency of the path.
In conclusion, our results show that in the monkey, the frequency of a target path does not influence the accuracy and precision of interceptive and catch-up saccades. An influence on presaccadic slow eye movement is observed, but only when the path is fully or highly predictable.

Disclosures: N. Orlando dessaints: None. **A. Montagnini:** None. **L. Goffart:** None.

Poster

144. Eye Movements: Saccades in Nonhuman Primates

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

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Topic: E.01. Eye Movements

Support: NSF Grant 1811543
NIH Grant EY026924
NSF Grant 1439221
NIH Grant EY014800
Research to Prevent Blindness Inc., New York, NY

Title: A statistical framework for characterizing the perisaccadic spatiotemporal sensitivity of visual neurons

Authors: *Y. ZAMANI[1], A. AKBARIAN[1], B. NOUDOOST[2], N. NATEGH[1,2];
[1]Electrical & Computer Engin., [2]Ophthalmology & Visual Sci., Univ. of Utah, Salt Lake City, UT

Abstract: Rapid eye movements, called saccades, alter responses of visual neurons to the visual stimuli presented around the time of saccades, and these changes emerge before the eye begins to move. These perisaccadic changes include the emergence of sensitivity to stimuli presented at locations outside the neuron's classical receptive field, or responses occurring at times past the usual latency of the neuron. In order to characterize these sensitivity changes, we devised a quantitative approach to trace changes in the neuron's perisaccadic sensitivity across space and time on the precise spatial and temporal scale at which they occur. In this study, we use a high spatiotemporal resolution experimental paradigm along with a statistical approach to precisely map the temporal evolution of the spatiotemporal receptive field of visual neurons in the middle temporal and V4 areas across a saccadic eye movement. We represent the neuron's sensitivity using a collection of spatially and temporally precise basis units defined across the time to saccade, time to stimulus, and space dimensions. We then develop a statistical framework which quantifies the contribution of each of these spatial and temporal bases to describing the statistics of the neuron's spiking activity at each time point with respect to the saccade time. We also

utilize this high-resolution discretized representation to build a model using an extension of the generalized linear models that can account for the neuronal response modulations across a saccade on a millisecond timescale. We then evaluate how much each basis component across time and space contributes to driving the instantaneous spiking responses. This approach enables us to characterize the changes in the spatiotemporal sensitivity of neurons with high spatial and temporal precision around the time of saccades, which is critical for understanding the complex, fast modulations of visual neurons' responses across a saccadic eye movement.

Disclosures: Y. Zamani: None. **A. Akbarian:** None. **B. Noudoost:** None. **N. Nategh:** None.

Poster

144. Eye Movements: Saccades in Nonhuman Primates

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 144.03/L41

Topic: E.01. Eye Movements

Support: NSFC 31871078

Title: How do monkeys perform double-step saccades tasks

Authors: *M. LI[1], B. LI[2], M. ZHANG[1];
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[2]Beijing Inst. of Basic Med. Sci., Beijing, China

Abstract: Human and non-human primates are able to accurately make sequential movements to interact with surrounding environment. There are at least two different theories proposed the mechanism of sequential movements, i.e., preplan theory and online updating theory. The preplan theory proposes that, prior to the initiation of the first movement, the sequential movements are pre-programed. In contrast, the online updating theory argues that the sequential movements are completed by updating the targets' locations following each movement. To test these two hypotheses, we trained monkeys to perform two double-step saccade tasks: **T1-T2 task**, after fixation point disappeared, target one (T1) and then target two (T2) sequentially flashed, and monkeys need to make two saccades to the locations of these two targets (firstly to T1 and then to T2); **T2-T1 task**, after fixation point disappeared, T2 and then T1 sequentially flashed, monkeys need to first make a saccade to T1 and then to T2. Trials in T1-T2 task could be separated into two groups according to whether the targets remained visible when monkeys made saccades. One group is memory-guided double-step T1-T2 saccades (the targets disappeared before first saccade), the other is visually-guided T1-T2 double-step saccades (the targets remained visible during saccades). We analyzed the first saccadic reaction time (FSRT) and inter saccadic interval (ISI). Our hypothesis is that, if the monkeys adopt the preplan strategy, the FSRT should be longer and ISI should be shorter. Conversely, if they adopt the

updating strategy, the FSRT should be shorter and the ISI should be longer. We found that: I. the first saccadic reaction time (FSRT) is greatest in the memory-guided T1-T2 trials, the shortest in the visually-guided T1-T2 trials, and the FSRT in T2-T1 trials is in between; II. The inter saccadic interval (ISI) is the shortest in the memory-guided T1-T2 trials, the greatest in the T2-T1 trials, and ISI in visually-guided T1-T2 trials is in between; III. ISI is negatively correlated only with FSRT in memory-guided T1-T2 trials, but not in visually-guided T1-T2 and T2-T1 trials. Such results indicate that monkeys dynamically alter the strategy to perform the memory-guided double-step saccades depending on the context of task. While the two saccadic targets appeared succession in time (T1-T2 task), monkeys primarily preplan two successive saccades before the initiation of the first saccade. In contrast, while the two saccadic targets appeared in sequence against the flowing of time (T2-T1 task), monkeys primarily update the spatial location of second saccade target after completing the first saccade.

Disclosures: **M. Li:** None. **B. Li:** None. **M. Zhang:** None.

Poster

144. Eye Movements: Saccades in Nonhuman Primates

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 144.04/L42

Topic: E.01. Eye Movements

Support: NIH Grant EY022854
NIH Grant EY024831

Title: Sources of endpoint position variability of saccades produced in the double-step task

Authors: ***C. BOURRELLY**[1], N. J. GANDHI[1,2];
[1]Dept. of Bioengineering, [2]Ctr. for the Neural Basis of Cognition, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Movements are inherently variable. Repeated attempts of an identical action exhibit substantial variability in both metrics (endpoint position) and kinematics (peak velocity, duration). This heterogeneity in behavior is largely due to variability in neural signals, not biological noise in endpoint effectors. More specifically, variability in the sensory representation of the stimulus is thought to contribute more significantly than variability in neural processes associated with motor planning and movement execution. Importantly, this interpretation is derived from statistical inference and computational models because, in the standard behavioral tasks used previously, the vectors representing stimulus location and required movement were identical. We instead sought to obtain behavioral evidence for sensory and/or motor sources of variability in movement generation. We used the saccadic eye movement as our model system. We employed a variation of the memory-guided double step paradigm, in which two targets were

presented successively around an imaginary circle, and a Rhesus monkey was trained to make eye movements to the remembered locations of these two targets in the same order as they were presented. Hence, the sensory vector associated with the second target was dissociated from the movement vector required to fixate that location. We analyzed the saccade endpoint variations as a function of saccade and target directions. Preliminary results point to contributions by both sensory and motor origins of the variability. They also emphasize the importance of motor variability relative to what has been proposed earlier. Thus, further evaluation of the contributions of the different sources of movement variability is required. More than just variable, movements are also compensated. After an imprecise movement to the first target, the motor plan for the second saccade needs to be updated to achieve accuracy. This compensation is thought to be based on the corollary discharge of the first motor vector. This suggestion is mostly based on average analysis, leaving unanswered the effect on individual trials. Thus, we analyzed saccade compensation across trials for the same behavioral task. Preliminary results suggest that the compensation is substantially variable across trials, highlighting inconsistencies in mechanisms based solely on corollary discharge. Together, these results are crucial to understanding how the brain controls movement generation.

Disclosures: **C. Bourrelly:** None. **N.J. Gandhi:** None.

Poster

144. Eye Movements: Saccades in Nonhuman Primates

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 144.05/L43

Topic: E.01. Eye Movements

Support: This work was funded by a grant of the Fonds de la Recherche Scientifique Médicale (FRSM) number 3.4.523.08.F (www.fnrs.be/)
Agence Nationale de la Recherche (ANR) referred to as ‘SHS 2 2012 PeDu’

Title: Ketamine reduces temporal preparation in the Rhesus monkey

Authors: S. BRULÉ[1], B. HERLIN[1], P. POUGET[1], ***M. MISSAL**[2];
[1]ICM,INSERM UMRS 975, CNRS UMR 7225, UPMC, Paris, France; [2]Inst. of Neurosciences, IONS - COSY, Univ. Catholique de Louvain, Brussels, Belgium

Abstract: Ketamine, a well-known general dissociative anesthetic agent that is a non-competitive antagonist of the N-methyl-D-aspartate receptor (NMDAr), has been shown to induce various dose-dependent psychoactive and psychiatric effects. It has also been shown that ketamine could reduce the readiness to act before a predictable event or temporal preparation. In the present study, we investigated the influence of ketamine on temporal preparation before saccades to a visual target. Rhesus monkeys were trained to make a saccade between a central

and an eccentric visual target appearing on a screen. The foreperiod (FP) between the extinction of the central target and the appearance of the eccentric one could take one of four values randomly with the same probability (400, 900, 1400 or 1900 ms in monkey S; 50, 167, 283, 400 in monkey L). During experimental sessions, a subanesthetic dose of ketamine (0.35 mg/kg i.m.) or a saline solution of the same volume was injected between the 1st and the 2nd block of trials. A total of twenty-two injections of ketamine were performed in monkey S (4780 saccades) and 6 injections in monkey L (2702 saccades). We found that in the control conditions (saline solution or no injection), saccadic latencies strongly decreased with elapsed time during the FP showing that temporal preparation built up before the appearance of the eccentric target. However, the influence of FP duration on saccadic latency was significantly less intense after ketamine injection in both monkeys. We conclude that ketamine reduces the influence of elapsed time on saccadic preparation.

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Poster

144. Eye Movements: Saccades in Nonhuman Primates

Location: Hall A

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Program #/Poster #: 144.06/L44

Topic: E.01. Eye Movements

Support: CIHR Foundation Grant
CIHR Fellowship
BrainsCAN Fellowship

Title: Single unit activities in the marmoset parietal cortex during a saccadic task

Authors: *L. MA, J. SELVANAYAGAM, L. K. H. SCHAEFFER, K. D. JOHNSTON, S. EVERLING;
Univ. of Western Ontario, London, ON, Canada

Abstract: Abnormal saccadic eye movements are characteristic for patients with several psychiatric and neurological disorders but are difficult to study in non-primate animals. The common marmoset (*Callithrix jacchus*) is a promising nonhuman primate model with a lissencephalic brain, allowing for accurate targeting of brain regions that are hidden in sulci in the macaque brain. We trained two marmosets on a task in which Gap trials (stimulus onset lagged fixation spot offset by 200ms) were interleaved with Step trials (the two events were simultaneous). Both marmosets showed a gap effect commonly observed in humans, which is a reduction in saccadic reaction times (SRTs) in Gap trials compared to Step trials. Both spiking activities and local field potentials were recorded during the task through 32-channel microelectrode arrays (Utah array) implanted in the posterior parietal cortex (PPC). Among 361

isolated units we found 56 gap-modulated cells (15.5%), the activities of which changed significantly from the pre-gap fixation period to the gap period. Among these cells, 46 (12.7%) were only modulated by gap and did not respond on Step trials. When the stimulus was presented contralaterally to the area recorded, activities of these cells were predictive of the subsequent SRTs, and whether the response would be an express (SRT<=104ms) or regular (SRT>104ms) saccade. We also found 143 cells (40%) that displayed a significant visual response 70-120ms after stimulus onset, the intensity of which predicted the SRTs in all trial types. Our findings suggest that the common marmoset has a PPC that plays similar functional roles as the lateral intraparietal area in macaque monkeys.

Disclosures: L. Ma: None. **J. Selvanayagam:** None. **L.K.H. Schaeffer:** None. **K.D. Johnston:** None. **S. Everling:** None.

Poster

144. Eye Movements: Saccades in Nonhuman Primates

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Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 144.07/L45

Topic: E.01. Eye Movements

Support: Deutsche Forschungsgemeinschaft CRC/TRR-135 – A1

Title: Encoding of interceptive saccades in parietal cortex of macaque monkeys

Authors: *J. CHURAN[1,2], D. I. BRAUN[3,2], K. R. GEGENFURTNER[3,2], A. KAMINIARZ[1,2], F. BREMMER[1,2];
[1]Philipps-University, Marburg, Germany; [2]Ctr. for Mind, Brain and Behavior, Universities of Marburg and Giessen, Germany; [3]Giessen Univ., Giessen, Germany

Abstract: Humans and non-human primates use a combination of saccades and smooth pursuit when observing a moving visual target. The initial saccades towards such a moving target (interceptive saccades) were shown to be very accurate; this requires to extrapolate the motion direction and speed of the target to bridge the durations of programming and execution of the saccade. The neuronal basis of this process is still under debate. Recordings from the intermediate layers of Superior Colliculus (SC) have shown that the spatial tuning of SC-neurons does not fully account for the trajectory of interceptive saccades since it does not take the motion of the stimulus fully into account. The Lateral Intraparietal Area (LIP) is a part of the saccadic system and is connected to other oculomotor centers like the Frontal Eye Field and the SC. Its neurons show peri-saccadic activation that is dependent on the saccade trajectory, but the spatial tuning is rather broad. We investigated the involvement of LIP in the generation of interceptive saccades by recording peri-saccadic activity from 196 LIP-neurons from two macaque monkeys during interceptive saccades towards targets moving in 8 different directions. In a first step we

measured and used the tuning of neurons towards stationary targets to create a probability map of saccade trajectories based on the peri-saccadic activity of each neuron. We then combined the probability maps of all individual neurons in our sample to predict the landing point of the saccade. We found that a combination of even a relatively small number of 50 tuned neurons predicted the saccade end-point with an accuracy of ~1°. In a second step we used the same probability maps to predict the landing point of interceptive saccades. We found that the neuronal activity in a time-window from 20 ms before to 60 ms after saccade onset predicted the saccade-endpoint of interceptive saccades considerably well. This prediction was less accurate (accuracy ~3°) than for the saccades towards stationary targets. Importantly, the distribution of predicted end-point probabilities did not show a shift towards 'earlier' stimulus positions as found in SC neurons. Instead it was centered on the end-position of the interceptive saccades or even represented saccades towards 'later' stimulus positions. We conclude that the position and the motion of a saccade target in full 2-D frontoparallel space are represented in neuronal activity in area LIP. This representation seems to be different from the results obtained in SC.

Disclosures: J. Churan: None. **D.I. Braun:** None. **K.R. Gegenfurtner:** None. **A. Kaminiarz:** None. **F. Bremmer:** None.

Poster

144. Eye Movements: Saccades in Nonhuman Primates

Location: Hall A

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Program #/Poster #: 144.08/L46

Topic: D.08. Visual Sensory-motor Processing

Support: Brain/MINDS project, AMED, Japan
Crick-Clay Professorship CSHL
H N Mahabala Chair IIT Madras

Title: Cortico-tectal projections in marmoset monkeys studied in a brain-wide neurohistological pipeline

Authors: *D. MATROV[1], M. LIN[2], C.-Y. CHEN[1], B.-X. HUO[2], M. HANADA[3], J. NAGASHIMA[3], J. A. BOURNE[4], P. P. MITRA[2], T. ISA[1];
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Abstract: Cortical area functionally identified as a "frontal eye field" (FEF) controls saccadic eye movements through several descending pathways, including a projection to the intermediate layers of the superior colliculus (SC). The anatomical boundaries of FEF in marmosets are not settled. The prevalent opinion equates FEF with the anatomical area 8aV. Herein we present

results of retrograde tract-tracing after AAV2retro-CAGGS-EGFP (titer 6 * 10^9 vg/µl) and Fast Blue injections into the superficial and intermediate layers of SC in 3 marmosets. The injection technique was modified to minimize the leakage of the tracer outside of the target area. The analysis of cortico-tectal connectivity was greatly enhanced by the recently established histologico-computational pipeline (Lin et al. 2019). Such pipeline allows to significantly improve the study of brain connectivity in smaller primates by eliminating many sources of histological distortion and bringing together post-mortem MRI, cytoarchitectural and connectivity data into the common reference space. The presentation of the projection patterns on the shared 3D template enables a reliable comparison between cases and makes effective the presentation of the results. We present the detailed analysis and visualizations from the 3 cases of SC injections. All 3 injections produced a bilateral pattern of cell labeling in the prefrontal cortex: the number of the labeled cells and the spatial extent of the projecting brain areas were larger in the ipsilateral hemisphere. The number of the marked cells in the prefrontal cortex was also proportional to the size of the injection bolus. The smallest injection was confined to the rostrolateral quadrant in SC, which corresponds to the lower half of central visual space. Correspondingly, cortical areas located in the lateral corner such as 47 and 45 exhibited the strongest connectivity. Only very ventral part of the area 8aV exhibited retrogradely labeled cells. As the centers of the injection sites moved more centrally in the coronal plane and caudally in the sagittal plane, the corresponding label in the prefrontal cortex expanded, mostly in the dorsolateral direction. In summary, the cortical oculomotor control likely involves additional architectonic areas besides A8aV, the prime candidates being areas 45 and 47.

Disclosures: **D. Matrov:** None. **M. Lin:** None. **C. Chen:** None. **B. Huo:** None. **M. Hanada:** None. **J. Nagashima:** None. **J.A. Bourne:** None. **P.P. Mitra:** None. **T. Isa:** None.

Poster

144. Eye Movements: Saccades in Nonhuman Primates

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 144.09/M1

Topic: E.01. Eye Movements

Support: Brain/MINDS from AMED
JSPS fellowship

Title: Mapping saccadic representation in the frontal cortex of common marmoset

Authors: ***C.-Y. CHEN**[1], D. MATROV[1], A. WAJD[1], K.-T. HO[1], T. ISA[1,2];
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Abstract: The frontal cortex in primates has several important subregions that are related to saccade generation, e.g., the frontal eye field (FEF) and supplementary eye field (SEF). These regions are also involved in higher cognitive functions, such as attention and decision making. However, in the marmosets, these areas are difficult to identify anatomically because their brain is lissencephalic. In the current study, we identified these regions by electrical microstimulation and calcium imaging in awake, behaving marmosets.We first identified candidate frontal cortical regions by retrograde labeling from the superior colliculus, a subcortical area known to be the target of FEF. We then applied electrical microstimulations with tungsten electrodes in these regions while two marmosets performing gap saccade task to control the baseline state of eye fixation. The stimulations were applied with biphasic current <= 50 μA at 250 Hz for 30 trains. We systematically varied the initial fixation locations to assess the position dependency of evoked saccades and identify whether the stimulated area was FEF or SEF. We also performed calcium imaging in similar frontal region using nVoke (Inscopix) while one separate marmoset was engaged in free viewing task using short video clips as stimuli. We successfully evoked saccades in area 8 and 45. In the lateral side of area 8v to area 45, we observed a systematic decrease in evoked saccade amplitude when the stimulation location moved from medial to lateral. If the stimulation moved from rostral to caudal, the evoked saccade changed direction from upper to lower visual field. The stimulation in a single site produced evoked saccades with the same amplitude and direction even if the initial eye positions were different. We identified these regions to be putative FEF. On the other hand, if the stimulation position was in the medial side of area 8v or area 8d, the evoked saccades converged to a single location in visual coordinate space. We identified these regions as putative SEF. By reverse correlating the neuronal activity to saccade trajectory, we could also identify saccade related neurons with calcium imaging in the same region. Taken together, we identified putative FEF and SEF in marmoset using microstimulation. We also confirmed that the neurons in this area contained saccade related activity. Because several important cortical areas related to oculomotor attention system are deep in the sulci of macaques, they are difficult to study. Using marmosets, we will not only advance our knowledge in the primate oculomotor systems, but also have easier access in these regions to study higher cognitive function using imaging techniques.

Disclosures: **C. Chen:** None. **D. Matrov:** None. **A. Wajd:** None. **K. Ho:** None. **T. Isa:** None.

Poster

144. Eye Movements: Saccades in Nonhuman Primates

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 144.10/M2

Topic: E.01. Eye Movements

Support: NSERC
CIHR

Title: Identification of the frontal eye fields in the common marmoset using microstimulation

Authors: *J. SELVANAYAGAM, K. D. JOHNSTON, D. J. SCHAEFFER, L. K. HAYRYNEN, S. EVERLING;
Western Univ., London, ON, Canada

Abstract: Studies in old world macaque monkeys have demonstrated that the frontal eye fields (FEF) play an important role in oculomotor control and visual attention. The macaque FEF, however, lies deep in the anterior bank of the arcuate sulcus, making it difficult to investigate the functional microcircuitry of this area. The common marmoset (*Callithrix jacchus*) with its lissencephalic cortex is a promising model for exploring FEF microcircuitry but the precise location of the FEF in marmosets and its functional properties remain largely unknown. Here we implanted a 96-channel Utah array (1mm electrode length, 400µm pitch), in the left frontal cortex of two adult marmosets targeting the border of area 8aD with area 6DR. We selected this area on the basis of resting-state fMRI, which revealed strong functional connectivity with the superior colliculus. Individual electrodes were stimulated with 100 ms biphasic pulse trains (300 Hz, 0.2-0.3 ms pulse duration) while the monkey was head-restrained and freely viewing a video clip. At sites in area 8aV, fixed vector saccades were evoked at short onset latencies. An effect of initial eye position was observed where microstimulation often failed to elicit saccades when gaze position was initially in the contralateral hemifield. Evoked saccades were contraversive with systematic variation in direction where downward saccades were elicited at more anterior sites and upward saccades at more posterior sites. Amplitude mapping was also observed at these sites with larger amplitude saccades evoked at anterior-medial sites and smaller amplitude saccades at more posterior-lateral sites. We also observed skeletomotor responses and saccades at more posterior sites. The majority of saccades were contraversive, though some ipsiversive saccades were also observed. Evoked saccades had a longer onset latency and tended to converge at single locations in craniocentric space. Here we demonstrate a similarity of organization between the premotor cortex and FEF in marmosets and macaques. These results suggest the FEF in the common marmoset is located in area 8aV on the border of area 8aD and 6DR. Taken together, our data suggest that the common marmoset is both an appropriate and advantageous primate model for exploring FEF microcircuitry.

Disclosures: J. Selvanayagam: None. **K.D. Johnston:** None. **D.J. Schaeffer:** None. **L.K. Hayrynen:** None. **S. Everling:** None.

Poster

144. Eye Movements: Saccades in Nonhuman Primates

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 144.11/M3

Topic: E.01. Eye Movements

Support: Grant-in-Aid for Scientific Research (s)
AMED Grant number JP19dm0107151

Title: Analyzing visual instrumental learning in blindsight monkeys with reinforcement learning model

Authors: *R. KATO[1], K. MAJIMA[2], A. MURAKAMI[2], A. ZEGHBIB[3], P. REDGRAVE[3], Y. KAMITANI[2], T. ISA[1];
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Abstract: Reinforcement learning is a basic mechanism that can acquire novel adaptive behavior in response to environmental change without the need for a supervisor. Its algorithm revises incessantly agent's actions to maximize the total amount of reward using feedback signals caused by their actions. Besides, some subjects with damage to the primary visual cortex (V1) can localize visual stimuli presented in the V1-lesion affected field, despite being subjectively unaware of the visual target. This phenomenon is termed 'blindsight'. Over the past several decades, residual visual abilities in blindsight subjects have been extensively investigated. However whether residual visual systems can support visual instrumental learning remains to be elucidated. In this study, we examined whether a visual cue presented in the blind field could act as a secondary reinforcer for instrumental learning paradigm in macaque monkeys with unilateral V1 lesions. We designed a hidden target area search task in which the monkeys were required to discover the location of a hidden target area (TA) by directing their gaze within a completely blank screen. Entry to the TA was informed by presentation of a visual cue at the edge of monitor. We tested whether the monkeys were able to learn to find the TA, even when the cue was presented in the blind field. Irrespective of whether the visual reinforcer was presented in the intact or the blind hemifield the monkeys were able to learn the location of the hidden TA. Initially, both search time and total length of eye movement trajectory during the search was long. Those were gradually reduced as the monkey recurrently experienced the cue presentations. The time course of these reductions were fitted well by a decaying exponential curve. We also noted that the final saccade that took the animals' gaze into the TA tended to converge on one or two particular vectors. The result suggest that the monkeys learned various aspects of saccades immediately before the cue presentation, not just the location of TA. To provide a quantitative understanding of the effect of the visual reinforcer on learning, we constructed a reinforcement learning model according to Chukoskie et al. (2013). We found that increases in the action value to locations within the TA and to the start locations of the final saccade, greatly surpassed the value of movements to other areas, which well explained the reduction in search time, and convergence of vectors of the final saccades. Altogether, those results suggest that the visual input bypassing the V1, which is considered to lack visual awareness, can act as effective secondary reinforcer to acquire new behaviors in the instrumental learning paradigm.

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Poster

144. Eye Movements: Saccades in Nonhuman Primates

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 144.12/M4

Topic: E.01. Eye Movements

Support: Intramural Research Program at the National Institutes of Health, National Eye Institute / EY000415-16

Title: Excitatory input to globus pallidus externus facilitates saccadic eye movement

Authors: *A. YOSHIDA[1], H. AMITA[1,2], M. TANAKA[3], O. HIKOSAKA[1];
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Abstract: The globus pallidus external segment (GPe) has been considered as the core of the indirect pathway in the basal ganglia. Its main function is thought to be the suppression of actions. Since the GPe receives inhibitory inputs from the striatum, it is assumed that most neurons in the GPe decrease their activity before actions. Indeed, we have shown that many neurons in caudal-ventral GPe (cvGPe) are inhibited by historically bad objects (i.e., previously associated with small reward), and this response is used to suppress saccades to the bad objects (Amita & Hikosaka, bioRxiv 2019). However, some GPe neurons (including cvGPe neurons) increase their activity before saccades, especially in response to good objects (i.e., previously associated with large reward).
Such an increase in activity may be caused by excitatory inputs or may be caused by disinhibition (especially in the basal ganglia). To test the first mechanism, we injected the glutamatergic antagonist (CPP (NMDA antagonist) + NBQX (non-NMDA antagonist)) into cvGPe while monkeys performed visually-guided saccade tasks. As a comparison, we did the same injection into rostral-dorsal GPe (rdGPe). The injection into cvGPe as well as rdGPe caused the same effect: Increase in saccade latency. The effect of cvGPe was only for saccades toward the contralateral side, whereas the effect of rdGPe was bilateral. These results were consistent with the preferred direction of saccade-related neurons in GPe: contralateral in cvGPe vs. bilateral in rdGPe. In addition, one monkey performed NoGo task during the injection in the rdGPe and the injection did not impair the ability to suppress saccades to the distractor.
These results suggest that excitatory inputs to GPe neurons facilitate saccades. This effect is opposite to what GPe neurons create as the core of the indirect pathway. The origin of the excitatory inputs may be the subthalamic nucleus (STN) or the parafascicular thalamic nucleus (Pf). These data together suggest that the STN/Pf-GPe pathway plays an important role in controlling behavior by switching the output of the basal ganglia.

Disclosures: A. Yoshida: None. **H. Amita:** None. **M. Tanaka:** None. **O. Hikosaka:** None.

Poster

144. Eye Movements: Saccades in Nonhuman Primates

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 144.13/M5

Topic: E.01. Eye Movements

Support: NIH Intramural Research Grant

Title: Functional characterization of inter hemispheric projections of superior colliculus

Authors: *A. GOPAL P A, O. HIKOSAKA;
Lab. of Sensorimotor Res., NIH, Bethesda, MD

Abstract: The subcortical circuit involving caudate tail, SNr, and SC helps in rapid detection of high value (good) objects present among low value (bad) objects in the visual field. Neuronal responses in these regions are mostly lateralized in that they represent the value of objects in the contralateral receptive field. Value information carried separately within these lateralized networks needs to be compared to guide behaviour involving the choice of the most valuable object among an array of lesser objects. Our previous work suggested that this value comparison occurs within the SC. We trained a monkey to choose between two objects of unequal (good-bad) or equal value (good-good or bad-bad) presented on opposite sides of the vertical meridian. We found that firing rates were higher when a good object in the RF was opposed by a bad object in anti RF, compared to a good object in the RF opposed by good objects in the anti RF. We hypothesized that the sensitivity of SC neurons to the value of objects present in the anti-RF may be mediated by mutual inhibition from the opposite hemisphere of the colliculus through the inter-collicular connection. To test this, we recorded in both hemispheres of SC simultaneously using linear arrays while the monkey performed the choice task. Spike-triggered local field potentials (stLFP) were computed separately for each channel on the linear array by averaging many traces aligned on single impulses. The laminar profile of stLFP traces thus obtained were then assessed with current source density (CSD) analysis to reveal the sinks and sources arising from the single neuron under investigation. These single neuron CSD profiles indicated that different neurons from one hemisphere of SC can exert excitatory or inhibitory influence as evidenced by current sinks/sources found on the other side. Moreover, we found that the strength of this inter-collicular influence was dependent on the type of choice (equal value or unequal value) that the monkey encountered. In another experiment, we injected low concentrations (0.2mg/ml) of muscimol in one SC hemisphere while monitoring the activity of intact hemisphere. This delayed the contralateral saccades. Visual neurons from the intact hemisphere decreased their firing rate and lost their sensitivity to the value information from the inactivated hemisphere. On the other hand, motor neurons increased their firing rate and maintained their sensitivity to the value of object in the inactivated hemifield. Taken together these results suggest

that inter-collicular projections maybe involved in comparing value information from lateralized circuits in both hemispheres to arrive at an optimal decision.

Disclosures: A. Gopal P A: None. **O. Hikosaka:** None.

Poster

144. Eye Movements: Saccades in Nonhuman Primates

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

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Topic: E.01. Eye Movements

Support: Intramural Research Program at the National Institutes of Health, National Eye Institute (project number: 1ZIAEY000415-16)
Japan Agency for Medical Research and Development (Grants JP18dm0307021 and JP18dm0207003)
Japan Science and Technology Agency (PRESTO Grant JPMJPR1683)

Title: Pathway-selective optogenetic modulation of amygdala-basal ganglia circuits in macaque monkeys

Authors: *K. MAEDA[1], K.-I. INOUE[2], M. TAKADA[2], O. HIKOSAKA[1];
[1]Lab. Sensorimotor Res., Natl. Eye Inst., Bethesda, MD; [2]Primate Res. Institute, Kyoto Univ., Inuyama, Japan

Abstract: The amygdala is uniquely sensitive to emotional events and contexts. Our previous study showed that amygdala neurons, mostly in the central nucleus of amygdala (CeA), encode emotional contexts (dangerous vs. safe and rich vs. poor) which were based on various environmental scenes (Maeda et. al., 2018, PLOS Biol.). We found that the activity of amygdala neurons was correlated with the reaction time of saccades to reward-associated objects. Importantly, the neuronal activity and the saccade reaction time, together, were modified by the emotional contexts, although the context effect varied somewhat across the monkeys. We then found that the temporary inactivation of CeA (by muscimol injection) suppressed saccades, selectively those directed to the contralateral side (Maeda et. al., 2019, bioRxiv). However, the underlying neural circuit mechanism was unknown. To address this question, we used an optogenetic method to manipulate the downstream neuronal circuits of CeA. We injected a viral vector (ChR2) into CeA of macaque monkeys. First, we optically stimulated CeA with ChR2 and found that saccades to contralateral side were facilitated, which was consistent with the CeA inactivation data (above). We then hypothesized that the saccade facilitation effect is caused by the connection from CeA to the substantia nigra pars reticulata (SNr), which is the final output area of the basal ganglia. To test this hypothesis, we optically stimulated SNr while recording single neurons in SNr using an optrode. We found that most SNr neurons were inhibited by the

optical stimulation (32/51). Importantly, the activity of these SNr neurons was modulated by the emotional contexts (described above) and/or by the objects associated with reward or no reward. These results suggest that the emotional context information in CeA is transferred to SNr, which then controls saccades to individual objects inside each context (i.e., environmental scene). Indeed, the optical stimulation in SNr facilitated saccades to the contralateral side while the monkey was freely watching a movie with social interactions. Our results demonstrate that the amygdala, especially CeA, modulates saccadic eye movements by regulating basal ganglia circuits.

Disclosures: **K. Maeda:** None. **K. Inoue:** None. **M. Takada:** None. **O. Hikosaka:** None.

Poster

144. Eye Movements: Saccades in Nonhuman Primates

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 144.15/M7

Topic: E.01. Eye Movements

Title: Every spike counts: Intra-saccadic superior colliculus visual bursts modify saccade metrics

Authors: *A. BUONOCORE, Z. M. HAFED;
Werner Reichardt Ctr. For Integrative Neurosci., Tuebingen, Germany

Abstract: Saccades and microsaccades are fast eye movements that align the fovea with salient regions of the visual field. Despite their ballistic nature, recent evidence suggests that these eye movements' kinematics can be strongly modulated by presenting strategically timed transient visual stimuli. Specifically, visual flashes occurring ~100 ms before saccade/microsaccade onset robustly alter the sizes and peak velocities of these movements (Buonocore et al., 2016; 2017), but the neural mechanisms underlying such alteration remain unclear. Here we tested the hypothesis (Buonocore et al., 2017) that visually-induced action potentials in the superior colliculus (SC) can occur intra-saccadically, thus adding predictable spatial "vectors" to the ongoing eye movements when the SC population activity is read-out by downstream oculomotor nuclei. This results in predictable alterations in movement kinematics. In two rhesus monkeys, we analyzed SC activity after presenting 2.22 cycle/deg vertical gratings of high contrast in the visual response fields (RF's) of recorded neurons. We also analyzed microsaccades occurring after grating onset. As expected (Buonocore et al., 2017), microsaccades occurring ~100 ms after grating onset were not only directed towards the gratings, but they were also much larger than baseline microsaccades. Neuronally, visual bursts during such microsaccades were robust, suggesting that visually-induced SC activity located at the RF locations (i.e. away from the movement bursts located rostrally for the microsaccades being generated) was present intra-saccadically. Critically, there was a consistent relationship between the altered microsaccade amplitudes and each added "visual" spike that was injected into the SC population activity by the

gratings: the more visual spikes that occurred intra-saccadically, the larger the microsaccades became. This effect was not due to movement-related bursts per se, because we ensured that the neurons that we recorded from were not exhibiting movement bursts for the ranges of eye movements that we analyzed. We also explored the detailed time course of the impact of each "extra" injected SC spike on movement amplitude: visual spikes injected into the SC from ~20 ms before movement onset to ~20 ms before movement end were most effective in altering the movements. This effect was eccentricity dependent, with the most peripheral injected spikes (in peripheral RF's) having the least impact on microsaccade amplitudes. Our results provide clear mechanistic insight on recently discovered robust effects of transient visual stimuli on saccade frequency (saccadic inhibition) and kinematics.

Disclosures: A. Buonocore: None. **Z.M. Hafed:** None.

Poster

144. Eye Movements: Saccades in Nonhuman Primates

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 144.16/M8

Topic: E.01. Eye Movements

Support: DST-INDIA
Department of Biotechnology-INDIA

Title: Dissociation of attentional and saccade-related activity in the frontal eye field

Authors: *N. SENDHILNATHAN[1], D. BASU[3], M. E. GOLDBERG[2], A. N. MURTHY[4]; [2]Neurosci., [1]Columbia Univ., New York, NY; [3]Brown Univ., Providence, RI; [4]Indian Inst. of Sci., Bangalore, India

Abstract: Do we make saccades in our environment only to facilitate attention? We investigated this question in the monkey frontal eye field (FEF), which distinguishes purposive, behavior-related saccades from spontaneous saccades made in total darkness without an obvious purpose or goal, exhibiting pre- and postsaccadic activity in the former (Bruce and Goldberg, 1985) but only postsaccadic activity in the latter (Bizzi,1968). Here we studied FEF activity associated with a non-task-related saccade made by the monkey after a task-related saccade or the second of two task-related saccades. Surprisingly, the spike rate of FEF neurons before these non-task-related saccades was not different from the spike rate before task-related saccades. However, there were three major differences in the neural activity: First, the variability in spike rate across trials decreased and the regularity of spiking within trials increased only for task-related saccades and not for non-task-related saccades. Second, beta band LFP power decreased only during task-related saccades. Third, the neural population activity in the PCA state space reorganized between two sequential purposive saccades but not when a non-task-related saccade followed a

task-related saccade, even though the kinematics of the two pairs of saccades were similar. Attention is well known to decrease variability in neuronal activity. The lack of decreased variability in the non-task related saccades suggests that these saccades were not made for purposes of overt attention. The concurrent high level of beta band activity suggests this oscillatory activity might represent an inhibition of attentional activity in FEF. Overall, our results report unexpected differences in neural signatures for task-related versus non-task-related saccades in a brain area previously thought to drive saccades for overt attention. They add a new dimension to the way we understand the computation of attention in the brain.

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Poster

144. Eye Movements: Saccades in Nonhuman Primates

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 144.17/M9

Topic: E.01. Eye Movements

Support: Duke Institute for Brain Sciences Germinator Award to Bohlen and Sommer
NIH R21 EY030278 to Sommer

Title: Anatomical characterization of virally-mediated transduction to extraocular and periorbital motoneurons in macaques

Authors: *M. O. BOHLEN[1], H. EL-NAHAL[2], M. HU[3], V. YUZIUK[4], E. KREMER[5], M. A. SOMMER[4];
[1]Biomed. Engin., Duke Univ. Hlth. Syst., Durham, NC; [3]Doctorate Program in Physical Therapy, [4]Biomed. Engin., [2]Duke Univ., Durham, NC; [5]Inst. de Génétique Moléculaire de Montpellier, Montpellier, France

Abstract: The vast majority of non-human primate optogenetics work has been performed in cerebral cortex, far from neuromuscular circuits and primary sensory neurons. Refining optogenetic methods in cerebral cortex is daunting due to its numerous recurrent pathways that may mask effects and result in subtle, unclear behavioral readouts of efficacy. An alternative testbed would be the extraocular motoneurons. Eye movements, which are easily and precisely measurable, could then be used as a highly sensitive indicator of viral transduction and the comparative efficacy of optogenetic parameters. Beyond technical refinements, optogenetic control of extraocular motoneuronal populations would help us to answer fundamental yet currently unresolved questions about brainstem gaze control. Toward this goal, we have been anatomically assessing the capacity of multiple viral vectors to transduce cranial nerve motoneurons with opsin genes following intramuscular injections in rhesus macaques. We have

tested several adeno-associated viral (AAV) serotypes, canine adenovirus-2 (CAV2) and a modified herpes simplex virus-1 (HSV). The research program has revealed two main findings. (1) We have discovered that one virus in particular, CAV2, effectively transduces fluorescent genes into motoneurons that innervate the injected muscles. We have replicated this result in multiple macaques and found that the volumes required are more reasonable compared to the volumes required for most other vectors. (2) Although AAVs have historically been the vector of choice, the elicited immunological response to peripherally injected AAVs appears to be ferocious and rapid. We have explored three methods aimed at improving AAV transduction following peripheral injections that have proven effective to varying degrees for buying time for injected AAVs to find their receptor and transduce their genomic payload. The first is a mild and transient immunosuppressant protocol, the second is to increase the volume being injected, and the third is to use decoy capsids to functionally occupy neutralizing antibodies. Our preliminary findings suggest all three approaches have promise and need to be explored further. The next step is to begin testing the physiological functionality of these vectors with optical stimulation of the transduced motoneurons.

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Poster

144. Eye Movements: Saccades in Nonhuman Primates

Location: Hall A

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Program #/Poster #: 144.18/M10

Topic: E.01. Eye Movements

Support: Duke Institute for Brain Sciences Germinator Award
NIH R21 EY030278

Title: Anatomical characterization of labeling in the claustrum following injection of rAAV2-retro into the FEF of rhesus macaques

Authors: *H. EL-NAHAL, M. BOHLEN, M. SOMMER;
Duke Univ., Durham, NC

Abstract: More than two centuries after the claustrum was first mentioned in the literature, its functions remain a mystery. Neurophysiological research on the claustrum, a telencephalic sheet of grey matter wedged between the external and extreme capsules, is complicated by its irregular shape and narrow mediolateral dimension. Previous anatomical work has shown that the claustrum is reciprocally connected to most cortical areas and several subcortical areas. Suitable tools for probing claustral function have been lacking, but viral labeling coupled with optogenetic control of claustral neurons could provide advances. As part of ongoing work, we

are characterizing viral vectors for optogenetic and behavioral experiments in rhesus macaques (*Macaca mulatta*) and have identified rAAV2-retro as a good candidate for claustral study. Injections of rAAV2-retro into the frontal eye field (FEF) resulted in retrograde labeling of predominantly ipsilateral claustro-cortical projection neurons. Preliminary data suggests that mainly type 1 large, spiny claustral neurons were labeled. Most of the labeled neurons were confined to the central third of the claustrum on the dorso-ventral axis. Additionally, they were most concentrated in the anterior claustrum, with numbers diminishing gradually towards the caudal end of the claustrum. These anatomical experiments are the first step towards potentially using rAAV2-retro to study the function of claustro-FEF projection neurons using phototagging, optogenetic control, and behavioral experimentation in monkeys. Isolation of the claustral influence on FEF would provide a defined circuit for understanding the role of claustrum in vision, eye movements, and related cognitive functions such as attention.
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Disclosures: H. El-Nahal: None. **M. Bohlen:** None. **M. Sommer:** None.

Poster

144. Eye Movements: Saccades in Nonhuman Primates

Location: Hall A

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Program #/Poster #: 144.19/M11

Topic: E.01. Eye Movements

Title: Asymmetric binocular control revealed by monocular pursuit on the midline

Authors: *S. J. HEINEN[1], J. B. BADLER[1], A. CHANDNA[1], S. N. WATAMANIUK[2]; [1]Smith-Kettlewell Eye Res. Inst., San Francisco, CA; [2]Psychology, Wright State Univ., Dayton, OH

Abstract: The vergence system rotates the eyes through equal but opposite-signed angles for gaze shifts in depth, separate from the conjugate system that rotates them equally in the same direction. The vergence system is thought to control both eyes symmetrically, using both sensory cues and internal drive from the accommodation system. We showed previously (Heinen et al., SfN 2018) that during periodic pursuit in depth on the midline, occluding one eye caused it to rotate inappropriately. For some individuals, it rotated in the same direction as the covered eye as if executing a conjugate movement, opposite to the expected vergence response. Accommodation remained appropriate in both eyes. Here we investigate the temporal profile of the covered eye rotations to determine if they reflect the addition of a conjugate component to a unitary vergence command. Observers pursued a motorized physical target (small letter “E”) moving periodically in depth on the midline, between 33.3cm (3.0 dpt) and 66.7cm (1.5 dpt) with peak velocity 30cm/s, acceleration 50cm/s^2 and total cycle time ~6 sec. Viewing was either

binocular or monocular with either eye. Eye movements and accommodation were measured from both eyes with a PlusOptix photorefractor. The temporal delay (phase lag) of each eye relative to target motion was computed using cross-correlation. Viewing eyes followed the target with near zero delay, as expected. Covered eyes, however, were usually desynchronized with target motion with delays up to 2.6 sec, and a temporal profile that fit neither vergence nor conjugate movement. Covered eye accommodation delays had a narrower range and were not correlated with gaze delays, suggesting accommodation did not drive the covered eye response. The results provide evidence that during monocular midline pursuit, the covered eye receives a separate vergence command that is not linked to accommodation.

Disclosures: S.J. Heinen: None. **J.B. Badler:** None. **A. Chandna:** None. **S.N. Watamaniuk:** None.

Poster

144. Eye Movements: Saccades in Nonhuman Primates

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 144.20/M12

Topic: D.08. Visual Sensory-motor Processing

Support: NIH Grant EY024831
NIH Grant EY022854
GAANN Fellowship P200A150050

Title: Population dynamics underlying sensorimotor transformation in the primate superior colliculus

Authors: *M. R. HEUSSER[1,2], U. K. JAGADISAN[1,2], N. J. GANDHI[1,2];
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Abstract: To correctly orient our eyes toward an object of interest, we must process information contained in our visual field and convert it into an appropriate motor command. The superior colliculus (SC) in the subcortex is essential for performing this sensorimotor transformation. Neurons in its intermediate layers, in particular, emit a burst of spikes when a stimulus is presented in their response fields, and they produce yet another burst that results in a saccadic eye movement to that stimulus. In the intervening period, SC neurons display low-frequency activity. These neural signatures have been well-characterized in an experimental setting through a delayed saccade task, which temporally dissociates the visual target presentation and the eventual eye movement. We investigated the dynamics of population activity during the delay period, with the objective to understand whether the activity remains largely static, or if it instead transitions gradually or alternates between visual and motor representations. Single trial neural

data collected with laminar probes were available from two adult male rhesus macaques (Macaca mulatta) performing a delayed saccade task to a target placed in the population's response field. We implemented dimensionality reduction (Gaussian Process Factor Analysis, GPFA) on each session's full-dimensional neural population activity (i.e., 16 or 24 channels) to visualize and characterize the temporal evolution of neural activity in state space during individual trials. We also quantified trial-averaged activity as visual-like and motor-like through a "proximity" metric (as in Dekleva et al., 2018). Visual proximity values tended to decline and motor proximity values tended to increase throughout the delay period. Trial-averaged analyses indicate that SC population activity exhibits a gradual transition from visual- to motor-like, but individual trials hint at a more nuanced neural trajectory throughout the delay period. Thus, delay period activity seems to encode a dynamic sensorimotor transformation process.

Disclosures: **M.R. Heusser:** None. **U.K. Jagadisan:** None. **N.J. Gandhi:** None.

Poster

144. Eye Movements: Saccades in Nonhuman Primates

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 144.21/M13

Topic: E.01. Eye Movements

Support: NIH Grant EY024831

Title: Independent component analysis of local field potential sources in the primate superior colliculus

Authors: A. H. DALLAL[1], Y. LIU[1], U. K. JAGADISAN[2,3], ***N. J. GANDHI**[2,3];
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Abstract: The superior colliculus (SC) is essential for transforming sensory signals into movement commands. It has a laminar organization - visual processing in superficial layers and increasingly more motoric signals in deeper layers - and structured anatomical connections both within the SC itself and for external inputs. These spatial attributes make SC ideal for investigating communication between the different layers when producing visually-guided eye movements (saccades). A first step in addressing this objective is to identify the loci of current generators ("sinks") involved in the transformation. The local field potential (LFP), the component of the extracellular potential attributed to dendritic processing, represents a combination of the various generators measured in the vicinity of each electrode, and we sought to estimate the spatial and temporal properties of each one. This is akin to the blind source separation problem addressable by independent component analysis (ICA). Neural data across the dorsoventral axis of SC was recorded with a multicontact laminar probe in Rhesus monkeys

performing a standard delayed saccade task. The LFP component was extracted with a low-pass filter. We used ICA to identify the generators contributing to the LFP, which is presumably composed of a weighted mixture of independent sources (ICs). The LFPs are binned into segments, and the ICs and their corresponding mixing weights are computed for each epoch. We also compared our results against current source density (CSD) techniques, which relies on the second spatial derivative of the mixing coefficients computed with a Laplacian of Gaussian kernel. We then reconstructed the CSD that corresponds to each component by multiplying that IC by the computed derivative of its coefficients. To identify the dominant ICs, we performed correlation analysis between each CSD reconstructed via a single IC against the ground truth CSD (the second spatial derivative of the raw LFP) and we kept only those ICs with a correlation score exceeding a predefined threshold. These dominant ICs correspond to the current generators in this time window, and the spatial distribution of the coefficients defines the depth at which this sink originates. Preliminary data indicate that the correlation between the IC based reconstruction and the ground truth CDS exceeds 90%. We consistently identified 2-3 generator sites, mostly confined near the boundary of superficial and intermediate layers. The strongest modulations occurred slightly after saccade onset and in response to target onset. Thus, the ICA method can accurately reconstruct the CSD, as well as identify and quantify the origin of current sinks.

Disclosures: **A.H. Dallal:** None. **Y. Liu:** None. **U.K. Jagadisan:** None. **N.J. Gandhi:** None.

Poster

145. Basal Ganglia: Neuromodulation

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 145.01/M14

Topic: E.03. Basal Ganglia

Support: ERC starting grant
Hjarnfonden
Vetenskapsradet
Wallenberg academy fellowship

Title: Disynaptic inhibition promotes synchrony between striatal cholinergic interneurons and is regulated by dopamine via D_2 receptors

Authors: *M. C. DORST[1], A. TOKARSKA[1], M. ZHOU[1], K. LEE[2], A. S. STAGKOURAKIS[3], C. C. BROBERGER[1], S. C. MASMANIDIS[2], G. SILBERBERG[1];
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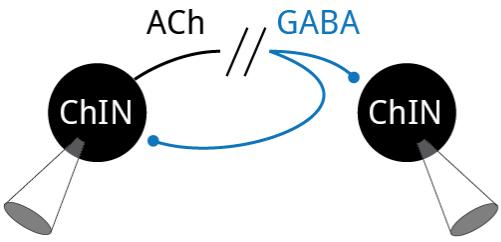
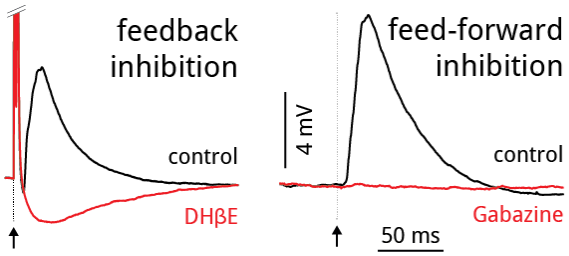
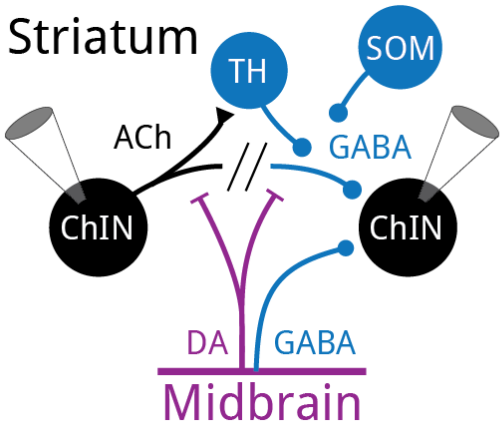
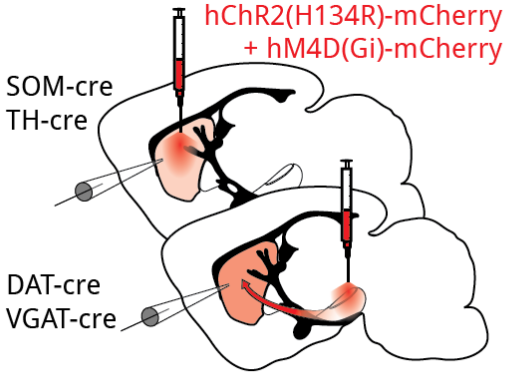
Abstract: Striatal activity is dynamically modulated by acetylcholine and dopamine, both of which are essential for proper basal ganglia function. Synchronized pauses in the activity of striatal cholinergic interneurons (ChINs) are correlated with elevated activity of midbrain dopaminergic neurons, whereas synchronous firing of ChINs induces local release of dopamine. The mechanisms underlying ChIN synchronization and its interplay with dopamine release are not fully understood. Here we show using multineuron patch-clamp recordings, voltammetry, optogenetics, chemogenetics, and *in vivo* recordings, that robust disynaptic inhibition between ChINs acts as an efficient synchronization mechanism. Inhibitory disynaptic responses were elicited by single action potentials in ChINs and showed a high degree of recurrence within the ChIN network. Disynaptic inhibition was attenuated by dopaminergic midbrain afferents acting on D_2 receptors. Our results present a mechanism supporting synchronization of activity and pauses across the ChIN population and a novel form of interaction between striatal acetylcholine and dopamine.









Disclosures: **M.C. Dorst:** None. **A. Tokarska:** None. **K. Lee:** None. **A.S. Stagkourakis:** None. **C.C. Broberger:** None. **S.C. Masmanidis:** None. **G. Silberberg:** None. **M. Zhou:** None.

Poster

145. Basal Ganglia: Neuromodulation

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 145.02/M15

Topic: E.03. Basal Ganglia

Support: FWO 1518519N

Title: Glycine receptor alpha 2 depletion induces an exaggerated dopaminergic response

Authors: *J. DEVOGHT[1], J. COMHAIR[1], G. MORELLI[2], J.-M. RIGO[1], E. PICCART[1], B. BRONE[1];
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Abstract: Tight control of the dopaminergic system is key to a proper brain function and its dysregulation is associated with several grave pathologies, i.e. schizophrenia, Parkinson`s disease and addiction. Our lab recently identified the glycine alpha 2 receptor (GlyRa2) as a tool to unveil the neurobiological mechanisms of hyperdopaminergic pathologies. We established that GlyRa2s are the only functional glycine receptors expressed throughout adulthood in striatal medium spiny neurons (MSNs). Activation of GlyRa2s induces an inhibitory chloride current when the cell becomes depolarized (> -55 mV), i.e. at times of high cortical input. As such the GlyRa2 might be uniquely positioned as a specific treatment target in pathologies with hyperdopaminergia such as schizophrenia. We aimed to link dopamine-mediated behavioral observations in GlyRa2 knockout (KO) mice to cellular mechanisms and neuronal network defects. GlyRa2KO mice exhibit enhanced amphetamine-stimulated locomotion (5mg/kg i.p.) and increased performance during an appetitive conditioning task (increased nose pokes at demanding reward schedules) compared to wild type littermates. In a first step of elucidating the cellular mechanisms related to exaggerated dopaminergic responses, we examined basal pacemaking and phasic burst activity of midbrain dopamine cells. Firing frequency measured in loose cell-attached voltage-clamp revealed no differences in pacemaking or burst activity, the latter induced by NMDA iontophoresis. Currently, we are focusing on the striatal integration of glutamatergic signals modulated by dopamine. For the determination of the modulatory effects of dopamine on MSN activity, GlyRa2KO mice were crossbred with a Cre-inducible channelrhodopsin-2 mouse line for optogenetic stimulation of dopaminergic terminals. MSNs are held in a resting downstate by whole-cell current clamp and brought to an active upstate by electrical stimulation of glutamatergic terminals, whether or not combined with optogenetic control of dopamine release from terminals. We can conclude that GlyRa2 depletion induces an exaggerated dopamine-mediated behavior independent of dopamine neuron activity, but striatal signal integration is yet to be investigated.

Disclosures: **J. Devoght:** None. **J. Comhair:** None. **G. Morelli:** None. **J. Rigo:** None. **E. Piccart:** None. **B. Brone:** None.

Poster

145. Basal Ganglia: Neuromodulation

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 145.03/M16

Topic: E.03. Basal Ganglia

Title: Pallido-nigral terminals express functional CB1 and CB2 cannabinoid receptors

Authors: *I. O. CONDE ROJAS, III[1], R. CABALLERO FLORÁN[2], R. SÁNCHEZ-ZAVALETA[3], G. B. FLORAN[4];
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Abstract: In general, it is accepted that pallidal neurons do not express CB1 receptors, whereas, recent studies, indicate the existence of CB2 receptors in external Globus Pallidus (GPe) of primates. In this study, we explored the existence of the mRNA of the CB1 and CB2 receptors in the mouse pallidal neurons as well as their expression and function in pallido-nigral terminals. We performed RT-PCR experiments on both slices of GPe and primary neuronal cultures from C57 mice 12-14 days old. Typical bands corresponding to CB1 and CB2 receptors were found. Immunochemical studies showed an intense mark corresponding to the CB1 and CB2 receptors colocalized in the same neuron. To evaluate the presence of receptors in synaptic terminals, we perform electrophysiological recordings in Substantia nigra pars reticulata (SNr) slices using the patch-clamp technique in whole cell configuration, registering eIPSC using a stimulus train protocol (5 pulses at 20 Hz) into GPe and analyze their function in the modulation of GABA release. GPe-SNr synapses were characterized by short term plasticity according Miguelez et al (2012). Once the synaptic terminals were characterized, we record the basal current for 5 minutes, then during 15 minutes, we administered the agonists of the CB1 receptor ACEA or for the CB2 receptor GW833972A observing a decrease in the amplitude of the eIPSC in almost 25 % for CB1 (n = 26) and 20% for CB2 (n = 16). The effect of ACEA and GW8339372A was prevented by AM251 and AM630 respectively. The effect is presynaptic since the quotient pulse amplitude of a pulse x/pulse 1 was modify significantly. This result indicates that the release probability of GABA at pallido-nigral terminals is modulated by presynaptic CB1 and CB2 receptors

Disclosures: **I.O. Conde Rojas:** None. **R. Caballero Florán:** None. **R. Sánchez-Zavaleta:** None. **G.B. Floran:** None.

Poster

145. Basal Ganglia: Neuromodulation

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 145.04/M17

Topic: E.03. Basal Ganglia

Support: DGAPA UNAM PAPIIT IN216019

Title: Differential calcium channel-mediated dopaminergic modulation in the subthalamo-nigral synapse of the rat

Authors: *A. A. ROBLES-GÓMEZ[1], J. A. BARRAL[2], G. B. FLORAN[3];
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Abstract: Dopamine (DA) modulates basal ganglia (BG) extrastriatal nuclei for proper motor functionality. Subthalamic-nigral synapse is particularly important for the integration of previously processed information in neostriatum and globus pallidus that is conveyed to substantia nigra pars compacta (SNc) and pars reticulata (SNr). D_2-like receptors inhibit glutamate release from subthalamic terminals to SNr, however this has not been described to SNc. The main effectors of this presynaptic modulation are unknown. As Ca^{2+} channels are thought to be key modulators of transmitter release. We analyze the D_2-like mediated DA modulation occurring in this synapse to both SNc and SNr neurons by identifying the presynaptic Ca^{2+} channels that serve as effectors in each case. We tested this by electrophysiological recording in rat brain slices. Whole cell patch-clamp recording of SNc and SNr neurons was carried out to measure excitatory postsynaptic currents (EPSCs), evoked by subthalamic stimulation, and paired-pulse ratio in presence of selective Ca^{2+} channels blockers alongside DA receptor's agonists and antagonists to determine changes in transmitter release. We found that Ca_V3/$Ca_V2.3$ family blockade occludes the inhibition of glutamate release observed by D_2-like receptors activation to SNc neurons, suggesting that these families are the main presynaptic effectors of DA modulation. However, $Ca_V2.1$ channel exert this function to SNr neurons. The activation of D_3 subfamily was enough to reproduce the D2-like mediated inhibition for both SNc and SNr. Assuming a homogenous subthalamic innervation to SN, we propose that functional role for this differential distribution of calcium channels that modulate glutamate release imply a fine tuning of the presynaptic ionic channel population specified for both postsynaptic neuronal classes. Dopaminergic inhibition of glutamate release from subthalamic terminals to (1) SNc dopaminergic neurons may be substantial to regulate DA levels within the SNc, SNr and the whole BG nuclei, whereas to (2) SNr neurons would have strong impacts on the inhibitory output to thalamic and brainstem target nuclei. These findings support a more

complex model of presynaptic modulation, with specified effectors as a function of the postsynaptic neuron type.

Disclosures: **A.A. Robles-Gómez:** None. **J.A. Barral:** None. **G.B. Floran:** None.

Poster

145. Basal Ganglia: Neuromodulation

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 145.05/M18

Topic: E.03. Basal Ganglia

Title: Calmodulin modulates signaling efficiency of presynaptic D2 receptors in striatopallidal terminals

Authors: *R. JIJÓN-LORENZO[1], I. CABALLERO-FLORAN[1], S. RECILLAS-MORALES[2], A. AVALOS-FUENTES[1], F. PAZ-BERMUDEZ[1], B. FLORAN-GARDUÑO[1];
[1]Ctr. de Investigación y de Estudios Avanzados del Inst. Politécnico Nacional, Ciudad de México, Mexico; [2]Univ. Autónoma del Estado de México, Estado de Mexico, Mexico

Abstract: D2-type dopamine receptors (D_2R) use different signaling pathways for the control of neuronal functions. The function of these receptors is modulated by different proteins that interact in the third intracellular loop, such as signaling proteins, coreceptors and calcium sensor proteins including Calmodulin (CaM). It has been reported that the interaction of CaM causes changes in the magnitude of signaling and coupling to the G protein of D_2R. However, the results are contradictory, observing both increase and decrease in the functionality of the receptors in cell cultures. We investigated whether CaM interacts with D_2R in striatopallidal synaptosomes of rats under normal conditions and its effect as a mediator of receptor signaling efficiency. We also evaluated the interaction in high potassium (depolarizing) conditions since it has been reported that the entry of calcium causes the dissociation of proteins. Our results indicate that CaM interacts with D_2R under basal conditions and when we depolarize the synaptosomes the interaction decreases due to the calcium input, which was tested when BAPTA/AM an intracellular calcium chelator was added. To see the functional effect of this decrease in the interaction, we studied the formation of second messengers in striatopallidal synaptosomes stimulated by the activation of the D_2R with its agonist Quinpirole. We found that the formation of IP_1 is induced and that the effect increases when we depolarize the striatopallidal terminals. In addition, we analyzed the effect of depolarization in cAMP formation assays in pallidal synaptosomes, finding that only when we depolarize with high K^+ (15mM), there is a significant decrease mediated by the activation of presynaptic D_2 receptors with the agonist Quinpirole. This data indicate that D_2R signal enhanced when calcium increase into synaptosomes and when Calmodulin is less associated with the receptor.

Disclosures: **R. Jijón-Lorenzo:** None. **I. Caballero-Floran:** None. **S. Recillas-Morales:** None. **A. Avalos-Fuentes:** None. **F. Paz-Bermudez:** None. **B. Floran-Garduño:** None.

Poster

145. Basal Ganglia: Neuromodulation

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 145.06/M19

Topic: E.03. Basal Ganglia

Title: Melatonin reduces dopamine release in the mouse caudate putamen

Authors: T. BORGELD, K. HUGHES, J. WYN, *E. RAMSSON;
Biomed. Sci., Grand Valley State Univ., Allendale, MI

Abstract: The caudate putamen is a subregion of the basal ganglia containing neural tracts important for cognition, reward learning, and voluntary motor function. Dopamine (DA) signaling received from the dopaminergic neurons of surrounding nuclei mediate locomotion; degradation of these inputs the characteristic neuropathology for Parkinson's disease (PD). PD is initially a motor disorder but can progress to include cognitive impairments as well. Sundowners syndrome (SS) has been observed in patient populations with neurodegenerative diseases, characterized by the decline of cognition in the evening hours. Due to melatonin influence on circadian rhythms, it likely plays a strong role SS. While melatonin has been observed to decrease DA release, the real-time measurement of acute melatonin exposure on DA release within the caudate has yet to be studied. Utilizing various waveforms of fast scan cyclic voltammetry (FSCV) in an *ex vivo* mouse model, we observed a decrease in DA release upon exposure to supraphysiological concentrations of melatonin. Results from these experiments support previous literature suggesting that activation of presynaptically expressed melatonin receptor 1 (MT1) plays an important physiological role downregulating DA release. Additionally, results demonstrate that 1-hour of MT1 activation is sufficient to decrease DA release.

Disclosures: **E. Ramsson:** None. **T. Borgeld:** None. **J. Wyn:** None. **K. Hughes:** None.

Poster

145. Basal Ganglia: Neuromodulation

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 145.07/M20

Topic: E.03. Basal Ganglia

Title: Characterizing the effects of Melatonin on striatal dopamine transmission

Authors: S. S. PAWELKO[1], J. FRIED[1], J. A. NADEL[1], E. RAMSSON[2], *C. D. HOWARD[1]; [1]Dept. of Neurosci., Oberlin Col., Oberlin, OH; [2]Biomed. Sci., Grand Valley State Univ., Allendale, MI

Abstract: Dopamine neurotransmission is necessary for both movement and reward-based learning. Accordingly, depletion of brain dopamine, which occurs in Parkinson's disease, results in severe motor deficits and circuit dysfunction the striatum. Parkinsonian patients and their caregivers report a 'sundowning' effect characterized by cognitive abnormalities during evening hours, which may reflect a circadian cycle of brain dopamine levels. Previous studies have confirmed that striatal dopamine concentrations, transporters, and release kinetics oscillate on a 24-hour cycle. In addition, dopamine neurons are known to express receptors for the neurohormone melatonin, which is involved in entrainment of circadian cycles and in the regulation of sleep-wake timing. The interaction between these systems, however, is largely unknown. We investigated the effects of melatonin on evoked dopamine signals in the mouse striatum using fast-scan cyclic voltammetry (FSCV). Melatonin has been shown to foul electrochemical electrodes under a variety of FSCV waveforms, adhering to the electrode surface and drastically impacting sensitivity. However, we were able to characterize melatonin fouling in vitro and in vivo using both a traditional FSCV waveform and a modified waveform recently used to detect melatonin. Our results suggest that melatonin may decrease evoked dopamine responses in the striatum, supporting the notion of hormone-driven circadian regulation of dopamine levels. Future directions include exploring the use of photometry to measure melatonin-induced changes in evoked striatal dopamine and kinetic modeling to determine melatonin's effects on dopamine release and uptake.

Disclosures: S.S. Pawelko: None. **J. Fried:** None. **J.A. Nadel:** None. **E. Ramsson:** None. **C.D. Howard:** None.

Poster

145. Basal Ganglia: Neuromodulation

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 145.08/M21

Topic: E.03. Basal Ganglia

Support: Brain research New Zealand
Neurological Foundation of New Zealand

Title: Changes in dopamine signaling and responses to drugs affecting dopaminergic neurotransmission in striatal and ventral midbrain slices from DAT-KO rats

Authors: *J. T. L. LLOYD[1], P. KALLINGAPPA[1], A. YEE[3], P. CHEUNG[1], R. N. KARUNASINGHE[2], A. JABED[1], P. S. FREESTONE[1], J. LIPSKI[1];
[2]Physiol., [1]Univ. of Auckland, Auckland, New Zealand; [3]Univ. of Colorado Denver, Aurora, CO

Abstract: Cessation of dopamine (DA) transmission largely depends on reuptake by the DA transporter (DAT) encoded by the Slc6a3 gene. DAT expression/activity is reduced in several neurological disorders and after exposure to drugs of abuse (e.g. cocaine, methylphenidate, amphetamine). Our aim was to characterize behavioral, neurochemical and electrophysiological effects of eliminating DAT activity in a novel DAT knockout rat generated using CRISPR/Cas9. As expected, DAT-KO rats displayed no DAT immunoreactivity in the striatum, increased basal locomotor activity, and paradoxical calming by amphetamine. Fast-scan cyclic voltammetry (FSCV) in brain slices demonstrated a large decrease in the clearance of electrically stimulated DA release in the dorsal striatum and to a lesser extent in the Substantia Nigra *pars compacta* (SNc). Cocaine increased the amplitude of DA release and slowed its clearance in slices from wild-type (WT), but not DAT-KO rats. Basal extracellular DA concentration ($[DA]_{out}$), measured with fast-scan controlled-adsorption voltammetry (FSCAV; Burrell et al., ACS Chem. Neurosci. 2015, 6:1802), was higher in DAT-KO rats compared to WT littermates, and was enhanced by L-DOPA, showing that DA release after L-DOPA is not due to DAT reversal. Baseline firing frequency of SNc neurons and $GABA_B$-mediated inhibition were similar in DAT-KO and WT rats. However, D_2-mediated inhibition (by both quinpirole and L-DOPA) was blunted in DAT-KOs, likely due to downregulation of D_2 receptors previously reported in DAT-KO mice. Amphetamine increased $[DA]_{out}$ in the dorsal striatum of WT rats, and this effect was strongly attenuated in DAT-KOs. Surprisingly, amphetamine increased $[DA]_{out}$ in the SNc of DAT-KOs, which did not differ to the response seen in WTs. The mechanism of this release is likely through reversal of low-affinity, high capacity uptake-2 transporters. These results not only validate our DAT-KO model, but also provide novel insights into the mechanism of DA releasing agents and form the basis for our future *in vivo* studies.

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Poster

145. Basal Ganglia: Neuromodulation

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 145.09/M22

Topic: E.03. Basal Ganglia

Title: Interaction between D2 and D3 receptors and its effect upon cAMP accumulation and GABA release in striato-pallidal terminals

Authors: *F. S. VILLALOBOS ESCOBEDO, A. AVALOS FUENTES, F. PAZ BERMÚDEZ, B. FLORÁN GARDUÑO;
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Abstract: D2 dopaminergic receptors (D2R) are present at striato-pallidal terminals, where modulate GABA release. The activation of D2R triggers the inhibition of adenylyl cyclase through the Gαi subunit and the stimulation of phospholipase C by the βγ subunit; these two mechanisms diminish the phosphorylation of calcium channels and thus inhibit neurotransmitter release. In heterologous systems was shown that the interaction of D2R with D3 receptors (D3R) enhances the activity of D2R by D3 activation. Immunohistochemical studies suggested the presence of D3R in aferences to globus pallidus and thus coexpression with D2R at nerve endings. Now, we study the interaction between D2 and D3R on cAMP accumulation and GABA release at striato-pallidal terminals. Coimmunoprecipitation assays showed that these receptors can interact physically. To evaluate the functional effects of this interaction both receptors were coactivated with the D2-like non-selective agonist Quinpirole, and the forskolin-stimulated cAMP accumulation and K^+ induced [^{3}H]GABA release were evaluated. The coactivation of D2 with D3R produced a modest inhibitory effect on forskolin-stimulated cAMP accumulation (10% one-way ANOVA, followed by Post-Hoc Tukey, n=5 **p<0.01), which was enhanced by depolarization since it removes Calmodulin interaction with D2R (Jijon et al. 2018). Due to the regulation of CAMKII on D3R activity (Avalos et al. 2013), the blockade of it with KN-62 , enhanced more the inhibitory effect of quinpirole (59% one-way ANOVA, followed by Post-Hoc Tukey, n=4 ***p<0.001), an effect that was mediated by D3R since blockade of them with GR 103691 prevented it. Also, similar effects of D3 receptors upon D2 activity were found when [^{3}H]-GABA release was analyzed. These data indicate that as in heterologous systems, D3R potentiate D2R effects on cAMP accumulation and GABA release in striato-pallidal terminals.

Disclosures: F.S. Villalobos Escobedo: None. **A. Avalos Fuentes:** None. **F. Paz Bermúdez:** None. **B. Florán Garduño:** None.

Poster

145. Basal Ganglia: Neuromodulation

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 145.10/M23

Topic: E.03. Basal Ganglia

Support: NIH R01NS099288
NSF 1354962

Title: Deciphering the circuit logic underlying dopamine release important to motor learning

Authors: *J. QI[1], M. G. KEARNEY[1], R. D. MOONEY[2];
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Abstract: The activity of midbrain dopamine (DA)-releasing neurons in the ventral tegmental area and substantia nigra pars compacta (VTA/SNc) is crucial for complex skill learning, movement initiation, and movement vigor. In songbirds, basal ganglia-projecting VTA neurons (VTA_{BG}) encode reinforcement signals sufficient to guide song learning. The neural mechanisms by which VTA computes these reinforcement signals remains poorly understood, partly due to the complexity of input-output relationships in the mammalian VTA. Songbirds represent a tractable system in which to decipher the circuit logic that enables these computations. VTA_{BG} neurons receive highly specialized inputs crucial for song learning, and recent work from our lab has established that two VTA-projecting regions-the ventral intermediate arcopallium (Aiv) and the ventral pallidum (VP) - operate in a push pull manner to affect song learning, with Aiv_{VTA} terminals suppressing VTA_{BG} activity and VP_{VTA} terminals exerting a net excitatory effect on VTA_{BG} neurons. To understand the circuit logic by which VTA_{BG} neurons integrate information from Aiv and VP, we combined *in situ* hybridization, immunostaining, and viral tracing. By retrogradely labeling Aiv_{VTA} and VP_{VTA} cell bodies and performing two-color fluorescence *in situ* hybridizations, we found the majority of Aiv_{VTA} cells selectively expressed VGLUT2 while not expressing VGAT, indicating that this projection to the VTA is predominantly excitatory. In contrast, most VP_{VTA} cell expressed VGAT but not VGLUT2, indicative of an inhibitory connection. To further explore the synaptic connections that Aiv and VP neurons make with different VTA cell types, we expressed GFP in Aiv or VP and analyzed axonal appositions onto TH or PV-expressing cells in the VTA. This approach revealed that Aiv_{VTA} axons in the VTA are studded with varicosities characteristic of en passant synapses mainly in apposition to PV+ cell bodies, while VP axons form a robust investment of GFP-labeled terminals encircling PV+ but not TH+ cell bodies in the VTA. Ongoing experiments are exploring the functional properties of the synapses that Aiv and VP axons make with different types of VTA neurons, and how these inputs function during song learning. Together, these results begin to elucidate the circuit logic by which by which local inhibition enables the computation of reinforcement signals in VTA_{BG} neurons.

Disclosures: J. Qi: None. **M.G. Kearney:** None. **R.D. Mooney:** None.

Poster

145. Basal Ganglia: Neuromodulation

Location: Hall A

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Program #/Poster #: 145.11/M24

Topic: E.03. Basal Ganglia

Support: Simons Foundation Grant 348880
NIH Grant K99 MH118412-01
NARSAD Grant BBRF
Bial Foundation Grant 413/14

Title: Sustained dopaminergic bursts and noradrenergic pauses favor exploitative behavioral states

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Abstract: We are constantly faced with the trade-off between exploiting past actions with known outcomes and exploring novel actions whose outcomes may be better. When environmental rewards are stable, it is preferable to perform actions known to be rewarding, but when the environment is changeable, it is adaptive to explore alternatives and revisit actions whose value may have changed. This balance between exploitation and exploration is thought to rely on two interacting systems, namely modulation of corticostriatal circuits by dopaminergic neurons of the substantia nigra pars compacta (SNc), and modulation of anterior cingulate cortex (ACC) processing by noradrenergic neurons of the locus coeruleus (LC). However, little is known about the dynamics of these systems during exploitative and exploratory states. Here, we investigate the ways in which dopaminergic and noradrenergic transmission evolve during exploratory and exploitative behavioral states. We developed a novel behavioral paradigm to capture exploratory and exploitative action selection in which mice explore an environment to discover a rewarded sequence of three nose pokes in order. The entropy of the distribution of sequences performed is initially high, suggesting that mice are exploring a range of possible actions, but entropy falls with training, showing that mice learn to consistently focus on the rewarded sequence. We then imaged the activity in genetically-identified dopaminergic neurons in SNc or noradrenergic neurons in LC during task performance. Exploitative behavioral states were marked by sustained increases in dopaminergic activity and decreases in noradrenergic activity. These effects cannot be accounted for by simple differences in reward rate. Together, these experiments clarify the role of dopaminergic and noradrenergic circuits in modulating behavioral variability, with important implications for coding in downstream circuits of the dorsal striatum and ACC.

Disclosures: A.C. Koralek: None. **R.M. Costa:** None.

Poster

145. Basal Ganglia: Neuromodulation

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 145.12/M25

Topic: E.03. Basal Ganglia

Support: ZIA AA000416
ZIA AA000407

Title: Sub-second dopamine measurement in cortex using a genetically-encoded fluorescent dopamine indicator (dLight)

Authors: *Y. MATEO[1], A. G. SALINAS[2], J. LEE[1], S. M. AUGUSTIN[3], T. PATRIARCHI[4], L. TIAN[5], D. M. LOVINGER[6];
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Abstract: Dopamine modulates cortical activity to mediate significant behavioral actions. Characterization of cortical dopamine release relevant to these functions has been mainly pursued with methodologies such as microdialysis that have low temporal resolution. Fast-scan cyclic voltammetry provides a direct measurement of catecholamine neurotransmission with precise temporal resolution but cannot discriminate between dopamine and norepinephrine, so it has been used mainly within the striatum where little to no norepinephrine is present. The recent development of optical sensors based on G protein-coupled receptors (GPCRs) has initiated a thriving line of research aimed at measuring neuromodulator extracellular levels in real time. One of these sensors, the genetically-encoded fluorescent dopamine biosensor, dLight, has a modified signaling-inactivated dopamine D1 receptor containing a circularly-permutated green fluorescent protein (cpGFP). Dopamine binding causes a structural change resulting in an increase in GFP fluorescence without activating downstream signaling cascades. Fluorescence emission can be captured with slice photometry for optical readouts and fast dynamics combined with physiological and pharmacological control . Using dLight in medial prefrontal cortex (mPFC) and motor cortical regions (M1 and M2), we found distinctive responses to bursts of electrical stimuli in different patterns suggesting that cortical dopamine terminal release properties differ from those of striatal terminals. Inhibition of the enzyme catechol-*O*-methyl transferase (COMT) by tolcapone reduced the dLight photometric responses primarily in mPFC. Activation of D2 dopamine receptors by quinpirole decreased photometric responses in mPFC and motor cortex. The role of the noradrenergic system regulating cortical dopamine release was also assessed. We found that activation of alpha-2 adrenoceptors with clonidine decreased cortical dopamine photometric responses. We are currently determining if responses are mediated solely by dopamine or if norepinephrine may also contribute to the fluorescence increases These signals were blocked by the D1 dopamine receptor antagonist, SCH23390. Our findings indicate the feasibility of using optical sensors to investigate precise temporal dopamine dynamics in cortical areas.

Disclosures: Y. Mateo: None. **A.G. Salinas:** None. **J. Lee:** None. **S.M. Augustin:** None. **T. Patriarchi:** None. **L. Tian:** None. **D.M. Lovinger:** None.

Poster

145. Basal Ganglia: Neuromodulation

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 145.13/M26

Topic: E.03. Basal Ganglia

Support: NIH Grant ZIA AA000407
Howard Hughes Medical Institute
NIH Grant K99 AA025991

Title: Real-time measurement of striatal acetylcholine release in brain slices

Authors: *D. M. LOVINGER[1], P. M. BORDEN[2], L. VOYVODIC-CASABO[3], J. S. MARVIN[2], A. G. SALINAS[4], L. L. LOOGER[2];
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Abstract: Acetylcholine (ACh) has numerous neuromodulatory actions in striatum that influence synaptic plasticity, action learning and decision making. Striatal ACh is also implicated in neurological disorders including Parkinson's Disease. Despite these many important roles we know little about the dynamics of ACh release and the time course of changes in extracellular ACh concentration in striatum. Recent development of the genetically-encoded intensity-based ACh sensing fluorescent reporter (iAChSnFR) biosensor has allowed us to directly monitor ACh dynamics in striatal brain slices under conditions in which we previously assessed ACh roles in striatal synaptic modulation and plasticity. Photometry in striatal slices from mice previously injected with an AAV encoding iAChSnFR was combined with electrical afferent stimulation to induce and measure ACh changes in dorsomedial striatum (DMS). Spontaneous fluorescence transients with durations of a few seconds were detected in some recordings. Single-pulse intrastriatal afferent activation produced increases in iAChSnFR-mediated fluorescence with subsecond onset, that showed biphasic transient decays with time courses of a few sec and >40 sec. These prolonged responses increased with increasing stimulus intensity and were blocked by reduced extracellular calcium and tetrodotoxin. These responses were not observed in brain slices expressing the iAChSnFR-Null mutant, which has much weaker (>1000x) ACh affinity. In contrast, stimulation and recording in M1 motor cortex revealed transients persisting for <20 sec. In striatum, ACh is mainly from cholinergic interneurons (CINs) that are sparse but have extensive intrastriatal axonal arborization. The prolonged responses in DMS appeared to be mediated by ACh release from cholinergic neurons as they were inhibited by a blocker of the vesicular ACh transporter and slowed/enhanced by acetylcholinesterase inhibition. Preliminary results indicate that the prolonged stimulus-induced increase in striatal ACh may be due to

"reverberating" activation of the local CIN network. High-frequency stimulation patterns that have been used to induce striatal synaptic plasticity produced increases in ACh that persisted for minutes. Our findings indicate that the intrastriatal ACh network is easily activated and that abundant and long-lasting ACh increases are induced by relatively modest synaptic activation in striatal slices, explaining why this neuromodulator has such an important role in striatal synaptic plasticity.

Disclosures: **D.M. Lovinger:** F. Consulting Fees (e.g., advisory boards); Alcohol Editorial Board, Elsevier Inc.. **P.M. Borden:** None. **L. Voyvodic-Casabo:** None. **J.S. Marvin:** None. **A.G. Salinas:** None. **L.L. Looger:** None.

Poster

145. Basal Ganglia: Neuromodulation

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 145.14/M27

Topic: E.03. Basal Ganglia

Support: The goverment of Japan

Title: Direct measurement of striatal cholinergic transmission with a specific genetically encoded fluorescent acetylcholine indicator in mice

Authors: *T. HERNANDEZ FLORES[1], Y. NAKANO[1], A. CARRASCO[2], M. GARCIA-MUNOZ[1], G. W. ARBUTHNOTT[1];
[1]Brain Mechanisms for Behavior Unit, [2]Neurobio. Res. Unit, Okinawa Inst. of Sci. and Technol., Okinawa, Japan

Abstract: The brain's highest level of acetylcholine (ACh) is in the striatum, a major motor-related nucleus. ACh is one of the major neurotransmitters modulating cognitive processes including motor control, learning, memory, action selection, and goal-directed behaviors. It is mostly locally produced by a small number of cholinergic interneurons (ChIs) characterized by their spontaneous activity and formation of a dense axonal network. This ensures a wide-ranging effect of released ACh through fast direct synaptic connections and slow non-synaptic volume transmission. Impairment of ACh transmission is related to brain disorders including Parkinson's disease, which directly affects striatal function. Our understanding of ACh-mediated transmission has been limited by a lack of available tools to monitor its presence with a high temporal and spatial resolution. However, recently with the creation of a stable, highly sensitive Genetically-encoded ACh (GACh2.0. Jing, M. et al., *Nat Biotechnol.* 36, 726, 2018) it has been possible to determine in real-time, the basal level of ACh and moreover, optogenetically manipulate its release. We used C57BL/6J and ChAT-Cre mice 25-30 days old with the aim of establishing relations between ChIs and neural networks within the dorsolateral striatum. The

experiments were performed following approval of the OIST Animal Experiment Regulations, Japanese laws, and in compliance with the NIH Policies on Use of Lab. Animals and Ethics. The striatum was injected with an adeno-associated virus (AAV) expressing the genetically-encoded ACh sensor GACh2.0. (pAAV-hSyn-GACh2.0, abbrev. citation: Addgene plasmid # 121921). Moreover, to selectively target ChIs, a Cre-dependent CrimsonR -red-shifted channelrhodopsin- was expressed in ChAT-Cre mice (AAV5. Syn-FLEX-rc[ChrimsonR-tdTomato], abbrev. citation: Addgene viral prep # 62723-AAV5). After a 2-week survival period, tissue slices were obtained and imaging recordings performed. Our results from 3 wild type controls (n= 4 tissue slices) and 4 experimental mice (n= 6 tissue slices) indicate that cells expressing GACh2.0 were able to produce fluorescent responses (ΔF/F0): 1- spontaneously, 2- by ChIs optogenetic activation, and 3- after delivery of ACh puffs close to the recorded area. In addition, bath application of cholinergic agents (e.g., ACh and eserine) increased the magnitude of the fluorescent responses, while atropine and tetrodotoxin) reduced them. With this study, we aim to further our understanding of the regulation and precision of striatal cholinergic signaling within its different neural networks.

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Poster

145. Basal Ganglia: Neuromodulation

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 145.15/M28

Topic: E.03. Basal Ganglia

Support: NIAAA/NIH K99 AA025991
NIAAA/DICBR/NIH ZIA AA000416

Title: Subsecond monitoring of acetylcholine dynamics in awake, freely-moving mice with a novel, genetically-encoded intensity-based acetylcholine sensing fluorescent reporter (iAChSnFR)

Authors: *A. G. SALINAS[1,2], P. M. BORDEN[3], L. L. LOOGER[3], D. M. LOVINGER[1]; [1]Natl. Inst. On Alcohol Abuse and Alcoholism, Rockville, MD; [2]Dept. of Bioengineering, George Mason Univ., Fairfax, VA; [3]Howard Hughes Med. Institute, Janelia Res. Campus, Ashburn, VA

Abstract: Acetylcholine (ACh) was the first neurotransmitter to be identified and studied. “Vagusstoff”, as it was termed by Otto Loewi due to its release from the vagus nerve, could regulate heart rate in isolated frog hearts. Since then, ACh has been found to be critical for alertness, cognition, and action control, and has been studied for decades. Recently,

microdialysis in combination with sensitive neurochemical detection methods has allowed for study of ACh release in freely-moving animals. This method, however, is limited by extremely low temporal resolution (typically 10-30-minute bins). Enzymatic/electrochemical electrode sensors were developed and allow for indirect measurements of ACh release on a faster time scale (tens of seconds) but are limited to single behavioral sessions before degradation of the enzymes impacts performance of the sensor; this timescale is also still orders of magnitude slower than neurotransmission. Such limitations have hindered the study of ACh dynamics and the role of ACh in specific behavioral events with any appreciable temporal resolution. Recently, genetically-encoded fluorescent biosensors have been developed that allow for better study of neurotransmitter release in head-fixed or freely-moving animals. Our lab has validated one of these novel sensors, iAChSnFR, to study ACh dynamics. We first characterized iAChSnFR *ex vivo* in striatal and cortical brain slices. We found that we could electrically evoke ACh release and that this release was stimulation duration- and intensity-dependent. We further found that we could dynamically, pharmacologically modulate the ACh signal with a muscarinic receptor agonist and a D2 dopamine receptor agonist. We have also confirmed the detection of photometry signals *in vivo* and found dynamic alterations in tonic and phasic ACh activity in freely-moving mice treated with either an inhaled anesthetic (isoflurane), an acetylcholinesterase inhibitor (tacrine), ethanol, or cocaine. Finally, we found that ACh dynamics vary across daily trials and between training days during the acquisition of a motor skill (rotarod) with the most phasic ACh activity occurring during the first three trials on the first day of training, with significantly less ACh activity on subsequent days.

Disclosures: A.G. Salinas: None. **P.M. Borden:** None. **L.L. Looger:** None. **D.M. Lovinger:** None.

Poster

145. Basal Ganglia: Neuromodulation

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 145.16/M29

Topic: E.03. Basal Ganglia

Support: ZIA AA000416

Title: Real-time *in vivo* monitoring of striatal GPCR signaling using FRET-based biosensors

Authors: *S. M. AUGUSTIN[1], J. O. LEE[1], Y. KIM[2], H. L. PUHL, III[2], S. S. VOGEL[2], J. KOUSSA[1], D. M. LOVINGER[1];
[1]Lab. for Integrative Neurosci., [2]Lab. of Mol. Physiol., Natl. Inst. on Alcohol Abuse and Alcoholism, Rockville, MD

Abstract: Striatal neuromodulators have critical roles in action selection/initiation, decision-making, and reward-related learning. These neuromodulators exert their functions through G-protein-coupled receptors (GPCRs) that can activate second messenger intracellular cascades to affect neuronal signaling and behavioral functions. The dorsal striatum is a critical basal ganglia region that integrates a variety of neuromodulatory inputs from cortex, thalamus, and midbrain, as well as intrinsic neuromodulators in the microcircuity, to control action learning and performance. These inputs form synapses onto indirect and direct projecting medium spiny neurons (MSNs). MSNs contain receptors that can oppositely regulate cAMP accumulation and PKA phosphorylation. The cAMP-PKA signaling pathway has been shown to be a key synaptic modulator not only in striatum, but throughout the brain. Little is known about real-time intracellular cAMP-PKA signaling following GPCR activation *in vivo*. We have optimized *in vivo* optical fiber photometry methods with time-correlated single-photon counting to assess intracellular cAMP accumulation and PKA phosphorylation using Förster Resonance Energy Transfer (FRET)-based EPAC (cAMP) and AKAR (PKA) biosensors. These sensors can measure activity-induced changes in cAMP accumulation and PKA activity, respectively. Fiber photometry was used to measure fluorescence lifetime to quantify FRET activity. In freely moving mice expressing AKAR in dorsal striatal neurons, there are movement related changes in fluorescent lifetime during several days of motor skill learning on the accelerating rotarod. These changes are more prominent on early training days compared to later in training. On-going work will assess cell-type-specific signaling changes during motor skill learning.

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Poster

145. Basal Ganglia: Neuromodulation

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Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 145.17/M30

Topic: E.03. Basal Ganglia

Support: National Institutes of Health, National Institute on Alcohol Abuse and Alcoholism, Division of Intramural Clinical and Biological Research

Title: Direct measurement of activity dependent endocannabinoid mobilization in brain slice using the novel genetically encoded fluorescent sensor $GRAB_{eCB}$

Authors: *D. J. LIPUT[1], A. DONG[2], K. HE[2], S. M. AUGUSTIN[4], H. L. PUHL, III[5], Y. LI[3], D. M. LOVINGER[6];
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Abstract: The endocannabinoid (eCB) system is a major source of synaptic modulation and thus involved in regulating neural functions and behavior. Although many mechanisms underlying eCB mobilization have been elucidated, direct measurement of eCBs on time scales supporting synaptic modulation has yet to be accomplished. Such ability will allow for many new investigations on eCB signaling logic as it relates to neural plasticity, behavior, and pathophysiology. Here we report on a novel genetically encoded fluorescent sensor GRABeCB, based on a CB1 receptor scaffold, which is being used with brain slice photometry to study eCB mobilization kinetics, neural activity rules supporting eCB generation, and neurochemical pathways underlying eCB synthesis and degradation. Preliminary data shows that, in the striatum, eCB signals can be generated by intrastriatal electrical stimulation and are blocked by CB1 receptor antagonists. The putative eCB transient is slow with rise and decay times spanning several seconds, consistent with conceptual models of eCB mobilization. An eCB transient can be generated by a single electrical stimulus and transients are altered by changes in stimulation frequency and duration. Transients evoked by train stimulation are attenuated by Group I mGluRs and iGluR antagonists, which is consistent with 2-AG synthesis through the Gq-dependent signaling cascade and an eCB synthesis pathway dependent on postsynaptic drive. We are continuing experiments characterizing the physiological and biochemical rules governing eCB generation in the basal ganglia and are initiating in vivo fiber photometry experiments to measure eCBs in the context of behavior.

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Poster

145. Basal Ganglia: Neuromodulation

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 145.18/M31

Topic: E.03. Basal Ganglia

Title: Dopamine D1 receptor/cAMP/PKA signaling is differently regulated in subregions of the striatum

Authors: *K. SUGIYAMA[1], M. KUROIWA[1], T. SHUTO[1], T. FUKUDA[2], A. NISHI[1]; [1]Pharmacol., Kurume Univ. Sch. of Med., Kurume, Japan; [2]Kumamoto Univ., Kumamoto, Japan

Abstract: In the striatum, dopamine regulates motor functions and reward systems. Dopamine acting on D1 receptors stimulates cAMP/PKA signaling, whereas dopamine acting on D2 receptors inhibits cAMP/PKA signaling. Recent studies revealed that structural organization and cortical innervation are different among subregions of the striatum. However, it is unknown whether dopamine signaling is differentially regulated in subregions of the striatum. Therefore, we investigated dopamine signaling in each subregion of the striatum. Mouse striatal slices were

divided into seven subregions: (1) rostral part, (2-1) intermediate medial part, (2-2) intermediate lateral part, (2-3) intermediate most lateral part, (3) caudal part, (4) most caudal part, (5) nucleus accumbens. Slices of seven subregions were treated with a D1 receptor agonist, SKF81297 (1 μM), or a D2 receptor agonist, quinpirole (1 μM), for 10 min, and the activity of cAMP/PKA signaling was evaluated with the phosphorylation of DARPP-32 (Thr34, PKA-site) and GluA1 (Ser845, PKA-site). The stimulatory effects of SKF81297 on the phosphorylation of DARPP-32 and GluA1 were the lowest in the subregion (3) in the rostrocaudal axis and in the subregion (2-3) in the mediolateral axis. However, the expression of D1 receptors in these subregions was not altered. The inhibitory effects of quinpirole on the phosphorylation sites were similar in all subregions. We next analyzed the expression of proteins that were involved in dopamine signaling, and found that the expression levels of phosphodiesterase (PDE) 10A and choline acetyltransferase (ChAT) were high in subregion (2-3 and 3) and subregion (3), respectively. Treatment of slices with a PDE10A inhibitor, papaverine (10 μM), alone induced the large increases in the phosphorylation of DARPP-32 and GluA1 in the subregions with low D1 receptor signaling as well as other subregions. Treatment with a muscarinic receptor antagonist, atropine (1 μM), or a selective M4 muscarinic receptor antagonist, MT3 (100 nM), enhanced the SKF81297-induced increases in the phosphorylation of DARPP-32 and GluA1 in the subregions with low D1 receptor signaling. In DARPP-32 knockout mice, the SKF81297-induced increases in the phosphorylation of GluA1 were attenuated in all subregions of the striatum as compared to wild-type mice, suggesting the contribution of DARPP-32 to PP1 inhibition in all subregions. Thus, dopamine D1 receptor/cAMP/PKA signaling is differentially regulated in each subregion of the striatum. In subregions with low D1 receptor signaling, dopamine D1 receptor/cAMP/PKA signaling is likely down-regulated by PDE10A and muscarinic receptors.

Disclosures: **K. Sugiyama:** None. **M. Kuroiwa:** None. **T. Shuto:** None. **T. Fukuda:** None. **A. Nishi:** None.

Poster

145. Basal Ganglia: Neuromodulation

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Topic: E.03. Basal Ganglia

Support: NSF1655365
SUNY Albany
SUNY Research Foundation

Title: Modulating excitation and inhibition in striatal medium spiny neurons: The role of neuronal glutamate transporters

Authors: *M. A. PETROCCIONE, L. D'BRANT, N. AFFINNIH, H. CHESBRO, S. ZAHID, A. SCIMEMI;
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Abstract: The dorsolateral striatum (DLS) is one of the brain regions with the most abundant expression of the neuronal glutamate transporter EAAC1. However, many of the fundamental mechanisms by which EAAC1 controls synaptic transmission in the DLS remain largely unknown. Our previous work showed that EAAC1 limits activation of metabotropic glutamate receptors and this promotes expression of D1 dopamine receptors in the DLS. Here we show that EAAC1 also reduces phasic excitation and limits extrasynaptic NMDA receptor activation. The magnitude of these effects varies across different populations of striatal medium spiny neurons (MSNs). In addition, EAAC1 strengthens phasic inhibition onto MSNs by increasing the quantal size of GABAergic mIPSCs. Once again, this effect varies across different classes of MSNs. These functional changes are associated with MSN remodeling. By using compartmental modeling, we show that small changes in local excitation of MNSs, coupled with small reductions in phasic inhibition, profoundly shape the firing output of MSNs and their ability to relay information out of the DLS. We believe these findings are of paramount importance to shed light on the molecular, cellular and circuit mechanisms of obsessive compulsive disorder, a neuropsychiatric disease associated with polymorphisms in the gene encoding EAAC1 and striatal hyperactivity.

Disclosures: M.A. Petroccione: None. **L. D'Brant:** None. **N. Affinnih:** None. **H. Chesbro:** None. **S. Zahid:** None. **A. Scimemi:** None.

Poster

146. Basal Ganglia: Behavioral Control

Location: Hall A

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Program #/Poster #: 146.01/M33

Topic: E.03. Basal Ganglia

Support: NIH/NIMH 1R01MH099114-06A1

Title: Altered spontaneous motor behavior in striatal mGluR5 knockout mice

Authors: *J. J. MARSHALL[1], J. XU[1], A. CONTRACTOR[2];
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Abstract: Striatal projection neurons (SPNs) play key roles in regulating voluntary movement and reinforcement learning. Imbalanced activity between SPNs in the the direct (dSPNs) and indirect (iSPNs) pathway circuits causes abnormal motor function, and is a feature of diseases

affecting the basal ganglia such as Parkinson's and Huntington's disease. Metabotropic glutamate receptor 5 (mGluR5) is critical in regulating plasticity at synaptic terminals formed by cortical pyramidal cells onto SPNs. Activation of mGluR5 is required for the induction of endocanabinoid mediated long-term depression (LTD) at these synapses. However, the role that mGluR5 dependent plasticity has in generating balanced activity between dSPNs and iSPNs, and ultimately affecting motor planning and coordination, is unclear. To address this question, we generated mice that lack mGluR5 selectively in either dSPN or iSPNs. Initial characterization of activity of these mice revealed opposing effects of mGluR5 loss in each of the pathway segregated neuron types. iSPN mGluR5 KO mice had increased spontaneous locomotor activity in the open field while conversely, dSPN mGluR5 KO mice had decreased locomotor activity. dSPN mGluR5 KO mice also demonstrated reduced spontaneous expression of simple motor behaviors such as digging and nest building. In ongoing work, we are using in vivo microendoscopy to determine how the selective loss of mGluR5 in these two pathways affects patterns of activity in populations of dSPNs and iSPNs that correlate with the expression of simple motor behaviors. The results from these experiments will provide a circuit level understanding of the effects of mGluR5 dependent plasticity on motor function.

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Poster

146. Basal Ganglia: Behavioral Control

Location: Hall A

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Program #/Poster #: 146.02/M34

Topic: E.03. Basal Ganglia

Support: Nu Rho Psi 2018-19 Undergraduate Research Grant

Title: Role of striatal patches in habit formation and the potential contribution of dopamine

Authors: *J. A. NADEL[1], S. P. PAWELKO[1], D. COPES-FINKE[1], M. B. NEIDHART[1], J. R. SCOTT[1], N. G. HOLLON[2], C. D. HOWARD[1];
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Abstract: Habits are inflexible, automated behaviors that are notoriously difficult to modify. Habitual behaviors are cognitively efficient but can also be maladaptive, as habit formation underlies Obsessive-Compulsive Disorder, drug addiction, and Tourette's Syndrome. The dorsal striatum, an input nucleus of the basal ganglia, is generally thought to be involved in action selection and control of behavioral strategies, including the formation and maintenance of habits. Interspersed throughout the striatum are neurochemically distinct subregions known as patches (or striosomes). Although these structures have been thoroughly characterized neurochemically and anatomically, their function is not fully understood. Notably, patches are embedded in limbic

circuits and may provide direct inhibitory input onto midbrain dopamine neurons in the substantia nigra pars compacta (SNc), which project to the striatum and are also heavily implicated in habit formation, addiction, and compulsivity. To examine this interaction between striatal patches, habit formation, and the dopamine system, we utilized a *Sepw1*-Cre line (Gerfen et al., 2013) which expresses Cre only in patches. We first injected mice bilaterally with an AAV encoding a modified caspase 3 virus, which causes Cre-dependent lesion. Lesioned and sham mice were then food deprived and underwent a variable interval (VI) operant schedule, which has been shown to induce strong habits. We found that patch lesions reduced habitual responding during probe trials. As patches may regulate habit formation by modifying dopamine, we next investigated patch regulation of dopamine release by performing simultaneous optogenetics and fast-scan cyclic voltammetry (FSCV) in anesthetized *Sepw1*-Cre mice expressing ChR2 in striatal patches. Activation of patches was sufficient to reduce hindbrain-evoked dopamine responses. Overall, our results provide support for the role of striatal patches in the regulation of habitual behavior, potentially via effects on dopamine.

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Poster

146. Basal Ganglia: Behavioral Control

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Title: Striosome and matrix coding in a probabilistic decision-making task

Authors: *B. BLOEM[1], R. HUDA[3], M. SUR[2], A. M. GRAYBIEL[1];
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Abstract: Central to our understanding of the striatum is its organization consisting of the direct and indirect pathways, which play opposite roles in reinforcement learning and action selection. Layered on top of this is a second level of organization based on the striatal compartments, the striosomes and matrix. Despite their discovery over half a century ago and the fact that they have

been observed in many species, we know very little about their functional roles in behaving animals. We previously developed a two-photon calcium imaging approach that allows us to simultaneously record the activity of neurons in visually identified striosome and matrix compartments. We, and others, have reported that striosomal activity reflects the value of cues in classical conditioning paradigms. Compared to matrix neurons, encoding of cue values becomes stronger and more specific in striosomal neurons as animals learn the task.
Despite these early findings, the specific contribution of striosomes to action selection and reinforcement learning remains unexplored. Hence, we developed a decision-making task in which head-fixed mice earn rewards by rotating a wheel in the clockwise or anti-clockwise direction, with each action linked to reward probabilities that change regularly.
Our initial results indicate that the mice learn this task well. They perform hundreds of trials and make decisions by incorporating previous choices and their outcomes. Using two-photon calcium imaging, we found a large overlap in the functional responses of both compartments, but also potentially important differences with respect to how rewards are encoded and linked to previous actions and future choices. These findings should be important for understanding the functional subdivisions of the striatum, including those related to basal ganglia disorders.

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Poster

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Topic: E.03. Basal Ganglia

Support: Canadian Institutes of Health Research (T.W.). CIHR Grant MOP_102482

Title: Cell class specific segregation of gamma and beta synchronous neuronal ensembles in the striatum

Authors: *K. BANAIE BOROUJENI[1], M. OEMISCH[3], S.-A. HASSANI[1], P. H. TIESINGA[4], T. WOMELSDORF[2];
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Abstract: The striatum is composed of inhibitory neurons that form different neuron classes. One hypothesis suggests that these neuron classes contribute differently to the routing of information through the circuit, but it has been unclear how this hypothesis can be tested during ongoing goal-directed behavior. Here we propose a strategy for achieving this link of cell-level to network-level phenomena and report its results. In a first step, we electrophysiologically recorded striatum neurons in nonhuman primates performing a demanding reversal learning task

to ensure that neuron activation reflects goal-directed behavior. We found that the majority of 350 isolated striatum neurons are modulated by the task. In a second step, we classified neurons into separate classes according to their action potential rise time and repolarization speed, as well as according to their first and second order firing rate statistics. Using data driven clustering this approach yields seven distinct striatal interneurons classes. Classes with broader action potential shape and irregular firing patterns were reminiscent of medium spiny neurons. Another distinct subclass of narrow spiking cells showed fast action potential dynamics and highly regular firing reminiscent of fast spiking interneurons.In a third step, we quantified how strong cells synchronize to the local field potentials recorded in the same striatal circuit as the cells. This approach utilizes a novel filtering method that prevents influences from artifactual field potential components. We then use the strength of phase synchronization of individual cells to predict the cell class it was assigned to. We apply support vector machine classification tools with randomization statistics to arrive at an unbiased prediction of the cell class given the phase synchronization at varying frequencies. Using these analysis steps, we found that neurons showing strong 25-40 Hz low gamma band synchrony are significantly more likely to be part of the neuron subclass that was identified as fast spiking neurons. In addition, we found that the strength of 12-25 Hz beta band synchrony distinguishes between the broad spiking neuron classes. Taken together, these results provide a link between cell class specific firing, action potential properties and cell-class specific neuronal synchronization at the beta and the gamma frequency band. This finding documents a successful mapping of cell-level to network-level activity. We believe these findings are a starting point to understand cell class specific contributions to information routing in the striatal network during complex, goal directed behaviors.

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Poster

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Support: CONACyT: FDC_1702 to P.R-O
UNAM-DGAPA-PAPIIT: IA201916, IA201018 to P.R-O

Title: Sensory representations in the dorsolateral striatum provide a temporal reference for learning and executing motor habits

Authors: A. E. HIDALGO-BALBUENA, A. Y. LUMA, A. K. PIMENTEL-FARFAN, T. M. PEÑA-RANGEL, ***P. E. RUEDA-OROZCO**;

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Abstract: The basal ganglia (BG) are classically associated with the execution of habits. Anatomical and electrophysiological evidence suggests that the dorsolateral striatum (DLS) integrates sensory and motor information from cortical and thalamic regions to produce automatic motor sequences. However, there is no direct evidence linking sensory processing in the DLS and complex behaviors. In this study, we specifically explored the role of the forelimb somatosensory flow in the DLS during the learning and execution of motor habits.First, we compared somesthetic responses in the DLS and primary somatosensory cortex (S1) of anesthetized rats. Under these conditions, mechanical stimulation of the forelimbs mimicking locomotion reliably produced rhythmic sensory representations in both regions. Yet, sequential and temporal stimuli contents were more strongly represented in the population dynamics of the DLS. Then, we used a behavioral protocol in which rats running on a treadmill under tight spatiotemporal constraints developed a stereotyped motor sequence. Functional disconnections of thalamic- and cortical-striatal pathways indicate that the somatosensory flow to the DLS is required to extract the temporal component of the task in both apprentice and expert animals, without affecting stereotypy or running speed. Correspondingly, optogenetic activation and inactivation of somatosensory pathways to the DLS bidirectionally and selectively biased the temporal component of expert execution. Our results provide direct evidence for the role of sensory information in the DLS, indicating that the somatosensory flow imposed by movement provides the temporal reference for the development and execution of motor habits. Our data support the notion that one of the main functions of the BG is to continuously monitor the inner state of the animal to guide behavior.

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Poster

146. Basal Ganglia: Behavioral Control

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BES-2011-049131

Title: Striatum encodes task-adaptive expectations and expectation-based choice biases

Authors: A. HERMOSO-MENDIZABAL[1,2], A. HYAFIL[2,3], P. E. RUEDA-OROZCO[4], D. ROBBE[3,4], ***J. DE LA ROCHA**[1];
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Abstract: Prior experiences shape the way we perceive the world by creating expectations, a reference frame for future decisions. Where these expectations are represented in the brain and where they are converted into perceptual or choice biases is still unknown. We trained rats in an auditory discrimination task where the probability to repeat the previous stimulus category was varied in trial blocks. Rats capitalized on the predictability of the stimulus sequence by consistently developing a tendency to repeat or alternate their previous response using an internal trial-by-trial estimate of the sequence repeating probability, a variable we called transition evidence. Surprisingly, the transition evidence was only effective biasing choices in trials after correct responses. After an error it was transiently inactivated but became effective again after the next correct response. Such behavior was captured by a reinforcement learning model whereby the accumulated transition evidence was flexibly transformed into a choice bias depending on the response outcomes. To investigate how these computations can be implemented in the brain, we performed population recordings in the dorsomedial striatum (DMS). A GLM analysis of neural activity revealed three populations of neurons representing all the relevant variables necessary to carry out the transformation. First, a large fraction of neurons (~40%, $P < 0.01$) encoded choice side from the response initiation until the next trial response. Importantly however, this encoding was only carried over to the next trial after rewarded responses. Second, a population of neurons encoded the transition evidence (~19 %, $P < 0.01$), maintaining this information after both correct and error responses. Finally, a third population (~13%, $P < 0.01$) encoded the Left-Right choice bias derived from the transition evidence. As this bias resulted from the combination of the transition evidence and the previous choice, it disappeared after errors, consistently with the animals behavior and the post-error vanishing of the neural representation of choices. Together, our results suggest that DMS dynamically encodes the relevant variables that are necessary to build and maintain expectations and use them to modulate behavior.

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Poster

146. Basal Ganglia: Behavioral Control

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Title: The role of the basal ganglia in context-dependent changes in vocal performance

Authors: *J. SINGH ALVARADO, J. GOFFINET, J. HATFIELD, M. BEN-TOV, R. MOONEY;
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Abstract: Basal ganglia (BG) circuits play a key role in modulating movement in a context-dependent manner. Songbirds have emerged as a powerful and experimentally tractable organism for studying the mechanisms by which the BG shapes behavior as a function of social context. Male zebra finches can rapidly control the spectral and temporal variability in their song depending on whether they are singing alone or to a nearby female. The isolated male's ''undirected'' song is variable across renditions, but in the presence of a female, he sings a ''directed'' song that is highly stereotyped across renditions. Previous work suggests context changes in song premotor activity could initially arise in the BG homologue Area X, but the mechanisms through which this process occurs, as well as how it manifests at a population level, remain unclear. To address these issues, we used single photon microendoscopy, closed-loop optogenetics and in vivo pharmacology in male zebra finches producing directed and undirected songs. Imaging calcium activity from populations of genetically identified spiny neurons (SNs) during undirected song revealed more complex dynamics than previously appreciated. Individual SNs displayed preferential activity during various landmarks of song, including individual syllables, introductory notes, bout onsets, and offsets. Surprisingly, the majority of SNs participated in less than half of song performances, with variable ensembles being recruited across song trials. During directed performance, SNs were globally suppressed, bringing calcium signals from the majority of SNs below detection threshold. Consistent with the idea that this context-dependent change in activity underlies changes in vocal performance, optogenetic inhibition of Area X activity in isolated males was sufficient to reduce spectral and sequence variability to directed song levels. Although variations in dopamine in Area X are proposed to be the main driver of changes in song variability, here we report that blocking noradrenergic (NA)

transmission in Area X increases directed song variability, while activating NA receptors in Area X lowers the variability of undirected songs. Together, these results causally implicate noradrenergic modulation of Area X in rapid and adaptive context-dependent changes in vocal performance.

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Poster

146. Basal Ganglia: Behavioral Control

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Topic: E.03. Basal Ganglia

Support: Center for Molecular and Behavioral Neuroscience Graduate Program
NIH R01 NS072950

Title: The role of the striatal THIN-LTSI circuit in operant behavior

Authors: *B. STANFIELD, M. ASSOUS, J. KAMINER, J. M. TEPPER, T. Z. KOOS; CMBN, Rutgers Univ., Newark, NJ

Abstract: The striatum is one of the main input nuclei of the basal ganglia and has been implicated in goal-directed and habitual behavior. Within the striatum is a rich diversity of interneurons with distinct molecular and physiological subtypes. Two of these subtypes, the tyrosine-hydroxylase containing interneurons (THINs) and low-threshold spiking interneurons (LTSIs), appear to form a distinct circuit, but the activity of these neurons in-vivo and their involvement in learning and behavior are poorly understood. Here, we use in-vivo calcium imaging to record THINs and LTSIs in mice during operant behavior. We used a TH-cre mouse line to target THINs and a SST-cre mouse line to target LTSIs in the striatum. To visualize the calcium signal in awake, behaving mice, we injected the calcium indicator gCAMP6f with a cre dependent viral vector into the striatum, then imaged the cells in a variety of behavioral contexts. First we trained mice a simple operant task were a nose poke leads to a sucrose solution reward. In THINs we found that, while performing a simple operant task, there is a complete inhibition of the calcium signal. This inhibition is observed both during a successful trial and unsuccessful attempts, as well as during the omission of an expected reward, indicating that this response is related to the action, not reward collection. Unlike THINs, LTSIs showed a heterogeneous response to the simple operant task. Some cells responded to reward delivery, while others were less active during task performance. The simple operant task reveals a role for the THIN-LTSI system in operant behavior, but the self-directed nature of the behavior creates limits in analysis. For this reason, we also train mice in a dual-choice operant task. Mice initiate the trial in the

center of the maze, and one of two tones plays to direct the mouse to a reward on either the left or right side. Preliminary analysis indicates that THINs are active during certain kinds of movement.

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Poster

146. Basal Ganglia: Behavioral Control

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Topic: E.03. Basal Ganglia

Title: A novel task for probing inhibitory control

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Abstract: Background: Much work investigating inhibition in the motor system has utilized the stop signal task (SST). In this task, participants respond to a go signal as quickly as possible on the majority of trials. For a subset of trials a stop signal is presented shortly after the go signal. One of the limitations of the standard SST is that stop signal reaction time (SSRT) is not directly observable, rather it is estimated based on the go signal reaction time, the stop signal delay, and the probability of correct responding on stop trials. Additionally, because this task requires the inhibition of incipient action, its utility for understanding cessation of continuous movement is questionable. Thus, how the brain terminates ongoing motor programs is not well understood. To address these limitations we developed a novel stop task that requires termination of ongoing motor programs, provides a direct measure of SSRT, and allows for the comparison of stopping behavior in planned and unplanned conditions. Methods: In this continuous movement stop task (CMST), subjects move a computer mouse in a continuous, circular motion while monitoring a countdown that appears on the screen in front of them. In the majority of trials (70%) the countdown ends and then the stop signal appears (planned stop condition). In the remaining trials (30%) the stop signal appears before the countdown ends (unplanned stop condition). Results: Preliminary evaluation (n = 8) suggests that the CMST is effectively able to dissociate stopping behavior in the planned (mean SSRT = 478.5 ms) and unplanned conditions (mean SSRT = 552.5 ms; p = 0.008). Discussion: Thus far, the data support the efficacy of the CMST for differentiating stopping behavior between conditions. Future directions will further investigate behavioral strategies participants may utilize during this task (for instance preparatory slowing) and neurophsyiological mechanisms underlying this context-dependent termination of ongoing motor programs.

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Poster

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T &C Chen Brain-machine Interface Center at Caltech

Title: Computational and behavioral mechanisms of action inhibition

Authors: *V. CHRISTOPOULOS[1], N. HASHOUSH[2], D. BABAYAN[2], M. MALEKMOHAMMADI[2], R. ANDERSEN[1], N. POURATIAN[2];
[1]Biol. and Biol. Engin., Caltech, Pasadena, CA; [2]David Geffen Sch. of Med., Univ. of California Los Angeles, Los Angeles, CA

Abstract: Amongst the most important components of action regulation is action suppression, the failure of which contributes to neuropsychiatric diseases, such as Parkinson's disease (PD). Understanding the brain mechanisms for action suppression has important implications for revealing the pathophysiology of these diseases and improving and developing novel interventions. Action suppression occurs in at least 3 ways: (a) decision between multiple options, (b) decision with conflicting information, (c) stopping an ongoing action. The basal ganglia (BG) and, in particular, the subthalamic nucleus (STN) have been functionally implicated in action suppression, but in association with distinct frontal areas. However, how action suppression during selection, conflict and stopping, map to distinct frontal-BG networks remains incompletely understood. Here, we explored the computational and behavioral mechanisms of action suppression by training 32 healthy individuals to perform 3 types of tasks that require action inhibition: (a) Decision-free task: Select between one or two targets presented simultaneously in both hemifields, (b) decision-conflict task: Respond to a target stimulus that is surrounded by 2 flanker stimuli on each side, (c) stopping task: Inhibit an ongoing action when a stop signal arrives. All tasks performed using a 2-dimensional reaching joystick. Preliminary data showed that participants had slower reaction time when they were presented with two options, instead of a single option. They also responded slower and had lower accuracy when they were presented with conflicting information. Finally, the probability to stop an action was inversely correlated with the stop signal delay (SSD). To understand the mechanisms of action inhibition, we modeled the 3 tasks within a neuro-dynamical framework that simulates the frontal-BG network using dynamic neural fields and stochastic optimal control. The core component of the framework is a pause field that simulates the STN-mediate pause function. The

pause field projects into an action preparation field to suppress its activity when action inhibition is required. Our model makes predictions consistent with the behavioral findings, and importantly suggests that the strength in which the pause field projects to the action preparation field is different among the 3 tasks. This prediction is consistent with the hypothesis of distinct patterns of STN activation for the 3 types of action suppression functions.

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Poster

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Support: NIH Grant NS094754
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Title: Precise coordination of 3D rotational kinematics by ventral tegmental area GABAergic neurons

Authors: *R. N. HUGHES[1], G. D. WATSON[2], N. KIM[1], E. PETTER[1], K. BAKHURIN[3], H. H. YIN[2];
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Abstract: The Ventral Tegmental Area (VTA) is a midbrain region implicated in a variety of motivated behaviors. However, the function of VTA GABAergic (Vgat+) neurons remains poorly understood. Here, using 3D motion capture, in vivo electrophysiology and calcium imaging, and optogenetics, we demonstrate a novel function of VTA^{Vgat+} neurons. We found three distinct populations of neurons, each representing head angle about a principal axis of rotation: pitch, roll, and yaw. For each axis, opponent cell groups were found that increase firing when the head moves in one direction, and decrease firing in the opposite direction. Selective excitation and inhibition of VTA^{Vgat+} neurons generate opposite rotational movements. The relationship between these neurons and head angle is degraded only at the time of reward consumption, at which point all head-angle related neuronal subpopulations show indistinguishable reward-related responses. Thus, VTA^{Vgat+} neurons serve a critical role in the control of rotational kinematics while pursuing a moving target. This general-purpose steering function can guide animals toward desired spatial targets in any motivated behavior.

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Poster

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Title: A striatal interneuron circuit for continuous target pursuit

Authors: *N. KIM, R. N. HUGHES, H. H. YIN;
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Abstract: Most adaptive behaviors require precise tracking of targets in space. In pursuit behavior with a moving target, mice use distance to target to guide their movement continuously. To determine striatal circuits underlying pursuit behavior, we developed a new tracking task in which the animal followed the target moving toward left and right continuously and monitored the animal and target movements simultaneously with recording striatal activity. We show that in the sensorimotor striatum, parvalbumin-positive fast-spiking interneurons (FSIs) can represent the distance between self and target during pursuit behavior, while striatal projection neurons (SPNs), which receive FSI projections, can represent self-velocity. FSIs are shown to regulate velocity-related SPN activity during pursuit, so that self-velocity is continuously modulated by self-target distance. Moreover, bidirectional manipulation of FSI activity can selectively disrupt performance by increasing or decreasing the self-target distance. In addition, we used optogenetics to manipulate dSPNs (direct pathway SPNs) and iSPNs (indirect pathway SPNs) during pursuit behavior, and demonstrated differential contributions of dSPNs and iSPNs to velocity control. These findings reveal a key role of the FSI-SPN interneuron circuit in pursuit behavior, and elucidate how this circuit implements distance to velocity transformation.

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Poster

146. Basal Ganglia: Behavioral Control

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Program #/Poster #: 146.13/N1

Topic: E.03. Basal Ganglia

Title: Distinct roles for cortico- and thalamo-striatal projections in motor skill learning and execution

Authors: *S. B. WOLFF, A. K. DHAWALE, R. KO, B. P. OLVECZKY;
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Abstract: The remarkable capacity of the brain to acquire and execute motor skills depends on a distributed motor network. While many individual components have been identified, less is known about their specific roles and how they interact during learning and execution of motor skills.
To address this, we train rats in a lever-pressing task which results in spatiotemporally precise movement patterns. Our previous finding that motor cortex is necessary for learning, but not for execution of this motor skill, suggests that motor cortex may act as a tutor for subcortical motor circuits during learning. A main candidate to receive this tutoring is the dorsolateral striatum, a major target of motor cortical projection neurons. In line with this hypothesis, we show that the striatum is indeed necessary both for the acquisition and execution of the motor skills we train. Furthermore, chronic and selective silencing of motor cortex's direct projections to the striatum, by viral and molecular strategies, prevented animals from learning the motor skill. In line with our previous lesion experiments, the same silencing did not affect skill execution when it was done after learning. We next tested the contribution to skill execution of striatum's other main input, that from thalamus. We found that chronic silencing of thalamo-striatal projections disrupted both skill execution and learning. These findings identify the striatum as a central player and suggest distinct roles for its cortical and thalamic inputs during motor skill acquisition and execution.

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Poster

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Title: Striatal activity and modulation of direct/indirect striatal pathways during go/no-go learning in mice

Authors: *P. MARTINEZ[1], O. BOTONIS[2], H. SMITH[2], M. DAWSON[2], J. WANG[2], W. F. ASAAD[3], I. OZDEN[2];
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Abstract: Dopaminergic neuromodulation in the basal ganglia system has been implicated in reward-based regulation of behavior and learning. It has been hypothesized that differential activations of two anatomically distinct dopaminergic pathways in striatum, namely striatonigral direct and striatopallidal indirect pathways, exert opposing effects, e.g. reward vs. punishment, Go vs. No-Go, or motivation vs. demotivation, respectively, during dopaminergic regulation of behavior. However, how the activation of these two pathways specifically contributes to the learning process, and what specific signals the neuronal populations in striatum carry to facilitate learning have not been well understood. To address these issues, we have performed two studies in mice learning an odor-based Go/No-Go task. In this task, mice learn to identify the identities of four odors delivered through an odor port. Two of the odors were used to instruct reward delivery through a water port (Go trial) and the remaining two to wait and not go to the water port (No-Go trial). In the first study, we primed one of each Go and No-Go odors with optogenetic stimulation of either the direct pathway or indirect pathway neurons in dorsomedial striatum during feedback period. For specific stimulation of direct and indirect pathways, we injected the virus AAV5-EF1a-DIO-hChR2(H134R)-EYFP into dorsomedial striatum bilaterally in D1-Cre and A2a-Cre mice, respectively. Our optogenetic stimulation data showed that neither the speed of learning, nor final task performance (>75% success rate) was significantly altered by optogenetic stimulation of direct or indirect pathways. However, stimulation of the direct pathway during correct No-Go trials slowed learning and during Go trials decreased reaction times. On the other hand, stimulation of the indirect pathway increased latency in initiating the subsequent trial. These results indicate that stimulation of direct or indirect pathway alone in the Go/No-Go task does not provide a reward or punishment signal, respectively. In the second study we introduced genetically encoded calcium indicator protein GCaMP6f in neurons of dorsomedial striatum and used a miniature head-mountable fluorescent microscope to monitor the functional calcium activity in neuronal populations during learning of the Go/No-Go task. Our data showed that a variety of signals related with the learning process are carried by the striatal activity. Our results contribute to knowledge in deciphering underlying neuronal mechanisms of reward-based learning in striatum.

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Poster

146. Basal Ganglia: Behavioral Control

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 146.15/N3

Topic: E.03. Basal Ganglia

Support: Strategic Priority Research Program of Chinese Academy of Sciences (grant No. XDB32010200)
Shanghai Municipal Science and Technology Major Project (grant No. 2018SHZDZX05)
National Natural Science Foundation of China (31571079, 31771151)

Title: Control of licking behavior by specific cell type in dorsolateral striatum

Authors: *Z.-Y. ZHANG, Z. CHEN, T. XIE, H. YAO;
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Abstract: Drinking in rodents is achieved by a highly stereotyped, rhythmic licking movements of the tongue and jaws. Basal ganglia as well as the cortex and brainstem are involved in the motor control of licking behavior. Previous studies have suggested that the ventral part of dorsolateral striatum (vDLS) may play an important role in tongue movements. However, the function of vDLS in licking and the role of specific cell type in regulating licking remain unclear. We found that optogenetic inactivation of the vDLS, but not the dorsal part of the DLS or the dorsomedial striatum (DMS), suppressed the instantaneous lick rate of self-initiated licking in head-fixed mice. The reduction of lick rate induced by vDLS inactivation was due to a reduction in the number of licks per bout rather than a change in lick duration or inter-lick interval. Inactivation of D1 receptor-expressing medium spiny projection neurons (D1-MSNs) in vDLS reduced the number of licks per bout, whereas inactivation of D2 receptor-expressing medium spiny projection neurons (D2-MSNs) increased the number of licks per pout. By performing extracellular recording from vDLS in licking mice, we found that the majority of MSNs exhibited lick-related activity. Using fiber photometry to record population activity of D1 or D2 MSNs, we found that D1- and D2-MSNs showed similar level of GCaMP6s fluorescence change during licking, but the onset latency of fluorescence change was shorter in D1-MSNs. Furthermore, optogenetic inactivation of D1-MSNs in vDLS before lick initiation delayed the lick latency. We are currently doing more experiments to examine how inactivation of D1- or D2-MSNs in the vDLS influences neuronal activity in the substantia nigra pars reticulata in licking mice.

Disclosures: Z. Zhang: None. **Z. Chen:** None. **T. Xie:** None. **H. Yao:** None.

Poster

146. Basal Ganglia: Behavioral Control

Location: Hall A

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Program #/Poster #: 146.16/N4

Topic: E.03. Basal Ganglia

Title: Neural correlates of reward-driven switching behavior in striatum

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Abstract: Animals avoid foraging regions with depleted resources in the forest. Humans take alternative and uncertain pathways in their lives. A major drive resulting in changes in strategy or motor behavior is to maximize the future reward outcome. A body of theoretical and experimental studies point towards the central role of dopamine signal, which provides a framework for explaining how reward shapes behavior in reinforcement learning. However, the neural mechanisms that support reward-based regulation of behavior are not well understood. In this study, we trained mice on a version of multi-armed bandit task, in which one out of three choices would lead to reward in a dynamically changing environment. We characterized their behavior by relating the response time and number of attempted trials to the reward history. Mice were sensitive to the reward by employing an exploration-exploitation strategy. Specifically, they started a trial-and-error phase when reward was absent and later committed to a new favorable choice. The response significantly slows down during the exploration and the performance improved with training, i.e. less number of exploratory trials before switching. We devised a descriptive model to capture the behavioral observation by taking into account both the recent reward and long-term learning. We genetically introduced calcium indicators (GCaMP6f) onto the neurons in dorsomedial striatum, a major target of midbrain dopaminergic signals. We implemented a miniature head-mounting fluorescent microscope to monitor the functional calcium activities during different phases of the switching behavior. We found that, at single cell and population level, both reward prediction error and value of the chosen action were present. When incorporates a counterfactual update, the model can better explain animals' behavioral and neural features than conventional reinforcement learning models. Our result indicates that an agent can infer the value of action that it has not taken and the striatum provides neural substrate for the value functions.

Disclosures: J. Wang: None. **I. Ozden:** None.

Poster

146. Basal Ganglia: Behavioral Control

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Program #/Poster #: 146.17/N5

Topic: E.03. Basal Ganglia

Support: Simons Collaboration on the Global Brain

Title: Revealing elements of naturalistic reinforcement learning through closed-loop action identification

Authors: *W. GILLIS[1], J. MARKOWITZ[1], M. JAY[1], R. CIESZKOWSKI[1], J. MURMANN[1], E. PETERSON[1], D. ALDARONDO[1], D. BRANN[1], S. LINDERMAN[2], B. SABATINI[1], S. R. DATTA[1];
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Abstract: Animals navigate complex environments by optimizing sequences of actions that best predict reward outcome. This process, known as reinforcement learning, involves producing an action sequence, evaluating the value of the resulting outcome, and preferentially weighting actions that lead to larger or more predictable rewards. Although many rodent studies have shown that the dopaminergic circuits are involved in learning and skill acquisition in highly artificial contexts such as lever-press and 2-AFC tasks, little is known about how dopamine (DA) regulates the expression of behavioral components or sequences during naturalistic behavior. To study reinforcement learning under more naturalistic conditions, we developed rt-MoSeq (real-time Motion Sequencing), a closed-loop system capable of fast and accurate identification of behavioral motifs called syllables. By optogenetically stimulating neurons in either the ventral tegmental area (VTA) or substantia nigra pars compacta (SNc) during the execution of a target syllable, we show that mice can rapidly and flexibly learn to up-regulate production of the target syllable. Learning is target specific — the production of other syllables, similar or otherwise, are not affected. Mice learn to up-regulate the target syllable within the first five minutes of the first session, and robustly up-regulate the target by the end of the second session. Stimulation of DA neuron afferents in either the nucleus accumbens core or the dorsolateral striatum show similar levels of target syllable up-regulation. These data demonstrate that unitary behavioral motifs can be robustly reinforced via a dopaminergic teaching signal; we are currently using the platform to explore the effects of targeted reinforcement on intra-syllable variability, syllable timing, syllable sequencing, and the relationship in time and space between reinforced and non-reinforced syllables.

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Poster

146. Basal Ganglia: Behavioral Control

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Topic: E.03. Basal Ganglia

Support: NIH Grant 1U01NS103489-01
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NIH 5R21EY027592

Title: Encoding of force in an isometric 2-action task in dorsal striatum

Authors: *I. RODRIGUES-VAZ, V. R. ATHALYE, D. S. PETERKA, R. M. COSTA; Columbia Univ., New York, NY

Abstract: Survival requires animals to perform the appropriate set of actions in order to access their necessary resources. The basal ganglia and its output loops have long been linked to selection, initiation, performance and organization of actions. There are two main pathways in this circuit: the indirect striatopallidal pathway (D2) and the direct striatonigral pathway (D1). From classical models it has been hypothesized that these two pathways have opposing roles in action selection and ongoing action modulation. However, recent studies have shown concurrent activation of both projection pathways when animal is performing both learned and naturalistic movements. Thus, there is still ongoing debate on the role of these two pathways during action learning, selection, initiation, and performance. Specifically, we want to understand how the coordination of D1- and D2-SPNs encodes action during action learning, selection, and performance. We developed an isometric force task to study how the striatum encodes force in the absence of overt movement. This task consists on a head-fixed isometric force task in which mice do not receive any cue besides their own proprioception and reward triggered by the correct action. The mice are required to perform either pulls or pushes on an immobile, pressure-sensitive joystick within an experimenter-defined duration and pressure range in a self-paced manner. This task enables us to study the learning of two skilled actions exclusively dependent on force - no movements are required to meet the reward criteria. Once the animals learn the two actions, that task elicits action selection by alternating reward contingency between the two actions. Thus, we can study how mice select one skilled action versus another, using only their proprioception and the task's reinforcement. In order to understand the role of D1- and D2-SPNs during learning of these skilled actions, we simultaneously recorded these neurons in dorsal striatum during the isometric force task using two-photon calcium imaging through a gradient-index (GRIN) lens. Our preliminary data suggests that both D1- and D2-SPNs encode actions signals and their neuronal activity seem to have increased correlation with force exerted by the animal as training progresses. One interesting possibility is that the D1 and D2 neuronal activity is coordinated to achieve reliable execution of the action. We analyze how both populations co-vary during action learning and during stable performance of the two actions.

Disclosures: I. Rodrigues-Vaz: None. **V.R. Athalye:** None. **D.S. Peterka:** None. **R.M. Costa:** None.

Poster

146. Basal Ganglia: Behavioral Control

Location: Hall A

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Topic: E.03. Basal Ganglia

Support: LSRF Fellowship

Title: Dissecting the action learning process with closed loop optogenetics

Authors: *C. TANG[1], V. B. PAIXAO[3], F. J. CARVALHO[4], A. SILVA[3], R. M. COSTA[2];
[2]Neurosci., [1]Columbia Univ., New York, NY; [3]Champalimaud Fundation, Lisbon, Portugal;
[4]Champalimaud Fndn., Lisbon, Portugal

Abstract: While it is well known that animals are capable of learning from the consequence of their actions, it is less clear how they hone in on the correct action, amongst many other possible actions, for reward. A chief limitation of conventional operant conditioning paradigms lies with confounding factors that obscure a detailed analysis of the learning process. Combining closed loop optogenetics with high-resolution behavioral clustering, we show that freely-moving mice learn to perform specific behavioral clusters for dopamine self-stimulation. Along with increases in frequency of the selected behavioral cluster, initial learning is also characterized by increases in similar but not reinforced behavioral clusters. Later in learning, frequency of the selected behavioral cluster remains high while similar but not reinforced behavioral clusters drop in frequency in a manner that relates to their similarity to the chosen cluster. Cluster composition and sequence analyses will be presented in this work. This system paves the way towards a high resolution analysis of the action learning process and reveals the way in which dopaminergic self-stimulation guides the animal to behaviorally hone in on specific actions.

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Poster

146. Basal Ganglia: Behavioral Control

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Topic: E.03. Basal Ganglia

Support: NIH NINDS K08-NS072183
NIH NINDS R56-NS109227

Title: Precisely-timed phasic dopamine signaling creates distinct kinematic representations of skilled movements

Authors: *A. BOVA[1], A. HURST[2], D. K. LEVENTHAL[2];
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Abstract: Dopamine is critical to normal motor function as evidenced by its importance in Parkinson Disease and related movement disorders (e.g., dystonia, tic disorders, chorea). Dopamine replacement therapy (DRT), however, only improves some aspects of motor function (e.g., bradykinesia) in Parkinson Disease, while coordinated multi-joint movements ("dexterous" skills) remain impaired. Furthermore, with disease progression, clinical responses to DRT fluctuate rapidly between "off" and "on" states. The reasons for incomplete DRT responses and motor fluctuations remain unclear, in large part because dopaminergic contributions to motor function are not well-understood. To study dopaminergic modulation of dexterous skills, we developed an automated skilled reaching task that allows precisely timed optogenetic manipulations of nigrostriatal dopamine neurons as rats grasp and retrieve sugar pellets. Applying a machine learning algorithm for markerless motion tracking ("deeplabcut") to high-speed video from multiple viewpoints enabled us to reconstruct 3-dimensional paw and digit trajectories. In well-trained rats, stimulation during, but not between, reaches gradually altered reach kinematics and impaired reach accuracy. Reach extension became progressively shorter, grasp aperture (distance between the 1st and 4th digits) narrowed, and variability of paw trajectories increased with repeated stimulation. This gradual alteration in forelimb kinematics suggests that precisely-timed phasic dopamine release drives plastic changes in motor circuits, mediating aspects of motor skill adaptation. Once dopamine stimulation-induced "bad reaches" were established, both reach kinematics and accuracy rapidly returned to baseline when stimulation was withheld. "Bad reaches" then returned within a single "stimulation" trial. The abrupt alternation of competent and incompetent reaching suggests that dopamine fluctuations gradually establish coexisting "distorted" and "normal" striatal representations of movement strategies, which are selected based on current dopamine levels. This work extends prior observations regarding dopaminergic influences on stimulus-response associations to the control of complex goal-direct movements, and suggests mechanisms by which rapid striatal dopamine fluctuations (as in treated Parkinson Disease) cause movement abnormalities.

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Poster

146. Basal Ganglia: Behavioral Control

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Topic: E.03. Basal Ganglia

Support: HHMI

Title: Associative learning without value: A policy-based neural network model of Pavlovian conditioning

Authors: *J. T. DUDMAN[1], L. T. CODDINGTON[2];
[1]Janelia Res. Campus, HHMI, Ashburn, VA; [2]Neurobio., HHMI Janelia Res. Campus, Ashburn, VA

Abstract: Paradigms such as Pavlovian conditioning inform diverse theories of associative learning. Canonical theories have interpreted the learning problem as one of understanding how an initially neutral sensory cue acquires an expected value. Common behavioral measures of learning have thus often been interpreted as a reflection of this inferred value (i.e. a "preparatory reflex"). However, in a careful examination of behavior during acquisition of a trace conditioning task, we recently discovered that anticipatory licking - the most common behavioral measure of learning in Pavlovian tasks - reduces the latency between the delivery of a water reward and its collection (Coddington & Dudman, 2018). Thus, we propose that the goal of an animal may be to collect a reward as soon as possible after it is available. From this normative perspective, anticipatory licking has a simple interpretation. If an animal is vigorously licking when water is delivered, then, on average, the latency to collect the reward will be ~60 ms (half a lick cycle). This approaches and often improves upon the reaction time for sensory-evoked initiation of licking. Thus, anticipatory licking is an adaptive 'policy' that minimizes the latency to collect an available reward.
This account reinterprets anticipatory licking as a form of policy-based reinforcement learning - even for Pavlovian designs where no action is explicitly required to obtain reward. To explore this question we implemented a neural network model based upon the influential ideas of policy gradient reinforcement learning. Here we show, using a biologically plausible learning rule (related to Miconi, 2016), that policy-gradient learning reproduces commonly observed patterns of licking behavior in mice during task acquisition. We have recently shown that dopamine activity during task acquisition is difficult to reconcile with value-based learning models. In contrast, here we show that if one views midbrain dopamine neuron activity as a derivative not of a value function, but of a policy, we can faithfully match quantitative properties of dopamine activity.

Disclosures: J.T. Dudman: None. **L.T. Coddington:** None.

Poster

146. Basal Ganglia: Behavioral Control

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 146.22/N10

Topic: E.03. Basal Ganglia

Title: Defining the causal structures between behavior, learning, and midbrain dopamine activity

Authors: *L. T. CODDINGTON[1], J. T. DUDMAN[2];
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Abstract: Mammalian midbrain dopamine (mDA) neurons exhibit phasic activity in short bursts of <200 ms in response to reward related stimuli and actions, with 50-90% of neurons encoding the most salient incentive events. This phasic activity is thought to underlie the essential ability of animals to pursue rewarding outcomes, although the functions of brief DA transients calibrated to physiological responses have rarely been directly studied.
Here we simultaneously recorded mDA activity in the ventral tegmental area and multiple striatal projection targets in mice using fiberometric measurements of calcium activity, and we did so longitudinally as animals learned that a cue predicted a sweetened liquid reward. Multiple continuous measures of behavior and physiology were used to independently estimate the progression of learning. Finally, we used manipulations of mDA activity calibrated to fiberometry signals in order to probe the causality of physiological levels of DA activity for learning and behavior.
Together, these results lay a foundation for understanding the interplay between trial-by-trial changes in mDA activity, neural populations that determine phasic mDA neuron activity, and quantitative behavior. We propose that the causal effects of phasic mDA activity are best described as a non-error-based teaching signal within a policy optimization reinforcement learning algorithm. In contrast, our data are difficult to reconcile with an error-based signal that directly updates an inferred value function.

Disclosures: L.T. Coddington: None. **J.T. Dudman:** None.

Poster

146. Basal Ganglia: Behavioral Control

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Topic: E.03. Basal Ganglia

Support: Parkinson's Foundation
HHMI

Title: A novel behavior for assessing motor sequence learning and execution in rodents

Authors: *T. RICCELLI[1,2], L. NARAYAN[1], J. ARNOLD[1], J. PARK[1], J. DUDMAN[1];
[1]Janelia Res. Campus, Ashburn, NY; [2]Mayo Clin., Rochester, MN

Abstract: The basal ganglia (BG) is an evolutionarily conserved descending pathway from telencephalon to mesencephalon that are critical for diverse aspects of voluntary, purposive movement. Conflicting clinical studies show that pathological disruption of BG circuitry, such as that caused by dopamine (DA) depletion in Parkinson’s disease (PD), may impair motor sequence learning and/or performance. These discrepancies could be a consequence of differences in pathology across cohorts or could reflect different evaluation of behavior. Mouse

models are particularly useful in this regard as they allow for specific, reproducible perturbation of BG function. However, available motor sequencing tasks for rodents also often confound aspects of performance (regulating kinematics) with learning (acquisition of a motor sequence representation). To address this issue, we have developed a behavioral apparatus ("climbing wall assay"; CWA) that consists of configurable, touch-sensitive rungs allowing for unique spacing sequences that mice must traverse to obtain a liquid reward. The tilt of the CWA can be changed thereby dissociating kinematics from the specific sequence of rung positions. In addition to touch sensor data received from rungs and lick tracking from reward ports, we are using marker less tracking of individual body parts to record detailed kinematics on the millisecond timescale. Finally, we have developed a motorized commutator that tracks with mouse position to allow for neural recording/perturbation in freely moving animals moving at a variety of angles relative to gravity without significantly impairing motor behavior. Using this apparatus we first demonstrate that wild type mice exhibit classical indicators of motor skill learning (i.e. a progressive increase in speed coupled with enhanced accuracy) as well as evidence for sequence specific learning - all within ~300 trials on our task. Additionally, a refined parkinsonian mouse model exhibits severe deficits on our task, reversible by L-DOPA treatment, indicating our task is a useful model for assessing sequence execution in PD rodent models. In addition to behavior measurements, we have also demonstrated that midbrain DA neurons can be recorded using fiber photometry and closed-loop optogenetic perturbation of specific cell types in the basal ganglia can be combined with this task. In summary, we describe a novel apparatus that dissociates the learning and performance of sequential movements, allowing us to more accurately determine the role of the dorsal striatum and dopaminergic neurons in the performance of complex motor sequences.

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Poster

146. Basal Ganglia: Behavioral Control

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Topic: E.03. Basal Ganglia

Support: NIH Grant 068283

Title: Sex- and duration- dependent neural circuit control of voluntary exercise behavior

Authors: *M. K. TANNER[1], K. BONAR[2], N. A. MOYA[1], J. K. P. DAVIS[2], J. JAIME[2], E. C. LOETZ[2], B. N. GREENWOOD[2];
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Abstract: Incidence rates of stress-related psychiatric disorders are increasing and affect females at nearly twice the rate of males. Exercise has beneficial effects on mental health and can protect against the development of stress-related disorders. Although the benefits of exercise are well known, participation in regular exercise is decreasing. Dopamine (DA) and the dorsal striatum are critical for movement, however, the specific DA-striatal circuits that motivate voluntary exercise are unknown. Identifying the neural circuits that motivate voluntary exercise behavior in both sexes could lead to novel strategies to increase exercise participation and reduce the effects of stress. Rats given access to running wheels demonstrate robust voluntary exercise behavior that follows a distinct pattern of two phases consisting of an acquisition phase, during which nightly running escalates, followed by a maintenance phase, during which running distance plateaus. The goal of this study is to identify the DA-striatal circuits that support the different phases of voluntary wheel running in both male and female Long-Evans rats. Using a pharmacological inactivation technique, we temporarily inactivated two dorsal striatum subregions, which are involved with different learning strategies, during the two phases of exercise. In males and females during phases of the estrous cycle other than proestrus, temporary inactivation of the dorsomedial striatum (DMS), a region important for goal-oriented behavior, reduced voluntary exercise during the acquisition phase, but not the maintenance phase. In contrast, temporary inactivation of the dorsolateral striatum (DLS), which is a circuit important for habit formation, reduced voluntary exercise during the maintenance phase, but had no effect on exercise during the acquisition phase. Interestingly, we observed that females in proestrus, the phase of the estrous cycle when estrogen is the highest, rely on the DLS to support the acquisition of wheel running, rather than the DMS. These data suggest that the neural circuits that support voluntary exercise behavior depend on both the phase of exercise and sex. Different striatal subregions could be targets for manipulations aimed at enhancing the initial acquisition vs. long-term maintenance of exercise behavior. We are currently assessing the role of DA in the dorsal striatum during the different phases of exercise by quantifying the activation of DMS and DLS neurons that express D1 receptors.

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Poster

146. Basal Ganglia: Behavioral Control

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Support: NIH Grant 068283
Undergraduate Research Opportunity Program

Title: Sex differences in voluntary exercise behavior

Authors: *A. A. HOHORST, R. R. WOOD, J. K. P. DAVIS;
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Abstract: While various physical and mental health benefits of exercise are well known, this knowledge has not yet translated into increased exercise behavior among humans. In fact, participation in physical activity is decreasing. Rats provide a useful translational model as they, like humans, engage in voluntary exercise behaviors. Additionally, the positive physiological impacts of wheel running on rats resemble many of the health benefits of exercise in humans. However, very little of this research has been conducted with female rats due to additional work and difficulty in controlling for the estrous cycle, which has a pronounced impact on physical activity. Our research aims to begin to address this deficit by characterizing sex differences in voluntary exercise behavior. Adult male and cycling female Long-Evans rats were singly housed with in-cage running wheels for 4 weeks. Females were lavaged daily to track estrous phase and running distance was collected nightly. Initial results suggest that female rats run greater distances than males starting from the first night of wheel access. This sex difference is most pronounced when females are in the proestrus phase of the estrous cycle, when estrogen levels are highest. Nightly running distance escalates more quickly in females than it does in males, reaching a plateau after only 1 week. The circadian pattern of wheel running behavior is similar between sexes, wherein the majority of running occurs in the active cycle. During proestrus, however, females engage in wheel running behavior prior to the start of the active cycle. Most interestingly, the estrous phase females are in during the start of wheel running has a dramatic effect on later exercise behavior. Females which start wheel running during proestrus display greater running distances during subsequent days, compared to females which started running during any other estrous phase. This difference is most pronounced during later proestrus, suggesting that starting wheel running behavior in proestrus produces a state-dependent priming of exercise behavior. Further analyses of running speed and circadian rhythms of exercise behavior are ongoing. Our data reveal novel sex differences in voluntary exercise behavior, and suggest that ovarian hormones have a profound influence on voluntary exercise. Further, these data suggest that hormones present specifically at the onset of an exercise regime may prime the neural circuits controlling later exercise behavior in a state-dependent manner.

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Poster

146. Basal Ganglia: Behavioral Control

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Title: A portable system to quantify movement stopping in patients with movement disorders

Authors: S. CHOUDHURY[1], A. ROY[1], B. MONDAL[1], R. SINGH[1], S. HALDAR[1], K. CHATTERJEE[1], M. R. BAKER[2], H. KUMAR[1], *S. N. BAKER[2];
[1]Inst. of Neurosciences Kolkata, Kolkata, India; [2]Newcastle Univ., Newcastle upon Tyne, United Kingdom

Abstract: An impairment of stopping inappropriate movement has been reported previously in patients with Parkinson's disease and focal dystonia. The ability to inhibit movement can be quantified by Stop Signal Reaction Time (SSRT), which is measured assuming a 'race model' in which stop and go processes race to complete; the first to finish dictates the behavioural response[1]. We have improved the conventional SSRT estimation process using a Bayesian statistical approach to yield 'optimal combination SSRT' (ocSSRT). We validated that this is correlated with the conventional measure, but has increased reliability. We also developed a portable system to measure SSRT, comprising a battery-powered device which subjects can hold comfortably in two hands. One red (stop cue) and one green LED (go cue) are positioned on the box front; beneath the LEDs is a press button. Above the LEDs is an LCD screen, which provides a textual display of the numerical values for the estimated SSRT. Subjects were asked to respond to a go cue, but to inhibit their responses on some trials if a stop cue appeared. In our study, ocSSRT was significantly prolonged in patients with Parkinson's disease and focal dystonia (one-way ANOVA<0.001). Receiver operating characteristic curves demonstrated a clear separation between healthy controls and patients (area under the curve >0.9; p<0.001). Administration of L-dopa significantly improved ocSSRT in PD patients (p<0.001). Similarly, the ocSSRT was significantly improved after postoperative optimisation of deep brain stimulation setting at bilateral subthalamic nucleus in PD patients (p<0.05). Administration of botulinum toxin significantly improved ocSSRT after a month of injection, in CD patients (p<0.05). ocSSRT is an easily-administered bedside neurophysiological tool for detection of PD and focal dystonia and has potential for objective estimation of treatment outcome.
1.Logan GD, Cowan WB, Davis KA. On the ability to inhibit simple and choice reaction time responses: a model and a method. J Exp Psychol Hum Percept Perform. 1984 Apr;10(2):276-91.
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Disclosures: **S. Choudhury:** A. Employment/Salary (full or part-time):; Institute of Neurosciences Kolkata, India. **A. Roy:** A. Employment/Salary (full or part-time):; Institute of Neurosciences Kolkata, India. **B. Mondal:** A. Employment/Salary (full or part-time):; Institute of Neurosciences Kolkata, India. **R. Singh:** A. Employment/Salary (full or part-time):; Institute of Neurosciences Kolkata, India. **S. Haldar:** A. Employment/Salary (full or part-time):; Institute of Neurosciences Kolkata, India. **K. Chatterjee:** A. Employment/Salary (full or part-time):;

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Poster

146. Basal Ganglia: Behavioral Control

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 146.27/N15

Topic: G.02. Motivation

Support: ABCD CC

Title: Using corticostriatal networks to disentangle reward value and salience

Authors: *J. B. DENNISON[1], T. NG[1], L. ALLOY[2], D. V. SMITH[3];
[1]Temple Univ., Philadelphia, PA; [2]Temple Univ., Philadelphia, NJ; [3]Dept. of Psychology, Temple Univ., Philadelphia, PA

Abstract: The striatum is sensitive to a wide range of rewarding and salient events (Cooper & Knutson 2008) and also has been implicated in psychopathologies involving aberrant reward processing (Alloy, Olino, Freed, & Nusslock, 2016). In this study, we aimed to separate different qualities of reward sensitivity (i.e., value and salience) using the striatum's highly integrated architecture with the cortex (Haber & Knutson 2010). We applied tensiorial independent component analysis -- an underutilized form of ICA that captures spatiotemporal patterns across participants (Beckman & Smith, 2005) -- to a subsample of the Adolescent Brain and Cognitive Development (ABCD) study (N = 140). Our analyses focused on the imaging data from the Monetary Incentive Delay task, which provides a robust probe of reward anticipation (Knutson, Westdorp, Kaiser, Hommer, 2000) and allows for separation of value and salience (Cooper & Knutson, 2008). We recovered independent, but partially overlapping, networks representing value (linear function of reward highlighting the dorsal striatum and dlPFC) and salience (a quadratic function of reward highlighting the ventral striatum and ventromedial prefrontal cortex). We next investigated the clinical relevance of these corticostriatal network maps by examining how they relate to mood disorders within the ABCD study. We identified three groups of participants within our sample of using the K-SADS: healthy controls (N = 46), unipolar depression (N = 48), and bipolar disorder (N = 46). We found that the salience and

value components had greater projected values in the healthy controls and unipolar depression group than in the bipolar disorder group. These results suggest that the striatum integrates value and salience information from independent networks and that disjunction within these networks may be responsible for the dysfunction in reward sensitivity apparent in a multitude of disorders.

Disclosures: J.B. Dennison: None. **T. Ng:** None. **L. Alloy:** None. **D.V. Smith:** None.

Poster

146. Basal Ganglia: Behavioral Control

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 146.28/N16

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Effects of retina with midbrain coculture system on the neuroprotection of retinal ganglion cells

Authors: *J.-A. KO, Y. KIUCHI;
Hiroshima Univ., Hiroshima, Japan

Abstract: Glaucoma is the most common optic neuropathy, the second most common cause of blindness and the most common cause of preventable visual disability. Furthermore, glaucoma is a progressive neurodegenerative disease of the retinal ganglion cells and their axons. Neuroprotection in glaucoma is aimed at protecting those neurons that are damaged or likely to be damaged in glaucomatous optic neuropathy. So, we have now examined the effects of midbrain on the retinal ganglion cells survival with coculture system.
Midbrain slices from 3days rats after birth and retina explantation, or primary retinal ganglion cells from rats were cocultured using 3D-transwell culture system. Using RT-PCR and immunoblot analysis, the expression levels of the several survival markers of retinal ganglion cells were studied. Also, the expression of neurites of retinal ganglion cells after coculture was examined by immunofluorescence analysis.
Furthermore, the cells were treated with 500 μM hydrogen peroxide (H_2O_2), the down-regulation of neurite extension was blocked in coculture with midbrain.
These results suggest that coculture of retinal ganglion cells and midbrain separated by transwell insert system provides an in vitro model for studies of the interaction between the signals and its targets in vivo. The some secreted factors from midbrain may play an important role in the regulation of retinal ganglion cells survival.

Disclosures: J. Ko: None. **Y. Kiuchi:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.01/N17

Topic: F.02. Behavioral Neuroendocrinology

Support: KAKEN 16K08531

Title: Effects of the experience of pregnancy-delivery-nurturing on excitatory synaptic inputs to tuberoinfundibular dopaminergic neurons

Authors: *M. FURUTA, C. KAKEHASHI, T. FUNABASHI;
Dept. of Physiol., St. Marianna Univ., Kawasaki, Japan

Abstract: Dopaminergic neurons located in the arcuate nucleus and adjacent periventricular region of the mediobasal hypothalamus, are called A12 tuberoinfundibular dopaminergic (TIDA) neurons which control prolactin (PRL) release from the anterior pituitary as an inhibitory factor. Intracerebroventricular administration of PRL induces maternal behavior in virgin female rats, suggesting that TIDA neurons are involved in controlling maternal behavior through PRL regulation. However, whether TIDA neurons per se play a role on maternal behavior remains unclear. We examined in the present study whether reproductive experiences affect the excitatory synaptic inputs to TIDA neurons which might be involved in maternal behavior in female mice. We previously determined that green fluorescent protein (GFP) was a reliable marker of TIDA neurons in transgenic mice expressed GFP under the control of the rat tyrosine hydroxylase gene (RBRC02095). In 16-weeks old primiparous and nulliparous transgenic mice (N=8-9), brain slices including TIDA neurons were made and trunk blood were collected in proestrous mice. They were subjected to whole-cell voltage- and current-clamp study, and PRL assay, respectively. TIDA neurons were identified by fluorescence microscopy. The frequency, but not amplitude, of miniature excitatory post-synaptic potential (mEPSP) was augmented in primiparous mice compared to nulliparous counterpart. Plasma PRL concentrations of primiparous mice were significantly lower than those of nulliparous mice. Resting membrane potentials and the number of action potentials induced by current injection were not significantly different between the groups. We revealed the numbers of excitatory synapses on TIDA neurons, expressing vesicular glutamate transporter 2, were not changed by reproductive experiences using the confocal laser scanning microscopy, regardless of the sites of synapses whether or not near the cell bodies of TIDA neurons. These results suggest that the excitatory presynaptic release probably glutamate, to TIDA neurons are changed by reproductive experiences.

Disclosures: M. Furuta: None. **C. Kakehashi:** None. **T. Funabashi:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.02/N18

Topic: F.02. Behavioral Neuroendocrinology

Title: The impact of adolescent oxycodone exposure on future maternal behaviors in the postpartum mouse

Authors: A. WATTERS, ***K. M. SEIP-CAMMACK**;
Psychology & Neurosci., The Univ. of the South, Sewanee, TN

Abstract: Females are uniquely sensitive to drugs of abuse at specific points in their reproductive cycle, yet little is known about how opioid drugs affect the maternal (postpartum) female. Females' endogenous opioid system contributes to both maternally relevant physiological functions and reward-related processes. While acute opiate exposure impairs maternal behavior, less is known about how a history of opiate exposure, prior to pregnancy, might affect postpartum females' future maternal behavior and motivation for her offspring (pups). The present study assessed the extent to which chronic exposure to oxycodone during adolescence, prior to pregnancy, altered a female's subsequent response to her pups, and whether this history affects postpartum females' preference for oxycodone. Adolescent female and male mice were exposed chronically to oxycodone or saline before entering spontaneous withdrawal. Females were bred and experienced a drug-free pregnancy. Following parturition, females' maternal behavior and motivation and their conditioned preference for oxycodone was assessed. No major sex differences emerged in mice's behavioral responses to oxycodone or degree of sensitization, or in the trajectory or severity of withdrawal. During the postpartum period, females' maternal behaviors did not differ as a result of adolescent oxycodone exposure, suggesting that relatively stimulus-bound maternal responses remain intact. However, oxycodone-exposed females showed mild deficits in the maternal motivation test, suggesting that the oxycodone history may have impacted the incentive value attributed to pups. The strength of conditioned place preference for oxycodone varied across individuals. Results suggest that opioid exposure in adolescence may alter the performance of some adaptive, motivated behaviors in adulthood.

Disclosures: K.M. Seip-Cammack: None. **A. Watters:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.03/N19

Topic: F.02. Behavioral Neuroendocrinology

Support: Gustavus Adolphus College Presidential Faculty-Student Collaboration Grant

Title: The effect of postpartum stress on maternal behavior in rats: A possible mechanism for offspring anxiety?

Authors: L. S. LOWE, A. V. LANDAVERDE, N. L. ANSELMO, J. L. MILLER, *K. C. DE LORME;
Gustavus Adolphus Col., Saint Peter, MN

Abstract: Intergenerational trauma is the transmission of the effects of trauma from one generation to the next, and has been studied in humans in a variety of cultural contexts. Parental trauma has been linked to predisposition to anxiety and depressive disorders, higher vulnerability to developing PTSD, and changes to the HPA axis. There are two routes one may investigate intergenerational trauma: social transmission and epigenetic inheritance. Social transmission includes changes in parental behavior towards offspring because of the trauma, and thus, can be studied using an animal model. We sought to investigate the social transmission of intergenerational trauma using an animal model by exploring the effects of postpartum stress on Sprague-Dawley rats. Six pregnant rats arrived in the lab and gave birth approximately one week later. The maternal stress paradigm occurred from postpartum days 8-10 with maternal behavior being digitally recorded two days prior and two days after exposure. First, the dams were taken from their home nest, brought into separate rooms, and put into an empty aquarium for a 5-minute acclimation period. They were then exposed to predator odor (Stressed) or control odor (Control) for 30-minutes. The offspring remained undisturbed in their home nests during the maternal stress paradigm. Pups were weaned at postnatal day 26 and tested for anxious behavior using the elevated zero-maze and light-dark box during adolescence or adulthood. We found that postpartum stress affects offspring behavior in both the elevated zero-maze and light-dark box, as previously reported. The purpose of the current study is to determine whether this effect on offspring anxious behavior is due to changes in maternal behavior towards the pups after the dams experienced postpartum stress. Behaviors that were quantified include nursing (arch-back, passive, and blanket), licking/grooming pups, self-grooming, and neglect. Preliminary 2 x 2 mixed factor ANOVAs were performed on a partial data set. There was no significant change in any of the maternal behaviors analyzed pre- and post-stress for either maternal group (Stressed, Control) nor an interaction between pre- and post-stress and maternal group. Thus, a change in these maternal behaviors in stressed dams may not account for the difference in anxious behavior

between the offspring of the two maternal groups. Alternative explanations include increased cortisol in stressed dams being passed to their offspring via nursing or changes in maternal behaviors that we did not quantify. However, further analyses will be performed on the entire data set to determine a more definitive conclusion.

Disclosures: L.S. Lowe: None. **A.V. Landaverde:** None. **N.L. Anselmo:** None. **J.L. Miller:** None. **K.C. De Lorme:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.04/N20

Topic: F.02. Behavioral Neuroendocrinology

Support: NSF grant IOS 1256572

Title: The selective dopamine β-hydroxylase inhibitor Nepicastat inhibits pup-directed behavior in virgin male California mice

Authors: *M. C. ACOSTA, W. SALTZMAN;
Univ. of California, Riverside, Riverside, CA

Abstract: The neural mechanisms underlying paternal care in biparental mammals are not well understood. The California mouse (*Peromyscus californicus*) is a monogamous rodent in which fathers participate extensively in all of the same parental behaviors as mothers, with the exception of nursing. While virtually all fathers are attracted to pups, virgin male California mice vary widely in their behavior toward unrelated pups upon exposure, ranging from attacking to avoiding to huddling and grooming pups. The difference in pup-directed behavior between virgin males and fathers suggests that the neurochemical control of pup-related behavior changes as males transition into fatherhood. The current study tested the hypothesis that norepinephrine (NE) facilitates the initiation of nurturant behavior toward pups in virgin male California mice, using the selective and potent dopamine β-hydroxylase inhibitor Nepicastat to inhibit NE synthesis. Nepicastat or vehicle solution was injected intraperitoneally 2 hours prior to exposing virgin male mice to a novel pup, estrous female, or inanimate, pup-sized, novel object for 60 min. Nepicastat significantly reduced the number of virgin males that approached pups, and decreased the duration of time spent sniffing or huddling/grooming pups, compared to vehicle. In contrast, Nepicastat treatment did not alter virgin males' interactions with an estrous female or a novel object, suggesting that Nepicastat-induced inhibition of interactions with pups was not mediated by changes in generalized neophobia, arousal, or activity levels. Therefore, our data indicate that NE may facilitate the initiation of paternal behavior in male California mice.

Disclosures: **M.C. Acosta:** None. **W. Saltzman:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.05/N21

Topic: F.02. Behavioral Neuroendocrinology

Support: MH119631

Title: The plasticity of corticotropin-releasing factor receptor 1 (CRFR1) within the anteroventral periventricular of the hypothalamus during the postpartum period

Authors: *R. M. DE GUZMAN, Z. J. ROSINGER, J. JACOBSKIND, D. G. ZULOAGA; Univ. at Albany, Albany, NY

Abstract: Corticotropin-releasing factor (CRF) signaling through CRF receptor 1 (CRFR1) regulates autonomic, endocrine, and behavioral responses to stress and CRF/CRFR1 activity has been implicated in the pathophysiology of several disorders, including anxiety and depression. Using a validated CRFR1 reporter mouse line (bacterial artificial chromosome identified green fluorescence protein (BAC GFP-CRFR1)), previous work in our lab reported a novel sex difference in CRFR1-expressing cells within the anteroventral/rostral periventricular nucleus (AVPV/PeN), which are prominent in female mice and largely absent in males. The AVPV/PeN is interconnected with many forebrain structures (e.g., preoptic area and paraventricular nucleus of the hypothalamus) involved with endocrine activity, stress, and reproductive behaviors. Recent studies have also indicated the AVPV regulates maternal behaviors and undergoes changes in gene expression during the maternal period. In the present study, we investigated whether AVPV CRFR1 levels change dynamically during the postpartum period. Within the AVPV/PeN, postpartum day 14 females showed an increased number of CRFR1-GFP cells and an increased number of restraint stress-activated CRFR1 cells as assessed by immunohistochemical co-localization of CRFR1-GFP and phosphorylated CREB (pCREB). On the contrary, tyrosine hydroxylase (TH) and TH/CRFR1-GFP co-localized cell number decreased in postpartum mice. Changes in CRFR1 expression were also assessed in two other brain regions that contribute to stress and maternal functions: paraventricular nucleus of the hypothalamus (PVN) and medial preoptic area (MPOA). Within the PVN, postpartum females did not differ in CRFR1-GFP or TH/CRFR1-GFP cell number, but had an increased number of pCREB/CRFR1-GFP co-localized cells. No significant differences were found in the MPOA. Together, these results indicate that CRFR1 cells in the AVPV/PeN change dynamically during the postpartum period and may contribute to an array of behavioral changes that occur during this period, including stress-associated (anxiety/depression) and maternal functions.

Disclosures: **R.M. De Guzman:** None. **Z.J. Rosinger:** None. **J. Jacobskind:** None. **D.G. Zuloaga:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.06/N22

Topic: F.02. Behavioral Neuroendocrinology

Support: CNPq Grant 141700/2017-3

Title: Inappropriate nutrition worsens maternal diabetes by impairing glucose tolerance and altering maternal care and offspring development

Authors: ***M. G. MARTINS**[1,3], A. G. CRUZ[1,3], G. P. OLIVEIRA[1], B. C. WOODSIDE[4], J. A. C. HORTA, Jr.[2], A. C. I. KISS[1,3];
[1]Physiol., [2]Anat., São Paulo State Univ. (UNESP), Inst. of Biosci., Botucatu, Brazil; [3]Physiol., Univ. of São Paulo, São Paulo, Brazil; [4]Concordia Univ., Montreal, QC, Canada

Abstract: The effects of maternal diabetes and diet manipulation have been thoroughly studied separately. Nonetheless, there is no evidence of how diet manipulations may aggravate behavioral and metabolic impairments on mothers with diabetes and their offspring. Since maternal metabolism and nutritional status are key factors for a healthy offspring intrauterine and postnatal development, the aim of the present study was to investigate the impact of mild hyperglycemia associated with inappropriate nutrition on maternal care and offspring development. Newborn female rats were divided in Control (citrate buffer, s.c.) or STZ (streptozotocin, 100 mg/kg, s.c.) groups. On postnatal (PND) 90, rats from both groups were mated and further subdivided in four groups: females fed with standard chow (Control, n = 22; STZ, n = 23); and females fed with standard chow plus potato chips and 1,5% sucrose solution from pregnancy day (PD) 0 to lactation day (LD) 14 (Control-snack, n = 23; STZ-snack, n = 23). An oral glucose tolerance test was performed on PD15. Food and caloric intake during pregnancy and lactation were measured, as well as litter size and sex ratio. Maternal behavior was assessed on LD 5-6 and 10-11. Newborns were classified according to birth weight as small (SPA), adequate (APA), or large for pregnancy age (LPA) and their body weight, length, and anogenital distance were measured. The inappropriate nutrition was effective to further impair glucose tolerance of hyperglycemic dams. This aggravated metabolic impairment led to changes in maternal food and caloric intake and offspring birth weight as well as impaired maternal behavior, especially related to lactation parameters. These results suggest that inappropriate nutrition was effective to aggravate glucose tolerance of hyperglycemic dams, but not in the normoglycemic ones, leading to metabolic and behavioral changes on the mother and also

altering offspring development. Further studies will analyze how these alterations may modify offspring behavior at different life stages.

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Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.07/N23

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH grant RO1HD057962

Title: Oxytocin receptor knockdown in the midbrain dorsal raphe disrupts postpartum maternal sensitivity to pups and increases the number of perineuronal nets in the primary somatosensory cortex

Authors: *Z. GRIEB[1], K. KRISHNAN[2], F. MANFREDSSON[3], J. S. LONSTEIN[4];
[1]Neurosci. Inst., Georgia State Univ., Atlanta, GA; [2]Biochem. & Cell. and Mol. Biol., Univ. of Tennessee at Knoxville, Knoxville, TN; [3]Neurosci. Program, Michigan State Univ., Grand Rapids, MI; [4]Neurosci Program, Michigan State Univ., East Lansing, MI

Abstract: Oxytocin (OT) is well-known for positively influencing mammalian maternal caregiving. OT acts in many brain sites to affect postpartum behaviors, but midbrain sites sensitive to OT, such as the dorsal raphe and ventrolateral periaqueductal gray (together termed the dorsomedial tegmentum) are rarely studied despite being known to be involved in motherhood. We previously found a ~250% increase in oxytocin receptor (OTR) autoradiographic binding and ~60% higher OT-immunoreactive fiber density in the dorsomedial tegmentum of postpartum rats compared to diestrus virgins. Additionally, we found that ~40% of serotonergic neurons in the female rat dorsomedial tegmentum express OTR immunoreactivity. These postpartum increases in OT measures in the dorsomedial tegmentum may affect serotonergic control of postpartum behaviors. Here we hypothesized that elevated OT signaling in the dorsomedial tegmentum influences the display of maternal socioemotional behaviors. To test this hypothesis, we created an adeno-associated virus expressing a short hairpin RNA targeted to OTR mRNA and a scrambled control short hairpin RNA. We found that knocking down OTRs in the dorsomedial tegmentum led to higher rates of postpartum infanticide, less nursing, and more non-pup directed behaviors. OTR knockdown also increased postpartum aggression, decreased postpartum anxiety, and increased dams' depressive-like behaviors. Because OTR knockdown in the dorsomedial tegmentum also decreased serotonin-immunoreactive fiber length in the primary somatosensory cortex (S1), we hypothesized that the

knockdown effects were due to disrupted S1 plasticity that optimizes maternal tactile sensitivity to offspring. In support, we found that the number of *wisteria floribunda agglutinin*-labelled perineuronal nets (PNNs) in the face and trunk representation of S1 was higher in OTR knockdown dams compared to controls. Given that PNNs are associated with decreased neural plasticity, this would suggest that OTR knockdown dams may be less able to undergo neuroplastic changes in their S1 in response to interactions with pups. These data indicate that OT signaling in the dorsomedial tegmentum is an understudied target where oxytocin acts to optimize maternal cortical plasticity and sensitive caregiving.

Disclosures: Z. Grieb: None. **K. Krishnan:** None. **F. Manfredsson:** None. **J.S. Lonstein:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.08/N24

Topic: F.02. Behavioral Neuroendocrinology

Support: NSF IOS 1455960
Start-up funds awarded to R.M. Calisi

Title: Single parenthood in a biparental species: Maternal versus paternal behavioral and mechanistic responses

Authors: *A. M. BOOTH[1], R. VIERNES[1], V. S. FARRAR[1], S. H. AUSTIN[2], R. M. CALISI[3]; [1]Univ. of California, Davis, Davis, CA; [2]Neurobiology, Physiology, and Behavior, Univ. of California Davis, Davis, CA; [3]Neurobiology, Physiol. and Behavior, Univ. of California - Davis, Davis, CA

Abstract: Many species have evolved biparental care strategies to maximize fitness. However, when disease, predation and other unpredictable situations result in the loss of a partner, how do single parents behaviorally compensate to raise young, and how might this differ between the sexes? Using the biparental model of the rock dove, *Columba livia*, we examined how single mothers and fathers alter parental care behaviors in this event, and how such behavioral changes are related to changes in gene transcription in a major reproductive control center of the brain, the hypothalamus. We found that single mothers and fathers express differences in behavioral compensation for offspring care compared to paired mothers and fathers. Single mothers also experienced higher ER-β expression as compared to paired mothers, which might play a role in suppressing their stress response. We are currently evaluating how single parenthood affects hypothalamic gene transcription in males. These data provide behavioral and mechanistic insight

into how an ecologically relevant challenge, the loss of a mate, differentially affects maternal and paternal care.

Disclosures: A.M. Booth: None. **R. Viernes:** None. **V.S. Farrar:** None. **S.H. Austin:** None. **R.M. Calisi:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.09/N25

Topic: F.02. Behavioral Neuroendocrinology

Support: MSU faculty retention funds to S.A. Burt

Title: Interactive effects of maternal lead exposure and restricted home cage bedding on females' caregiving behaviors and central serotonin

Authors: S. A. BURT[1], E. M. VITALE[2], M. E. DAVIS[2], E. G. FORD[2], A. C. MOODY[2], ***J. S. LONSTEIN**[2];
[1]Clin. Psychology Program, [2]Behavioral Neurosci. Program, Michigan State Univ., East Lansing, MI

Abstract: In humans, early-life lead exposure is associated with neurodevelopmental deficits including hyperactivity, impulsivity, anxiety, and social dysfunction. While the effects of early-life lead exposure on offspring behavioral outcomes have been well established, much less is known about the effects of lead exposure during pregnancy and postpartum on the maternal brain and subsequent alterations in maternal caregiving that may contribute to developmental derailments in the offspring. Furthermore, because lead exposure during pregnancy and thereafter is strongly associated with low socioeconomic status (SES) in women, the alterations in caregiving may be particularly relevant to study under low-resource conditions. In the present study involving laboratory rats as a model, we hypothesize that lead exposure during pregnancy and postpartum combined with low maternal home cage resources will particularly derail maternal caregiving behaviors and the neurochemical systems involved in motherhood. Pregnant female rats were given 1% lead in drinking water, or regular distilled water, beginning the day after insemination. At parturition, dams in both water conditions received either a normal amount of home cage bedding (1000 mL bedding material), or reduced bedding (500 mL), to model low maternal resource availability. Dams continued to receive either lead water or regular water until pups were weaned on postnatal day (PND) 21. Undisturbed maternal behavior observations were conducted on postpartum days (PPD) 3, 6, 9, 12, and 15. On PND 21, pups were weaned and dams sacrificed and their blood and brains collected. Because chronic lead exposure is known to affect aspects of the serotonergic system, and serotonin signaling is necessary for normal

maternal care, brains from the mothers are being processed and analyzed for expression of serotonin-related mRNAs in the midbrain raphe nuclei, nucleus accumbens, and medial preoptic area. Our ongoing project studying these mothers and their offspring will provide important neural and behavioral information about how the interaction between early-life heavy metal exposure and resource restriction may synergize to threaten child development.

Disclosures: **S.A. Burt:** None. **E.M. Vitale:** None. **M.E. Davis:** None. **E.G. Ford:** None. **A.C. Moody:** None. **J.S. Lonstein:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.10/N26

Topic: F.02. Behavioral Neuroendocrinology

Support: FVF_084. D2C2
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ANII

Title: Are gonadal hormones responsible for the reorganization of the extracellular matrix of medial preoptic area during motherhood?

Authors: ***N. URIARTE**[1], M. FERREÑO[2], J. POMI[2], J. NOGUEIRA BORDE[3];
[1]Lab. of Neurosci., [2]Facultad de Ciencias, Univ. de la República, Montevideo, Uruguay;
[3]Facultad de Medicina, Univ. de la República, Montevideo, Uruguay

Abstract: A remarkable reorganization of the extracellular matrix (ECM) occurs in the medial preoptic area (mPOA) of female rats during motherhood. Specifically, we have shown that perineuronal nets (PNNs) - aggregations of ECM enwrapping neurons- are expressed and dynamically change during this period. PNNs are proposed to play key roles in neural plasticity. As pregnancy and lactation are characterized by a significant increase in neuroplasticity, as well as by sustained levels of gonadal hormones, our objective was to explore the role of gonadal steroids in the reorganization of ECM. With this aim, we analyzed the expression of PNNs in the mPOA of female rats using the glycosaminoglycan label obtained with the lectin of *W.floribunda* (WFA) in: 1) Ovariectomized rats treated with a hormone-simulated gestation protocol: daily injections of estradiol (E) (days 1-16), E+Progesterone (P), (days 17-21) or vehicle. 2) Virgin females in diestrous, Primiparous dams on lactation day 5(primL5) and one week after weaning the pups (in diestrous) (primW), and multiparous dams on lactaction day 5 (multiL5). Results showed that the hormone-simulated treatment mimicked the PNNs expression described previously in pregnancy, demonstrating that E+P exposure is sufficient to induce an organization

of PNNs. On the other hand, primL5 presented high levels of PNNs, while in primW, the PNNs intensity faded to low levels that were higher than diestrous females; interestingly multiL5, that were re-exposed to gestation endocrine levels- showed significantly higher levels of PNNs label than primL5. The presence of this remnant ECM following weaning suggests that once a female undergoes through a reproductive cycle, a molecular label persists, that may lead to a stronger expression follow a new hormonal exposure in a second gestation. Taken together, these results provide evidence for a hormonal mediation in the reorganization of ECM during the female reproductive cycle. In addition to these studies, we are currently analyzing the functional significance of this structural change. Hence, ongoing work in our laboratory aims to determine if the integrity of PNNs during gestation is needed for the physiological adaptations of motherhood as well as for the expression and maintenance of maternal behavior. Also, we are currently studying the identity of PNNs expressing neurons and whether they are active during the expression of maternal behavior.

Disclosures: **N. Uriarte:** None. **M. Ferreño:** None. **J. Pomi:** None. **J. Nogueira Borde:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.11/N27

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant HD097085

Title: Autoradiographic binding density of central serotonin 1A, 2A, and 2C receptors across female reproductive states and after repeated variable stress during pregnancy in female rats

Authors: *E. M. VITALE[1], J. S. LONSTEIN[2];
[1]Psychology, [2]Neurosci. Program, Michigan State Univ., East Lansing, MI

Abstract: Mammalian mothers show a unique suite of behavioral responses beginning around parturition, including offspring caregiving, maternal aggression, and low anxiety. The neurotransmitter serotonin (5-HT) regulates these behaviors, and our lab has found in female rats that there are reproductive state-dependent changes in expression of central 5-HT receptors that may be responsible for peripartum behavioral responses. Specifically, we found less serotonin 2C receptor (5-HT2C) mRNA in the midbrain dorsal raphe (DR), more serotonin 2A receptor (5-HT2A) mRNA in the medial preoptic area (mPOA), and more serotonin 1A (5-HT1A) in the nucleus accumbens (NAc) in females sacrificed at parturition and early lactation compared to females sacrificed as diestrus virgins. Additionally, repeated variable stress (RVS) during pregnancy disrupts caregiving and increases depressive-like behaviors, and we found these behaviors correlated with 5-HT receptor mRNA in the mPOA and DR. The aim of the current

study is to now determine whether 5-HT receptor binding (an indication of receptor protein dynamics) is affected by female reproductive status and repeated variable stress during pregnancy. Receptor autoradiography is being used to determine binding density of 5-HT1A in the NAc, 5-HT2A in the mPOA, and 5-HT2C in the DR. In Experiment 1, three female reproductive states are being compared - diestrus virgin, day of parturition, and postpartum day 7 (PP7). In Experiment 2, binding density of 1A, 2A and 2C receptors will be examined in postpartum rats exposed to RVS during pregnancy. Consistent with our previous PCR results, we predict that 5-HT1A binding will be higher in the NAc at parturition, 5-HT2A binding will be higher in the mPOA at parturition and PP7, 5-HT2C binding will be lower in the DR at both of these time points, and that stress during pregnancy will derail the normative postpartum expression of these three receptors. This work may reveal a contribution of 5-HT receptors to the stress-induced maladaptions in maternal caregiving and affective behaviors, with implications for human postpartum depression.

Disclosures: E.M. Vitale: None. **J.S. Lonstein:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.12/N28

Topic: F.02. Behavioral Neuroendocrinology

Title: The effects of a maternal defense test on the development of a conditioned place preference

Authors: *A. R. SELKE, K. L. D'ANNA-HERNANDEZ;
Psychology, California State Univ. San Marcos, San Marcos, CA, CA

Abstract: Maternal defense (MD) behavior is associated with hormonal changes accompanying late pregnancy and lactation, a period when contact with offspring is rewarding. MD may share certain qualities with male aggressive behavior, that has previously been linked to reward and is dependent on an intact mesolimbic dopaminergic system. It is unknown the extent dopamine (DA) may be involved in MD, or if MD can be rewarding to dams. DA plays a role in other maternal behaviors (e.g., licking and grooming of pups) and is upregulated in response to pup stimuli and pup-related behaviors. The present study serves as a preliminary analysis of whether MD is rewarding to mothers using a conditioned place preference paradigm. Dams (n=11) were exposed to MD or an empty control chamber. MD was evaluated for any changes in attacks or anogenital sniffing over the course of 3 conditioning days. A RM-ANOVA showed that of the dams who displayed aggressive behavior, there was no change in time spent in either the control chamber, $F(1,4)=.999$, $p=.374$, or in the MD paired chamber $F(1,4)=3.87$, $p=.121$.Total time dams spent attacking the male intruder did not change over time, $F(2,10)=3.133$, $p=.088$. Time

dams spent sniffing male anogenital region changed significantly over time, F(2,6)=5.49, p=.044, decreasing significantly from day 6 to day 7 postpartum, t(4)=2.31, p=.082. This change did not occur from day 7 or 8 postpartum, t(3)=1.1, p=.351. Thus, overall, maternal defense was not associated with reward. One explanation for these results could be due to a floor effect in maternal defense behavior. Furthermore, dams may have become habituated to the male intruder following the first exposure on day 6 postpartum. Future research will delineate whether dams are responding to the opportunity to interact with a male via this increase in anogenital sniffing, or if dams are engaging with the male intruder to determine if he poses a threat to her litter. Tyrosine hydroxylase activity will be analyzed in maternal and mesolimbic brain structures to understand whether the observed decrease in interaction with the male intruder is due to alterations in DA transcription following MD and male social interaction stimuli.

Disclosures: **A.R. Selke:** None. **K.L. D'Anna-Hernandez:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.13/N29

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH GM 08807

Title: Hypocretin antagonism's influence on postpartum anxiety and pup retrieval in mice

Authors: *J. K. KUSKE, K. D'ANNA-HERNANDEZ;
California State Univ. San Marcos, San Marcos, CA

Abstract: Postpartum anxiety is a detrimental condition that causes disruptions in maternal care to offspring and impairs a mother’s ability to care for herself and her offspring. However, the underlying neurobiological causes of postpartum anxiety remains unknown. We do know that high levels of hypocretin (HCRT, also called orexin), an arousal related peptide, have been associated with increased anxiety in non-lactating rodents, and that there is greater HCRT activity during lactation in dams. Because antagonizing HCRT receptor 1 (HCRTr1) decreases anxiety in non-lactating rodents, we hypothesized that HCRTr1 antagonism would decrease anxiety, and that the decrease in anxiety would increase pup retrieval behaviors in lactating mice. Using two doses of HCRT receptor 1 (HCRTr1) antagonist SB-334867 (10 mg/kg and 20 mg/kg) with a vehicle control, and a modified light/dark box protocol that includes a pup retrieval task, we saw no difference between our drug groups in time spent in the light [$F(2, 28) = 1.68$, $p = 0.21$], nor was there a difference in the number of pups retrieved between our three drug groups [$F(2, 28) = 0.36$, $p = 0.70$]. However, we saw that low doses of HCRTr1 antagonism reduced the frequency of stretch attend postures in dams, [F(2, 28) = 3.59, p = 0.04] (M = 6.73, SEM = 1.37)

compared to our vehicle group (M = 13.30, SEM = 1.37). Because stretch attend postures are important risk-assessing behaviors that rely on an increase in vigilance and arousal, we suspect to find similar results in HCRT receptor 2 antagonism. Currently, our results indicate that the role HCRT may have on anxiety behaviors could differ in the postpartum period compared to non-lactating mice.

Disclosures: J.K. Kuske: None. **K. D'Anna-Hernandez:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.14/N30

Topic: F.02. Behavioral Neuroendocrinology

Title: Determining the levels of maternal behavior and peer interaction in communal nesting

Authors: *G. H. LEE[1], K. PRESNELL[1], Y. CABRERA[1], M. CHAVEZ[1], K. L. D'ANNA[2]; [1]Psychology, California State Univ. San Marcos, San Marcos, CA; [2]Psychology, California State University, San Marcos, San Marcos, CA

Abstract: Communal nesting, a type of social enrichment in mice, has been found to lead to reduced vulnerabilities to social stressors in mice, such as depressive-like behavior in adulthood. The mouse communal nest (CN) involves several mice mothers and their offspring combined, whereas the standard/single nest (SN) involves simply one mice mother and her offspring. It has also been suggested that mothers in the CN may have higher lifetime reproductive success than those in the SN. Furthermore, It has been classically thought that increased levels of maternal behavior in the CN has been driving these benefits, as maternal behavior has been shown to increase in the CN and also associated with reduced depressive-like behavior. However, as there are an increased number of both mothers and peers, it is unclear if maternal behavior or pup peer interaction has more of an influence on the increased resilience to stressors that result from communal nesting. The present study investigated the benefits of the communal nest on mothers and found that lactating dams in the CN exhibited more maternal behavior and less depressive-like behavior. Eleven dams were placed in CN and 14 dams were placed in SN. On postnatal day 4, these subjects were tested on a tail-suspension test for six minutes and a subsequent maternal behavior task for 15 minutes. There was a trend for CN females licking/grooming more often than SN females, $p = .055$. This suggests that the CN increases maternal care. However, SN mothers spent more time on nest and self-grooming than CN mothers, $t(15.35) = -2.14$, $p = .049$ and $t(13.09) = -2.18$, $p = .048$, respectively. There was also a trend for CN mothers spending more time off nest than SN mothers, $p = 0.05$. This may suggest that CN mothers provide less maternal care because these behaviors can be shared with other mothers. Additionally, CN mice spent more time struggling and less time hanging than SN mice, $t(42) = 2.78$, $p = .008$ and $t(42)$

= -2.75, p = .009, respectively. CN mice showed less depressive behavior than SN mice, suggesting that the CN increases resilience against stress. Thus, the social enrichment paradigm of communal nesting appears to provide benefits to both the mom and pups, but more research is needed to elaborate and confirm these findings. Additionally, it is unclear whether peer interaction is also increased and possibly influence these results. Ongoing follow-up studies aims to confirm the differences in maternal care and depressive-like behavior as well as investigating the differences of pup peer interaction in the CN and SN.

Disclosures: **G.H. Lee:** None. **K. Presnell:** None. **Y. Cabrera:** None. **M. Chavez:** None. **K.L. D'Anna:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.15/N31

Topic: F.02. Behavioral Neuroendocrinology

Title: Hypocretin/orexin receptor 1 blockade increases maternal exploration in a T-Maze

Authors: *A. ALAYOUBI[1], K. L. D'ANNA-HERNANDEZ[2];
[1]California State Univ. San Marcos, San Marcos, CA; [2]Psychology, California State University, San Marcos, San Marcos, CA

Abstract: Postpartum depression can often involve lack of motivation to perform important maternal behaviors. Hypocretin (HCRT) is a neuromodulatory peptide involved in arousal, stress, and reward, all essential components of successful maternal care. A blockade of HCRT receptor 1 (HCRTR1), but not HCRTR2, decreases drug seeking and maternal behavior. HCRTR1 agonism has also been shown to increase maternal behavior. We anticipated that theHCRTR1 antagonist, SB-334867, would decrease maternal motivation in a T-Maze. Lactating dams (HSD:ICR) were deprived of pups for 2 hours and then given an intraperitoneal injection of SB-334867 (15 mg/kg, N = 12; 30 mg/kg, N = 11) or vehicle (N = 7), thirty minutes before being placed in a T-Maze for pup-retrieval observations. SB-334867 significantly decreased latency to leave the goal box (F(2, 27) = 6.47, p = .005) such that the 30 mg/kg dose (t = -3.57, p = .001) and low dose (t = -2.58, p = .016) were significantly quicker to leave the goal box than the vehicle; the two doses did not differ (t = -1.19, p = .244). SB-334867 also significantly reduced latency to approach pups (F(2, 27) = 4.59, p = .019) such that the 30 mg/kg dose (t = -3.018, p = .006) and 15 mg/kg dose (t = -2.08, p = .047) were significantly quicker to leave the goal box than the vehicle; the two doses did not differ (t = -1.12, p = .271). SB-334867 had no effect on pup retrieval (F(2, 27) = .201, p = .819). Considering experimental stressors, SB-334867 may have increased exploration by possible stress inhibition.

Disclosures: **A. Alayoubi:** None. **K.L. D'Anna-Hernandez:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.16/N32

Topic: F.02. Behavioral Neuroendocrinology

Support: UGRP (Internal Grant from SVSU)

Title: Effects of prenatal exposure to a mixture of endocrine disrupting compounds on parturition and early postnatal neuromuscular development in the Norway rat

Authors: M. FLATTERY, E. HILL, M. SMITH, C. SHELTON, M. ZAKY, *G. M. LANGE; Saginaw Valley State Univ., University Center, MI

Abstract: In classic work by William C. Young's laboratory, it was established that organization of mammalian brain morphology is guided by expressed gonadal hormones *in utero*. It has been further established that specific enzymatic alteration of gonadal hormones can occur in the undifferentiated neuron.Therefore, the genetic sex of an organism drives phenotypic development of sexual morphology and the brain.

Experiments involving manipulation of embryonic environments in oviparous avian and fish models has demonstrated phenotypic sex expression opposite that of genotype is possible. This phenotypic expression is possible both in body morphology and in brain organization. However, there are currently no viviparous organisms where induced phenotypic sex opposite of genotype has been demonstrated, most likely due to chemical complexities associated with internal gestation.

Here, we report on effects of prenatal exposure to a chemical cocktail, specifically use of a mixture of exemestane, letrozole, vinclozolin, and triclosan where exposures are each at environmentally relevant levels that may potentially influence early development by reshaping the mammalian intrauterine environment enough to permit phenotypic expression of sexual morphology opposite that of genotype in mammals.

We present a detailed look at our experimental design in the Norway rat. In this research model, sexually indifferent morphology is maintained through gestational day 10 following fertilization. Our chemical mixture has been introduced to our subjects beginning within this undifferentiated developmental stage of sexual organization and continued through parturition. Parturition difficulties were observed in our dams exposed to this chemical cocktail. Administration of this chemical mixture to the intrauterine environment appears to have impacted the timing of gestation by delaying parturition and have altered anogenital differences in offspring.

In current work, we evaluate pup performance from parturition through maturity on a variety of post-natal behavioral and morphological tests relevant to sex differentiation. By comparing

control and treatment populations at the neural, morphological, reproductive, and behavioral levels, we hope to gain deeper insight into the mechanisms driving sexual differentiation in mammals.

Disclosures: M. Flattery: None. **E. Hill:** None. **M. Smith:** None. **C. Shelton:** None. **M. Zaky:** None. **G.M. Lange:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.17/N33

Topic: F.02. Behavioral Neuroendocrinology

Title: Preventing the accumulation of ΔFosB in the nucleus accumbens of female mice during a hormone-simulated pregnancy alters anxiety-like behavior

Authors: A. B. GIBBONS[1], A. K. SINGH[1], D. R. GEARINGER[1], A. A. VALENTINE[1], E. J. NESTLER[2], ***L. E. BEEN**[1];
[1]Haverford Col., Haverford, PA; [2]Icahn Sch. Med. At Mount Sinai, New York, NY

Abstract: Peripartum mood disorders are the most common complication associated with childbirth, yet the neural mechanisms remain poorly understood. In particular, peripartum anxiety receives less attention despite being at least equally as prevalent as peripartum depression. We have previously found that ΔFosB, a transcription factor associated with long-term neural plasticity, is increased in the nucleus accumbens (NAc) of female mice following a hormone-simulated pregnancy. What's more, this neural plasticity is associated with alterations in anxiety like behavior. Here, we test the hypothesis that preventing the accumulation of ΔFosB during a hormone-simulated pregnancy will ameliorate peripartum anxiety behavior. Female mice were ovariectomized and given bilateral stereotaxic injections into the NAc of an adeno-associated virus containing ΔJunD, a dominant negative inhibitor of ΔFosB, or a control vector. Following recovery, all females underwent a hormone-simulated pregnancy, in which they were administered daily injections of estrogen and progesterone that approximate early and late pregnancy. After 21 days, one group of females was withdrawn from estrogen, simulating postpartum estrogen withdrawal, while the other group continued to receive estrogen injections. During this time, the behavior of all females was assessed in the open field and elevated plus maze. Preliminary data indicate an interaction between hormone condition (estrogen withdrawn vs. sustained) and virus injection (ΔJunD vs. control). Preventing the accumulation of ΔFosB during a hormone-simulated pregnancy did not change the high-anxiety phenotype following estrogen withdrawal. However, it did prevent the low anxiety phenotype during simulated pregnancy. This suggests that increased hormone levels during pregnancy may increase the

accumulation of ΔFosB in the NAc, resulting in a low anxiety behavioral phenotype. However, increased ΔFosB may not be related to postpartum anxiety following estrogen withdrawal.

Disclosures: **A.B. Gibbons:** None. **A.K. Singh:** None. **D.R. Gearinger:** None. **A.A. Valentine:** None. **E.J. Nestler:** None. **L.E. Been:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.18/N34

Topic: F.02. Behavioral Neuroendocrinology

Support: UNAM-DGAPA-PAPIIT-IA202218
UNAM-DGAPA-PAPIIT-IN202315
SECRETARIA DE SALUD-MEXICO

Title: Prolactin treatment early in life delays puberty onset on female mice and alters olfactory bulb responses

Authors: R. CORONA, M. DEL VALLE, L. LUNA, ***T. MORALES**;
Inst. for Neurobio. - UNAM, Queretaro, Mexico

Abstract: Puberty onset sets the physiological changes necessary to initiate the maturation needed for reproduction. This period begins with the activation of the gonadotrophin releasing hormone (GnRH) neurons by kisspeptin neurons (neurons that express the Kiss1 gene) that can be modulated by prolactin (PRL) as they express high levels of prolactin receptors. During lactation, a period with high levels of PRL, the Kiss1 mRNA and GnRH are decreased, the estrus cycle modified, and the ovulation compromised. Similar effects can be observed in animals with chronic PRL infusion. On the other hand, olfactory system is determinant for reproduction and sexually relevant odors can accelerate the puberty onset. In the present work we investigated the effect of high levels of PRL in infantile stages on the puberty onset and the maturation of the olfactory bulb (OB). By using female and male CD1 mice treated chronically (from 11 to 21 postnatal days) with daily injections of 5 mg/kg of PRL, 500 µg/kg of cabergoline (a D2 dopamine receptor agonist and PRL inhibitor) or saline (control) we determined the puberty onset by identifying secondary sexual characteristics. Once they were adults and before sacrifice, we exposed females to clean or male bedding (from sexually experienced males) or males to clean and female bedding (from reproductive experience females) to verify the activational responses (as measured by the expression of cFos) of the OB cells. Infantile/juvenile PRL treatment delayed puberty onset exclusively in females. Granular cells (Gr) of the accessory OB of females showed equal expression of cFos when exposed to male or clean bedding, suggesting that PRL treatment alters the basal Gr-AOB activity in females. Males treated with PRL showed

that the Gr cells of the main OB have higher expression on cFos when exposed to female bedding compared to the control group, indicating a greater sensitivity of those cells. Overall our results show dimorphic effects of PRL in the process of puberty onset and maturation of the OB.

Disclosures: **R. Corona:** None. **M. Del Valle:** None. **L. Luna:** None. **T. Morales:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.19/N35

Topic: F.03. Neuroendocrine Processes

Support: NIH Grant R01DA0396062-01
NIH Grant 1R24MH114815-01A1

Title: Sex-specific transcriptional networks in the medial amygdala underlie differences in expression of juvenile social play

Authors: *A. E. MARQUARDT[1], A. C. SHETTY[2], S. A. AMENT[3], M. M. MCCARTHY[4]; [1]Program in Neurosci., [2]Inst. for Genome Sci., [3]Inst. for Genome Sci. and Dept. of Psychiatry, [4]Pharmacol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Social play behavior, or rough-and-tumble play, is a characteristic pattern of social behavior exhibited by mammals during the juvenile period which likely contributes to the development of social and emotional skills needed throughout life. Importantly, there is a sex difference in expression of social play, with males exhibiting greater intensity and frequency of play than females, though as with any behavior, there is individual variability. To identify the transcriptional signatures associated with play in both a sex-dependent and sex-independent manner, we performed RNA sequencing (RNA-seq) of the medial amygdala (MeA), the site of masculinization of play, in high- and low-playing males and females at the juvenile age. As social play is a dynamic behavior likely produced by complex interactions among many genes, we then utilized a network approach, Weighted Gene Co-expression Network Analysis (WGCNA), focusing on 4,261 genes that showed nominal differences in expression related to sex or play. We identified 22 gene co-expression modules, many (11 modules with $p < 0.05$) of which are sex-specific in expression, as they are correlated with expression of play behavior in one sex but not the other. Future analysis will identify and validate “hub” genes driving differences in play in particular modules of interest. Additionally, we will integrate this analysis with published single cell RNA sequencing experiments (scRNA-seq) to explore potential cell-type-specific expression of these modules. Together, these novel analyses will greatly improve our understanding of how differential transcriptomic regulation in the medial amygdala drives sex differences in MeA circuitry and social play.

Disclosures: **A.E. Marquardt:** None. **A.C. Shetty:** None. **S.A. Ament:** None. **M.M. McCarthy:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.20/N36

Topic: F.03. Neuroendocrine Processes

Support: R01DA039062

Title: Understanding endogenous and exogenous cannabinoids in the development of sex differences in brain and behavior

Authors: *J. W. VANRYZIN[1], K. R. MONTGOMERY[2], A. E. MARQUARDT[2], M. M. MCCARTHY[1];
[1]Dept. of Pharmacol., [2]Program in Neurosci., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: The brain's endogenous cannabinoid system, or endocannabinoids (eCBs), are a family of signaling molecules essential for normal brain development. By acting on cannabinoid receptors (either the CB1 receptor or CB2 receptor), eCBs promote progenitor proliferation and differentiation, axonal pathfinding, and regulate synaptic transmission. We previously found that eCBs also program sex differences in brain development with lasting impacts on sex-specific behaviors in rats. In the developing male amygdala, a region responsible for integrating social information, neonatal androgens induce an upregulation of eCBs for the first few days after birth. This increased eCB content triggers microglia to become highly phagocytic and engulf newborn astrocytes. In contrast, the developing female amygdala- not having experienced an androgen surge- will have a lower eCB content, fewer phagocytic microglia, and in turn, more surviving astrocytes through development. By the juvenile age, the sex difference in astrocyte number influences neuronal excitability in response to social stimuli; males having fewer astrocytes, will have greater neuronal excitability and more robust expression of rough-and-tumble play. Marijuana is the most commonly used illicit substance by pregnant women during pregnancy. The primary psychoactive ingredient, delta-9-tetrahydrocannabinol (or THC), exerts its effects by acting on the same receptor system as eCBs. As western society continues to increase the medicalization, decriminalization, and legalization of marijuana, it is imperative we understand the biological mechanisms by which brain development can by perturbed by exogenous cannabinoid exposure in a sex-dependent manner. Thus, our present research aims to determine if THC exposure to pregnant rats and newborn pups can disrupt the basic biological processes of sexual differentiation. Moreover, we will examine the influence of THC exposure on the development of social behavior, as well as other behavioral domains, to determine the sex-dependent and sex-independent effects THC may impart on the developing brain.

Disclosures: **J.W. VanRyzin:** None. **K.R. Montgomery:** None. **A.E. Marquardt:** None. **M.M. McCarthy:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.21/N37

Topic: F.03. Neuroendocrine Processes

Support: NIH R01 MH52716-21
NIH F32 HD097816-01
NIH F31NS093947

Title: Exploring sex differences in the neuroimmune profile of the developing rat

Authors: ***E. L. REINL**, L. A. PICKETT, M. M. MCCARTHY;
Pharmacol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Development of both the body and brain are strongly influenced by the biological variable of sex. In the course of elucidating mechanisms by which sex influences brain development we have found the male rat brain consistently exhibits higher levels of inflammatory signaling molecules, immune cells and gene expression profiles indicative of immune activation compared to female brains. We hypothesize this increased inflammatory tone is a cause of the increased risk to males of neurodevelopmental and neuropsychiatric disorders, including autism and early onset schizophrenia, diseases that are also associated with maternal immune activation. This has prompted us to now consider how peripheral immune cells may be participating in normal neonatal brain development and how this contribution may differ between males and females. We recently reported that neonatal male rats have more mast cells residing within the neuropil of the preoptic area (POA), a highly sexually dimorphic region (Lenz, Pickett et al., J. Neurosci 2018). Conversely, females have a greater number of mast cells in the meninges surrounding the POA than in the neuropil. Assessment of the major peripheral immune cell subtypes in the entire meninges (leptomeninges and dura) and choroid plexus (CP) of PN0 rat pups by flow cytometry found no sex difference in the percentage of each cell type. Of those immune cell subtypes that could be confidently phenotyped, B cells represented the overwhelming majority, and they were present at a significantly higher proportion in the meninges and CP than in the blood. These data suggest that immune cell infiltration overall is tightly controlled, and that at least at this superficial level, immune cell populations are regulated to be the same between sexes during this critical period of sexual differentiation. It also suggests that any sex differences in immune cell migration, such as that observed with mast cells, are specific to brain regions and/or immune cell functional state.

Disclosures: **E.L. Reinl:** None. **L.A. Pickett:** None. **M.M. McCarthy:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.22/N38

Topic: F.03. Neuroendocrine Processes

Support: R01MH109471

Title: Effect of neonatal hormone exposure on electrophysiological properties of caudate-putamen medium spiny neurons in prepubertal rats

Authors: J. CAO, ***J. MEITZEN**;
North Carolina State Univ., Raleigh, NC

Abstract: Steroid sex hormones and genetic sex regulate the phenotype of neurons in the striatal brain regions, including the caudate-putamen and nucleus accumbens core (AcbC). These brain regions are of particular interest to neuroendocrinologists given that the role of the sex steroid hormone 17β-estradiol in modulating striatal dopamine and glutamatergic systems and the presence of membrane estrogen receptors. Indeed, output neurons of the striatum, the medium spiny neurons (MSNs), exhibit region and developmental-specific estradiol sensitivity and sex differences in electrophysiological properties. Our laboratory previously demonstrated that the electrophysiological properties of rat caudate-putamen medium spiny neurons (MSNs) vary by sex, including female MSNs exhibiting increased intrinsic excitability compared with male MSNs in prepubertal rat. These data suggest that neonatal masculinization via sex hormone action may regulate MSN electrophysiological properties in caudate-putamen, similar to previously published findings that neonatal estradiol exposure masculinizes female rat nucleus accumbens core MSNs. Here, we test the hypothesis that neonatal estrogen exposure also sex-specifically regulates the electrophysiological properties of caudate-putamen MSNs. In order to test this, we first exposed female and male rats to either 17β-estradiol or vehicle on the first two days after birth. We then recorded the electrophysiological properties of MSNs 16-23 days after birth, and found that neonatal estradiol exposure eliminates sex differences in MSN electrophysiological properties. We are currently investigating whether exposure to estrogen receptor agonists likewise masculinizes MSN neurons. Further data analysis is ongoing and these data will help elucidate the underlying mechanisms by which sex differences in caudate-putamen MSN properties are generated.

Disclosures: **J. Meitzen:** None. **J. Cao:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.23/N39

Topic: F.03. Neuroendocrine Processes

Title: Stress and altered pubertal timing: Is the limbic brain the key?

Authors: *D. IVANOVA, X. LI, C. MCINTYRE, C. GRIFFIN, C. MULETIER, K. O'BYRNE; Dept. of Women and Children's Health, Fac. of Life Sci. and Med., King's Col. London, London, United Kingdom

Abstract: Post-traumatic stress (PTSD) is associated with altered pubertal timing in humans and rodents. Predator odour is a classical rodent PTSD model. Extra-hypothalamic kisspeptin neurones in the posterodorsal sub-nucleus of the medial amygdala (MePD) are thought to modulate pubertal timing, as well as anxiety and emotional processing. We test the hypothesis that psychosocial stress, processed by the MePD, is relayed to the hypothalamic gonadotropin-releasing hormone (GnRH) pulse generator to delay puberty. Female C57BL/6 mice were exposed to predator odour, 2,4,5-Trimethylthiazole (TMT), for 14 days from postnatal day (pnd) 21. Anxiety was tested before (pnd 19-20), during (pnd 27-28) and after TMT-exposure (pnd 40-41) using the Elevated Plus Maze (EPM), Light/Dark Box (LDB) and social interaction (SI). The effect of TMT-exposure on pre-pubertal luteinizing hormone (LH) pulses was measured, at pnd 26 and 29. In addition, kisspeptin-cre mice were bilaterally injected with hM3Dq-DREADD AAV in the MePD at pnd 14. From pnd 21 they were administered CNO via drinking water for 14 days and the onset of puberty monitored. The TMT-exposed mice showed a significant delay of 5 days to first estrous (FE; marker of puberty; Kruskal-Wallis analysis $p<0.001$; control n=10, TMT n=14) without affecting body weight (BW). TMT-exposed mice spent less time in the open arm of the EPM on pnd 28 (13±3 s) and pnd 41 (5±2 s) compared to control (pnd 28 32±5 s, pnd 41 31±6 s). They spent more time in the dark compartment of the LDB (TMT 180±12 s vs controls 121±13 s) and less time socially interacting (TMT 26.8±2.8 s vs controls 47.7±8.8 s) on pnd 27. The TMT-exposed group exhibited a reduction in LH pulse frequency on pnd 26 (TMT 0.1±0.1 pulses/2h vs control 0.9±0.3 pulses/2h) and 29 (TMT 0.2±0.2 pulses/2h vs control 2±0.7 pulses/2h). DREADD activation of kisspeptin neurones in the MePD advances FE (Kruskal-Wallis analysis $p<0.05$; control n=3, DREADD n=4) without affecting BW. Early exposure to predator odour delays puberty in female mice, reduces GnRH pulse generator frequency and has long-term consequences of enhanced anxiety behaviour, while selective chemogenetic activation of the kisspeptin system in the MePD advances puberty.

Disclosures: D. Ivanova: None. **X. Li:** None. **C. Mcintyre:** None. **C. Griffin:** None. **C. Muletier:** None. **K. O'byrne:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.24/N40

Topic: F.03. Neuroendocrine Processes

Support: NIMH Grant 10018215

Title: An estradiol mediated sensitive period in cerebellar development is disrupted by TLR4 but not TLR3 mediated inflammation

Authors: *A. HOLLEY, M. PEREZ-POUCHOULEN, M. M. MCCARTHY;
Univ. of Maryland Baltimore, Baltimore, MD

Abstract: Purkinje neurons, the primary cells of the cerebellum, mature over the first three postnatal weeks. Previous work in our lab has identified the second postnatal week as a critical period in Purkinje development during which inflammation induced Prostaglandin E2 (PGE2) production stimulates aromatase expression, resulting in excessive estradiol production. Through mechanisms not yet understood, excessive estradiol at this time stunts Purkinje dendritic tree development. Previously, a bacterial mimic, LPS, was used to trigger inflammation via a TLR4 mediated pathway. We now seek to determine if TLR3 mediated inflammation, induced by a viral mimic (Poly I:C) produces the same Purkinje neuronal phenotype as LPS. This turned out not to be the case as there was no detectable impact of Poly I:C treatment on Purkinje cell development. This led us to compare TLR4 vs. TLR3 mediated inflammation in the developing cerebellum. We began with in vitro cultures of brain derived microglia stimulated with Poly I:C or LPS and observed distinct cytokine profiles. LPS treated cells produced significantly more IL-1B and TNF-A than Poly I:C or vehicle treated cells. We next explored the difference in vivo using a customized Nanostring panel to assess gene expression in a targeted set of genes involved in inflammation, sexual differentiation, and neurotransmission. In LPS treated animals, but not Poly I:C, we observed sexually dimorphic patterns of gene expression. Animals were treated at PN11 were examined either within 24h of exposure or at PN30 to explore both acute and long-term changes in gene expression. We observed up regulation of multiple genes in males and down regulation in a non-overlapping sets of genes in females when comparing the acute versus chronic response, indicating that males and females divergently respond to TLR4 mediated perturbations. At PN11 males treated with PolyI:C exhibited a significant increase in CCL5 production within the cerebellum. CCL5 is a chemotactic cytokine that calls eosinophils and basophils to the site of inflammation. We are currently characterizing the immune cell profile of the developing cerebellum and whether it differs in males and females.
Funded by: National Institute of Mental Health, Project ID: 10018215

Disclosures: A. Holley: None. **M. Perez-Pouchoulen:** None. **M.M. McCarthy:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.25/N41

Topic: F.03. Neuroendocrine Processes

Support: NINDS T32NS063391
NIDA R01DA039062

Title: Investigating developmental origins of sex differences in the rewarding properties of juvenile rat social play

Authors: *S. E. ASHTON[1], M. M. MCCARTHY[2];
[1]Program in Neurosci., [2]Dept. of Pharmacol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Social play is widely expressed across mammalian juveniles, including human children. As the primary social behavior that occurs pre-puberty, social play is important for the development of social cognition that will guide an animal to flexibly interact with others in a variety of contexts throughout life. Disrupted social play is a core symptom of male-biased neuropsychiatric disorders with origins in development, such as autism spectrum disorder and early-onset schizophrenia, which compels us to understand how the brain processes social interaction at younger ages when deficits generally appear. Social play is a highly rewarding activity that involves components of the mesolimbic dopamine (DA) system, particularly a population of DA neurons in the ventral tegmental area (VTA) that project to regions including the nucleus accumbens and lateral septum; these DA neurons become activated in response to social play in juveniles and their selective inactivation reduces the expression of play behavior. Sex differences in the rewarding properties of other stimuli, such as drugs of abuse, are well characterized, and a recent study in Syrian hamsters demonstrated that adult females find same-sex social interaction more rewarding than males (Borland et al., *Neuropsychopharmacology*, 2018). We thus hypothesize that sex also impacts the reward value of juvenile social play in rats. To test this, we will use a conditioned place preference design in which one chamber contains a sex- and age-matched play partner during conditioning and the other chamber is empty. Though males play more frequently and more intensely than females, we predict that females will develop a greater preference for the socially paired chamber compared to males following conditioning. We will next quantify DA cells in the VTA of juvenile males and females by immunolabeling histological sections for tyrosine hydroxylase (TH), a marker of DA cells. If there is a sex difference in the number of TH+ cells, we will further quantify DA cells across early postnatal development to identify the sensitive period during which sex shapes this difference that is apparent by the juvenile age. Future experiments will explore how VTA DA

cell number is programmed by sex during the sensitive period of development and whether this underlies sex differences in the rewarding properties of juvenile social play.

Disclosures: S.E. Ashton: None. **M.M. McCarthy:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.26/N42

Topic: F.02. Behavioral Neuroendocrinology

Support: NSF Grant IOS-1122074

Title: Mu-opioid receptors within the developing amygdala mediate juvenile social play in a sex-dependent manner

Authors: *H. L. SMYTH[1], L. CHANG[1], S. V. CAPATI[1], A. M. PANDL[1], C. ZHAO[2], L. V. RITERS[2], A. P. AUGER[1];
[1]Dept. of Psychology, [2]Dept. of Integrative Biol., Univ. of Wisconsin-Madison, Madison, WI

Abstract: Juvenile social play is an affiliative behavior that is crucial for typical neural and behavioral development, as deprivation of play during the juvenile period has been shown to lead to enduring negative outcomes on social development. The amygdala and the opioid system have both been implicated in the regulation of juvenile social play. Prior research on play has focused primarily on males, despite evidence that sex differences exist in various aspects of the behavior, the most prominent being overall play levels, with males demonstrating higher levels of total play than females. Previous research from our lab demonstrated that amygdalar mu-opioid receptor (MOR) protein levels negatively correlate with social play levels of juvenile females but not males, suggesting an inhibitory effect of amygdalar MOR on female social play. To further investigate the role of amygdalar MOR on juvenile social development, short hairpin RNA (shRNA) infusions were used to reduce MOR mRNA levels in the central and basolateral nuclei of the amygdala of neonatal male and female rats. To examine the effects of amygdalar MOR shRNA knockdown on behavioral development, behavioral tests were conducted during the juvenile period. MOR shRNA treatment increased female juvenile social play levels to male-typical levels, while male play levels were unaffected. Juvenile sociability was not altered by MOR knockdown, suggesting that disrupting MOR signaling influences social play but not overall sociability. Furthermore, results from the elevated plus maze test revealed that males and females who received the MOR shRNA treatment exhibited significantly lower levels of anxiety-like behaviors than controls. This is consistent with previous research that found that antagonizing MOR within the central amygdala decreases anxiety-like behavior in male rats. Our data suggests that amygdalar MOR is involved in the development of juvenile anxiety-like

behavior in both males and females. In contrast, the present study identifies a sex-specific effect of amygdalar MOR reduction on female juvenile social play. This study contributes to our knowledge of female social development and highlights the importance of considering biological sex differences when developing treatments for social disorders.

Disclosures: H.L. Smyth: None. **L. Chang:** None. **S.V. Capati:** None. **A.M. Pandl:** None. **C. Zhao:** None. **L.V. Riters:** None. **A.P. Auger:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.27/N43

Topic: F.02. Behavioral Neuroendocrinology

Support: NSF Grant IOS-1122074

Title: Examining the relationship between mu-opioid receptor protein levels and juvenile social play in male and female rats

Authors: *A. M. PANDL, L. CHANG, A. NINO DE GUZMAN RAMIREZ, E. K. H. KNEP, H. L. SMYTH, S. V. CAPATI, A. P. AUGER;
Univ. of Wisconsin-Madison, Madison, WI

Abstract: Juvenile social play is an affiliative behavior that is conserved across species including rats and humans. It is critical for typical behavioral and neural development, as deprivation of social play during the juvenile period can lead to atypical patterns of social behavior later in life. The opioid system has been implicated in regulating juvenile social play, and high levels of opioid receptors can be found in regions shown to mediate social play, such as the amygdala, the nucleus accumbens, the prefrontal cortex, and the hippocampus. Furthermore, biological sex differences are commonly observed in juvenile social play, with males playing at higher levels than females. While opioid regulation of social play has been well characterized in males, its involvement in female play has been less studied. The current study quantified mu-opioid receptor (MOR) protein levels in brain regions associated with regulation of social play and correlated them with play levels of male and female juvenile rats. Our data replicate previous findings of the sex difference in play levels between male and female juvenile rats. When correlating amygdala MOR levels and juvenile behaviors, no significant relationships were found in analyses that combined sexes. In analyses separated by biological sex, no correlations were found for amygdala MOR levels and juvenile behaviors in males. However, play behaviors in females negatively correlated with MOR levels in the amygdala, suggesting that increases in MOR reduces juvenile social play within females. This sex-specific relationship between amygdala MOR protein levels and juvenile social play provides potential insight into female

regulation of social play, when previous research has focused primarily on males. Our current findings suggest the possibility that MOR within the amygdala may negatively regulate female juvenile social play. We are in the process of quantifying and correlating MOR protein levels within other regions with male and female social play levels, including the nucleus accumbens, the prefrontal cortex and the hippocampus.

Disclosures: A.M. Pandl: None. **L. Chang:** None. **A. Nino de Guzman Ramirez:** None. **E.K.H. Knep:** None. **H.L. Smyth:** None. **S.V. Capati:** None. **A.P. Auger:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.28/N44

Topic: F.03. Neuroendocrine Processes

Support: NIH Grant RO1 MH114994
Good Nature Institute
Florida State University

Title: Electrophysiological activity of oxytocin receptor expressing neurons in the mouse endopiriform nucleus

Authors: *L. BIGGS, E. A. D. HAMMOCK;
Psychology, Neurosci., Florida State Univ., Tallahassee, FL

Abstract: The neuropeptide oxytocin (OXT) modulates social behaviors across species and may play a developmental role for these behaviors and their mediating neural pathways. Previous results from our lab using OXT receptor (OXTR) ligand binding have shown that OXTR are located in the oronasal cavity as well as the endopiriform nucleus in neonatal mice. The endopiriform nucleus integrates olfactory and gustatory input and has bilateral connections with several limbic areas. Thus, the endopiriform nucleus could play a role in the development of social behavior based on perinatal exposure to OXT. In these experiments, OXTR localization in the endopiriform nucleus was evident by an EGFP reporter in transgenic OXTR-EGFP mice shortly after birth (postnatal day zero; P0) through postnatal day 28 (P28). EGFP could be visualized in live cortical tissue slices that included the endopiriform nucleus collected from both male and female mice (P10-P28). Using whole cell slice electrophysiology, electrophysiological responses of OXTR-EGFP(+) and OXTR-EGFP(-) neurons to bath applied OXT (200 nM) were collected. In most of the OXTR-EGFP(+) neurons and some OXTR-EGFP(-) neurons, bath application of OXT increased the excitability seen as a depolarization of the membrane potential. In the presence of tetrodotoxin, the OXT-induced depolarization persists in OXTR-EGFP(+) neurons, but is abolished in OXTR-EGFP(-) neurons. Combined, these data suggest there may be

both a direct and an indirect effect of OXT in the endopiriform nucleus. Bath application of (THR4,GLY7)-Oxytocin (TGOT, 200 nM), a selective OXTR agonist, also results in an increase in the excitability of both OXTR-EGFP(+) and OXTR-EGFP(-) neurons, seen as an increase in the frequency of action potential firing. These results suggest an OXTR specific network effect, but do not rule out a possible effect of OXT acting on vasopressin 1a receptors in the OXT bath application. Acute responses of endopiriform neurons to OXT exposure during development may be important for the development of appropriate social behaviors based on olfactory, gustatory and other orofacial sensory input.

Disclosures: L. Biggs: None. **E.A.D. Hammock:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.29/N45

Topic: F.02. Behavioral Neuroendocrinology

Support: NARSAD Young Investigator Grant from the Brain & Behavior Foundation award to Dr. Mariana Pereira

Title: Network analysis of brain transcriptome profiles in new mother rats exhibiting depression-related parenting disturbances

Authors: *S. B. WINOKUR, M. PEREIRA;
Dept. of Psychological and Brain Sci., Univ. of Massachusetts Amherst, Amherst, MA

Abstract: Postpartum depression is a highly prevalent and debilitating disorder that impacts millions of mothers and their children worldwide. Depression in new mothers is associated with cognitive and motivational deficits, as well as disturbances in parenting, which can cause life-long adverse outcomes for their children. However, the molecular mechanisms by which parenting abilities are impacted by postpartum depression are currently unclear. The goal of the present study was to gain novel insight into the molecular basis of depression that most impacts parenting. To this aim, we used postpartum Wistar-Kyoto (WKY) rats, who, compared to Sprague-Dawley (SD) controls, demonstrate cognitive, motivational, and parenting deficits representative of postpartum depression symptomatology. Following maternal behavior phenotyping, RNA sequencing of regions relevant to depression and parenting in early postpartum SD and WKY mothers was used to identify gene expression networks underlying deficits in parenting associated with a depressive phenotype. Regions of interest included the nucleus accumbens (NA), the medial prefrontal cortex (mPFC), and the medial preoptic area (mPOA). Analysis of the mPOA revealed 305 differentially expressed genes (DEGs) between WKY and SD mothers ($p<0.05$, fold change>1.5). Functional annotation and pathway analyses

revealed that these DEGs were predominantly enriched for synaptic transmission, signal transduction, and neuronal plasticity. Further analysis of trait severity narrowed down the mPOA candidate genes to 25 DEGs mediating severity of parenting disturbances, including altered expression of galanin, oxytocin, kisspeptin, and nerve growth factor receptor. Ongoing bioinformatic analyses utilizing mPOA, NA and mPFC transcriptional profiles suggest networks of co-regulated genes associated with depressive-like parenting disturbances. Together, results from this study provide important insights into the transcriptional mechanisms of parenting disturbances associated with postpartum depression and contribute to bettering the understanding of the pathophysiology of depression and its impact on parenting.

Disclosures: S.B. Winokur: None. **M. Pereira:** None.

Poster

147. Maternal and Adolescent Behavior and Physiology

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 147.30/N46

Topic: F.03. Neuroendocrine Processes

Support: NIMH Intramural Research Program project ZIA MH002852-14

Title: Positron emission tomography (PET) imaging of phosphodiesterase 4 in brain and peripheral organs of McCune-Albright syndrome

Authors: *L. A. STOLZ, L. D. WEIDNER, Y. WAKABAYASHI, M. COLLINS, A. BOYCE, S. ZOGHBI, V. PIKE, R. INNIS;
NIH, Bethesda, MD

Abstract: Objective:
This study seeks to evaluate changes in the cyclic adenosine monophosphate (cAMP) cascade both in brain and peripheral organs of individuals with McCune-Albright syndrome (MAS). We accomplished this by using [^{11}C]*(R)*-rolipram which binds to phosphodiesterase-4 (PDE4) and reflects cAMP cascade activity. In MAS, a mutation in the *GNAS* gene results in constitutive activation of $G_s\alpha$ leading to increased cAMP signaling. This increase in signaling is thought to be responsible for the peripheral symptoms, such as fibrous dysplasia, however this has yet to be confirmed in vivo. In addition, it is unknown if cAMP signaling is increased in the brain compared to healthy controls (HC). Therefore, we sought to determine whether MAS patients have increased cAMP activity compared to HC, and if these increases in activity correlate with known areas of fibrous dysplasia. We hypothesized that subjects with MAS would show greater rolipram binding than HC in areas known to affected by the disorder.
Methods:
Subjects underwent whole-body (n = 3 MAS, n = 6 HC) and/or brain (n = 3 MAS, n = 7 HC)

scans using [^{11}C]*(R)*-rolipram. Both male and female subjects aged 31±10 were scanned. Radioligand binding in the brain was quantified as distribution volume (V_T) from arterial blood sampling as the input function.

Results:

[^{11}C]*(R)*-rolipram binding was increased in MAS patients in known areas of fibrous dysplasia (Fig 1). There was no difference in uptake in the peripheral organs between MAS and HC. V_T was higher in HC than in MAS across all the regions, but there was no significant difference (whole brain V_T : 0.52±0.12 vs 0.48±0.11, p>0.05).

Conclusion:

Increased binding of [^{11}C]*(R)*-rolipram correlated with areas of known fibrous dysplasia, indicating higher levels of phosphorylated PDE4. This in turn means that there were increased levels of PKA and cAMP in these regions. These results are significant as it is the first study to show these results in humans in vivo. Continuing this study, we will perform whole-body blocking scans using PDE4 inhibitor roflumilast to determine the specificity of the binding.

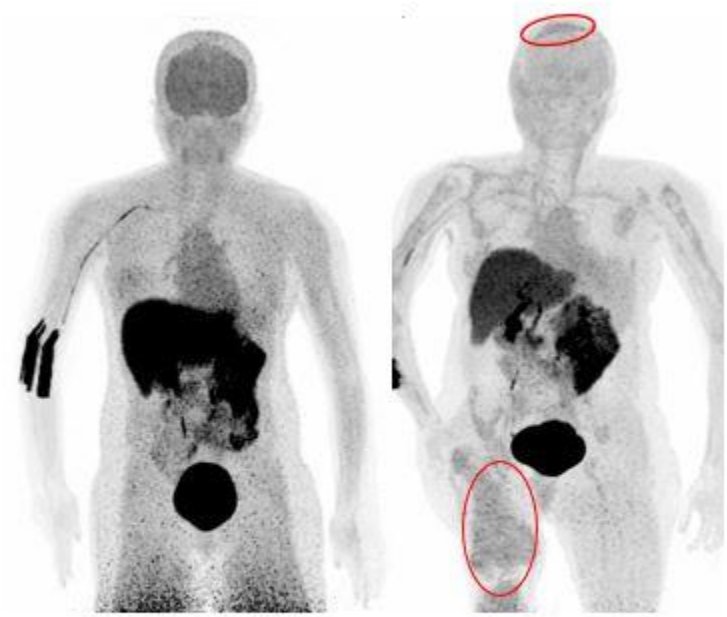

Fig 1: Maximum intensity projections (averaged)

Disclosures: **L.A. Stolz:** None. **L.D. Weidner:** None. **Y. Wakabayashi:** None. **M. Collins:** None. **A. Boyce:** None. **S. Zoghbi:** None. **V. Pike:** None. **R. Innis:** None.

Poster

148. Somatic Influences on the Brain and Vice Versa

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 148.01/O1

Topic: F.04. Stress and the Brain

Title: Tumor necrosis factor alpha and depression

Authors: *F. SANCHEZ-LADRON DE GUEVARA[1], D. HERNANDEZ-BALTAZAR[2], M. MELGAREJO-GUTIERREZ[1], X. CORTIJO-PALACIOS[3], T. CIBRIAN-LLANDERAL[3]; [1]Facultad de Medicina-Universidad Veracruzana, Xalapa, Mexico; [2]CONACYT- Inst. de Neuroetologia-Universidad Veracruzana, Xalapa, Mexico; [3]Neurofisiologia y Neurobiologia de la Conducta, Inst. de Neuroetologia-Universidad Veracruzana, Xalapa, Mexico

Abstract: Since 1975, thanks to the discovery and hard work of Dr. Lloyd Old in his document published in "Proceedings of the National Academy of Sciences" is that we know the tumor necrosis factor (TNF) as such, has been studied since the late seventies for playing a key role in inflammation and immunity.
Several studies have focused on recognizing tumor necrosis factor alpha (TNF-α) as an endogenous pyrogen, which can cause inflammation, apoptotic cell death and mediate the release of various cytokines such as interleukins (IL-6, IL-8, and IL-1β) by stimulation of macrophages deregulation, especially the overproduction of TNF-α, has been found in a wide variety of human diseases, including depression.
The inflammatory hypothesis proposed by Smith in 1991 called "macrophage" theory of depression has underlined from then the importance of psycho-neuroimmunological dysfunction when there is a stimulation of the immune system. Certainly, patients with depression have an abnormal peripheral immune system with weak cellular immunity and high levels of proinflammatory cytokines such as TNF-α. In addition, they have shown that proinflammatory cytokines have an effect on the pathophysiological domains, such as neuroendocrine function, regional brain activity and the metabolism of neurotransmitters, all of which contribute to the pathogenesis of depression.
Over the years through studies in animal models, administering TNF-α is that it was possible to observe the development of symptoms such as decreased social behavior and locomotor activity, anhedonia, suppression of food intake, sleep abnormalities, fatigue and alterations in cognition. These symptoms are collectively known as "disease behavior," which is similar to patients with human depression.
Undoubtedly, recognizing the influence of TNF-α on neurotransmitter systems and obtaining a better understanding of the consequences of altered inflammatory responses can support not only patients with depression but also chronic degenerative diseases in which depression coexists.

Disclosures: **F. Sanchez-Ladron de Guevara:** None. **D. Hernandez-Baltazar:** None. **M. Melgarejo-Gutierrez:** None. **X. Cortijo-Palacios:** None. **T. Cibrian-Llanderal:** None.

Poster

148. Somatic Influences on the Brain and Vice Versa

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 148.02/O2

Topic: F.04. Stress and the Brain

Title: The relationship between the anti-rhinitis and aversive effects of coffee aroma in mice

Authors: ***Y. HAYASHI**[1], M. TSUJIMOTO[1], A. SAIKI[1], J. TANAKA[2];
[1]Dept Foods & Human Nutr., Notre Dame Seishin Univ., Okayama, Japan; [2]Special Needs Educ., Naruto Univ. of Educ., Naruto, Japan

Abstract: Previous studies have shown that coffee aroma elevates alertness and cognitive function in humans while also having a relaxing effect, depending on the type of coffee bean. These findings suggest that coffee aroma might control the stress level of animals. In contrast, various stressors have been reported to affect the symptomatic manifestations of allergic diseases, such as atopic dermatitis and allergic rhinitis. The influence of stressors on allergic diseases varies depending on their type, strength, and duration. This study was carried out to clarify the effects of coffee aroma on allergic rhinitis using an ovalbumin-induced rhinitis model. In addition, we conducted a behavioral preference test to assess whether coffee aroma acts as a stressor in mice. To prepare the rhinitis model, female BALB/c mice were sensitized with an intraperitoneal injection of 200 μL of physiological saline containing ovalbumin (1 μg) and alum (100 μg) as an adjuvant on days 0 and 5. Then, local sensitization was performed every day, starting from day 14, by administering ovalbumin in saline (100 mg/ml, 2 μL) into the bilateral nasal cavities. Brazilian or Guatemalan coffee powder was used to evaluate the anti-rhinitis effects of coffee aroma. Female BALB/c mice were exposed to the aroma of coffee in a plastic container for 15 min. Just after exposure, 2 μL of ovalbumin solution was administered into the nasal cavities using a micropipette, and the frequencies of sneezing and nasal rubbing were counted for 10 min. Pretreatment with Brazilian coffee aroma dose-dependently decreased the number of sneezes and nasal rubbings, whereas Guatemalan coffee aroma had no effect. The preference test revealed that mice avoided the aroma of Brazilian coffee, but not that of Guatemalan coffee. These results suggest that Brazilian coffee aroma acts as a stressor for mice and that suppression of rhinitis symptoms was linked to aversion to the aroma.

Disclosures: **Y. Hayashi:** None. **M. Tsujimoto:** None. **A. Saiki:** None. **J. Tanaka:** None.

Poster

148. Somatic Influences on the Brain and Vice Versa

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 148.03/O3

Topic: F.04. Stress and the Brain

Support: NSERC CGSM

Title: Expression of CRH, ACTH and ACTH receptor in mouse lymphoid organs

Authors: *M. SALEHZADEH[1], J. E. HAMDEN[1], M. X. LI[2], H. BAJAJ[2], C. MA[2], K. K. SOMA[2];
[1]Zoology, [2]Psychology, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: The hypothalamic-pituitary-adrenal (**HPA**) axis is critical for responses to stressors. The hypothalamus releases corticotropin-releasing hormone (**CRH**), which stimulates pituitary secretion of the 39 amino acid hormone, adrenocorticotropic hormone (**$ACTH_{1-39}$**). $ACTH_{1-39}$ binds to its receptor, melanocortin 2 receptor (**MC2R**), on the adrenal glands and stimulates secretion of glucocorticoids (**GCs**). GCs travel through the blood to orchestrate changes in organismal physiology, including neural, metabolic, and immune function. GCs have been thought to be synthesized only in the adrenal glands. However, it is now clear that GCs are also locally-produced in immune organs, such as the thymus, spleen and bone marrow. It remains unclear what factors drive these immune organs to produce GCs locally. Interestingly, there is some evidence for a local HPA axis "homolog" in immune organs, including a shorter form of ACTH (**$ACTH_{1-24}$**). $ACTH_{1-24}$ is biologically active but is not produced by the anterior pituitary and is not found in the blood. We hypothesized that mediators of the HPA axis are expressed within immune organs, perhaps to regulate local GC synthesis. Here, we examined adult C57BL/6 mouse thymus, spleen and bone marrow, and performed qPCR to determine gene expression of proopiomelanocortin (**POMC**), the precursor polypeptide of ACTH, and MC2R. Preliminary data suggest that *Pomc* and *Mc2r* are expressed in the thymus and spleen, and only *Mc2r* is expressed in the bone marrow. Future studies will examine the expression of *Crh* and compare *Crh*, *Pomc* and *Mc2r* expression across development (post-natal day 5, 20 and 90). We will also determine the form of ACTH present in these organs using mass spectrometry. These data raise the exciting possibility that mouse lymphoid organs can regulate local GC levels independently of the HPA axis. These data are a crucial first step for understanding how local GC production is regulated in immune organs.

Disclosures: M. Salehzadeh: None. **J.E. Hamden:** None. **M.X. Li:** None. **H. Bajaj:** None. **C. Ma:** None. **K.K. Soma:** None.

Poster

148. Somatic Influences on the Brain and Vice Versa

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 148.04/O4

Topic: F.04. Stress and the Brain

Support: Early Postdoc.Mobility (Swiss National Science Foundation)
Walter and Gertrud Siegenthaler Postdoctoral Fellowship
RO1 MH090264
RO1 MH104559

Title: Interactions between peripheral monocytes and the neurovasculature in stress disorders

Authors: *F. CATHOMAS, Y. SHIMO, H.-Y. LIN, A. RAMAKRISHNAN, K. CHAN, L. F. PARISE, L. LI, K. B. LECLAIR, F. DESLAND, M. MERAD, L. SHEN, S. J. RUSSO;
Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Psychosocial stress is an important risk factor for many neuro-psychiatric disorders, including major depressive disorder (MDD). While evidence from both pre-clinical animal models and clinical studies suggests that chronic stress leads to profound changes in the immune system (e.g. increased levels of pro-inflammatory cytokines) that elicit behavioral changes relevant to MDD, the detailed mechanisms of how the peripheral immune system acts on the brain (and *vice versa*) remains to be elucidated. The present project aims to characterize how social stress affects immune cell populations in both blood and brain, and investigate the role of blood-brain barrier endothelial cells as an important interface between these two compartments. In a murine model of repeated social defeat stress (RSDS), we performed single cell, high-dimensional analysis using mass cytometry (CyTOF) of both the blood and brain. In blood, we show that RSDS leads to a significant stress effect, altering leukocyte subpopulation frequencies in of the myeloid and lymphoid lineage. Cell-type specific RNA sequencing of the major dysregulated leukocyte-subpopulations revealed that most genes were dysregulated in Ly6c^{high} monocytes. The majority of all up- or downregulated genes were uniquely dysregulated in susceptible mice. Interestingly, increased expression of several genes involved in monocyte-endothelial interactions occurred specifically in susceptible mice. Indeed, RSDS led to an increase in monocyte frequencies in the brains of susceptible but not resilient or control mice. This further supports the hypothesis that stress leads to an increased migration of peripheral monocytes to the neurovasculature of brain regions important in the etio-pathogenesis of MDD. Investigating the mechanisms underlying interactions between the peripheral immune and central nervous systems will yield important insights into the etio-pathophysiology of MDD and could lead to potential novel therapeutic targets.

Disclosures: **F. Cathomas:** None. **Y. Shimo:** None. **H. Lin:** None. **A. Ramakrishnan:** None. **K. Chan:** None. **L.F. Parise:** None. **L. Li:** None. **K.B. LeClair:** None. **F. Desland:** None. **M. Merad:** None. **L. Shen:** None. **S.J. Russo:** None.

Poster

148. Somatic Influences on the Brain and Vice Versa

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 148.05/O5

Topic: F.04. Stress and the Brain

Title: Alterations in resting-state amplitude of low-frequency fluctuation in patients with Crohn's disease in remission

Authors: J. HOU[1], V. A. NAIR[1], S. SAHA[2], *V. PRABHAKARAN[1];
[1]Radiology, [2]Med., Sch. of Med. and Publ. Health, Univ. of Wisconsin-Madison, Madison, WI

Abstract: In a recent study we observed differences in resting state fMRI connectivity in patients with Crohn's disease in remission, compared to healthy controls (Hou et al. accepted). In this study we investigated the brain functional difference between CD patients in remission (CDs) and age-matched healthy control (HC) using the measure of resting state amplitude of low-frequency fluctuation (ALFF), which reflects the intensity of regional spontaneous brain activity (Zang et al. 2007). 20 CDs (11 males and 9 females, mean age = 35.80, SD = 15.81) and 20 HC (11 males and 9 females, mean age = 38.60, SD = 18.21) were tested. Five minutes eyes closed resting-state functional data was collected on a 3T GE scanner with parameters: TR/TE/θ = 2600 ms/22 ms/60°, FOV = 100 × 100 mm, slice thickness = 3.5mm. A BRAVO anatomical scan was acquired with parameters: TR/TE/θ = 8160 ms/3.18 ms/12°, FOV = 100 × 100 mm, in-plane resolution = 1 x 1mm2, slice thickness = 1 mm. Data Processing and Analysis of Brain Imaging (DPABI) toolbox V3.1 (Yan & Zang, 2010) was used for data preprocessing (slice timing, realignment, normalization, smoothing, regressing out head motion parameters). Unpaired 2-sample t-test was performed to test ALFF difference between CDs and HC. Gaussian random field (GRF) with voxel $p < .001$, cluster $p < .05$ was used for multiple comparison correction. CDs were not different in age ($p = .61$), handedness ($p = .20$), education ($p = .77$). For ALFF results, compared to HC: (1) CDs had increased values in certain regions within the sub-lobar, frontal, temporal and limbic lobes; (2) CDs had decreased values in certain regions within the sub-lobar, temporal, parietal and occipital lobes . ALFF is a marker of individual differences in the strength or intensity of low frequency oscillations. Our results indicate significant regional differences in ALFF in CD patients in remission compared to healthy controls. These differences in brain function could likely be caused by differences in the regulation of chronic pain and affect in the CD patients. Other factors such as disease duration, medication, and disease burden could also cause changes in spontaneous brain activity and needs further investigation.

Reference: Hou, J. (2019). 'Alterations in resting-state fractional amplitude of low-frequency fluctuation (fALFF) in patients with Crohn's disease in remission', Scientific Reports, accepted. Yan, C. (2010), 'DPARSF: A MATLAB toolbox for pipeline data analysis of resting-state fMRI', Frontiers in Systems Neuroscience, vol. 4, pp. 13 Zang, Y. (2007), 'Altered baseline brain activity in children with ADHD revealed by resting-state functional MRI', Brain Development, vol. 29, pp. 83-91.

Disclosures: **J. Hou:** None. **V.A. Nair:** None. **S. Saha:** None. **V. Prabhakaran:** None.

Poster

148. Somatic Influences on the Brain and Vice Versa

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 148.06/O6

Topic: F.04. Stress and the Brain

Support: IOER funds

Title: Effects of chronic corticosterone exposure on gut microbiome diversity in rats

Authors: W. E. KIVLIN, C. FUNG, S. BANSAL, M. RUSLING, Z. REHMAN, B. POPE, ***L.-L. YUAN**;
Physiol. and Pharmacol., Des Moines Univ., Des Moines, IA

Abstract: Exposure to chronic stress can cause many serious health problems, and has been linked to structural and functional changes in the *brain-gut-microbiome axis*. However, it is unclear whether sustained elevation in corticosterone (CORT), the primary stress hormone in many nonhuman animals, is able to induce similar changes in gut microbiota and its communication with the brain. We utilized a chronic oral CORT exposure protocol to test this hypothesis. Rats were exposed to a drinking solution of CORT for three weeks (Gourley & Taylor, 2009). This protocol, followed by a wash-out period, is effective in inducing pro-depressive like behaviors in rats including anhedonia and helplessness. Fecal samples were collected at various time points of the procedure. DNA wasextracted and amplified for the 16s V4 region using 806R/515F primers, sequenced with Illumina and analyzed with QIIME2. Sequencing yielded a frequency of 57,292 ± 10,499 sequences/sample. Filtering samples with frequencies below 2 standard deviations of the mean yielded 58,419 ± 7,130 sequences/sample. Alpha rarefaction showed an asymptotic approach, supporting that adequate sampling depth was obtained. Shannon Alpha diversity between CORT and water groups was not significantly different, demonstrating that oral CORT exposure did not significantly change microbiome richness. When evaluating beta diversity with PCOA, increased microbiome variability in the CORT group was observed. The control microbiomes appeared clustered, showing qualitatively less within-group variation. While statistical evaluation of within-group evenness showed no

significant difference between groups, between-group phylogenetic distance by PERMANOVA was nearly significant. Especially, the S24-7 family of the *Bacteroidales*order was significantly affected by CORT treatment (ANCOM, w=499). The S24-7 family, with high prevalence in control animals, was not present in any CORT treated microbiome. Given S24-7's involvement in many biological processes including inflammation, infection, and butyrate production, further studies are currently underway to address the functional consequence of eliminating S24-7 family from gut microbiota.

Disclosures: W.E. Kivlin: None. **C. Fung:** None. **S. Bansal:** None. **M. Rusling:** None. **Z. Rehman:** None. **B. Pope:** None. **L. Yuan:** None.

Poster

148. Somatic Influences on the Brain and Vice Versa

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 148.07/O7

Topic: F.04. Stress and the Brain

Support: NIH Grant 1R01MH113892-01A1
AHA Grant 15SDG22430017
BBRF Grant 26809
VA Grant I21 BX002085

Title: A role for microglia in the behavioral and cardiovascular response to social stress in female rats

Authors: B. S. POPE[1], E. N. HARRINGTON[1], C. G. MORGAN[1], B. M. CALATAYUD[1], *S. K. WOOD[1,2];
[1]Univ. of South Carolina Sch. of Med., Columbia, SC; [2]WJB Dorn VA Med. Ctr., Columbia, SC

Abstract: Social stress is a common risk factor for anxiety and cardiovascular disease (CVD) and women are more likely than men to suffer from comorbid anxiety and CVD. While the underlying neural mechanisms linking these pathological conditions remain unclear, the central nucleus of the amygdala (CeA) and the locus coeruleus (LC) are two stress-sensitive regions which facilitate both behavioral and cardiovascular responses to social stress. Neuroinflammation has been shown to play a causative role in generating anxiety-like behavior. However, the contribution of microglial cells to stress susceptibility in a female population remain unclear. Here we demonstrate that in both the LC and CeA, 25 μg clodronate (CLD) results in a 50% reduction in microglia. The current study aimed to examine, separately, the effects of partial (~50%) microglial depletion in the CeA and the LC on behavioral and cardiovascular susceptibility in female rats. Following recovery from surgical implantation of cardiovascular transmitters (HD-S11) and bilateral CeA or LC cannulae, rats were treated with

either intra-CeA liposomal CLD injections (25 µg/side, 5µl) or empty liposomal vehicle (0µg/side, 5µl). Female rats were then subjected to witnessing an aggressive social defeat encounter between a male intruder and a novel male resident for 15 min on 5 consecutive days. Anxiety-like burying and cardiovascular responses were compared between witnesses and controls treated with vehicle or CLD in either the CeA or LC. These studies identified that witness stress exposure evoked increased burying duration compared with controls. Intra-LC CLD attenuated this burying response; however, there was no effect of CLD on burying in the CeA. Witness stress exposure also induced hypertensive responses that were affected by microglia in the LC and CeA in distinct manners. Intra-CeA CLD dampened the pressor response to acute stress, but had no effect on stress-induced blood pressure upon the 5^{th}exposure to stress versus vehicle-treated witness stress rats. Alternatively, intra-LC CLD had no cardiovascular effect upon the first exposure to stress, yet promoted the pressor response following repeated stress exposure (day 5). These findings suggest that despite a lack of effect on behavior, microglia in the CeA may play a role in facilitating the hypertensive response to acute social stress. In contrast, microglia in the LC mediate the anxiety-like response during acute and repeated social stress, but mitigate the pressor response to repeated social stress exposure in females. Taken together, these studies reveal a novel role for microglia in regulating behavioral and cardiovascular responses to social stress.

Disclosures: B.S. Pope: None. **E.N. Harrington:** None. **C.G. Morgan:** None. **B.M. Calatayud:** None. **S.K. Wood:** None.

Poster

148. Somatic Influences on the Brain and Vice Versa

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 148.08/O8

Topic: F.04. Stress and the Brain

Support: NIA PPG 2P01HL071643-06A1
NHLBI R01 HL128194

Title: Unraveling the complex roles of vimentin intermediate filament in CNS and during aging

Authors: *J. Y. HU, F. GONZALEZ, J. DAVIS, Y. CHENG, M. CIESIELSKI, K. M. RIDGE; Pulmonary and Critical Care Med., Northwestern Univ., Chicago, IL

Abstract: Vimentin is a type III intermediate filament (IF) that primarily found in mesenchymal cells and plays important roles in epithelial-mesenchymal transition (EMT).IFs were originally thought to be a relatively static structure that mainly functioned as mechanical support. However, in the past decades, it has been shown that IF networks are highly dynamic structures and their roles have expanded from maintenance of cell shape and integrity to cellular migration,

adhesion, and cancer metastasis. Vimentin knockout mouse was originally reported to have no overt phenotype, leading to the conclusion that vimentin IF represented an unnecessary cytoskeletal system. Interestingly, our group recently found that acute lung injury (ALI) is attenuated in mice lacking vimentin when challenged with either LPS, asbestos, or bleomycin. Moreover, these studies demonstrated vimentin to be a key player in the activation of the NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome, an intracellular protein complex that is activated in response to stimuli such as bacterial and viral pathogens, including influenza A virus. In this study, we thoroughly characterize young and aged vimentin knockout mice behaviorally using battery of neurocognitive tests and show that there are age-dependent changes in neuromuscular, neurocognitive, and metabolic lung functions distinctively from the wild type mice. We also use RNA sequencing as an unbiased approach to study transcriptional changes and identify a significant number of differentially expressed genes between the two groups in various cell types and with aging. Additionally, histological analyses from multiple organs of these mice also suggest a crucial role of vimentin in maintaining cellular structures and functions during aging. In conclusion, our data suggests that vimentin intermediate filament plays dynamic roles in multiple organ systems and during aging.

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Poster

148. Somatic Influences on the Brain and Vice Versa

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 148.09/O9

Topic: F.08. Biological Rhythms and Sleep

Support: NIH R01HL122390

Title: The intestinal bacterial metabolite, butyrate, promotes sleep

Authors: *E. SZENTIRMAI, N. S. MILLICAN, A. R. MASSIE, L. KAPAS;
Dept. of Biomed. Sci., Washington State Univ., Spokane, WA

Abstract: Emerging evidence suggests that the intestinal microbiota is a source of sleep-promoting signals. Bacterial metabolites and components of the bacterial cell wall are likely to provide important links between the intestinal commensal flora and sleep-generating mechanisms in the brain. Butyrate is a short-chain fatty acid produced by the intestinal bacteria by the fermentation of nondigestible polysaccharides. We tested the hypothesis that butyrate may serve as a bacterial-derived sleep-promoting signal.
We investigated the effects of oral administration, direct intraportal injection, as well as systemic injection of butyrate and tributyrin, a butyrate-yielding pro-drug, in mice and rats. C57BL/6 (n =

15) and Sprague-Dawley (n = 10) rats were instrumented for sleep, body temperature and motor activity recordings. On the control days, the animals received intraperitoneal or intraportal 0.3 ml saline injections or gavaged with 0.3 ml water to obtain baseline values of sleep-wake activity, body temperature and motor activity data. On the treatment days, rats were treated with intraportal or subcutaneous injections of 1 g/kg sodium butyrate in a volume of 2 ml/kg. Mice were gavaged with 0.3 ml tributyrin (n = 8) or received intraperitoneal injections of 20, 50 or 100 mg/kg sodium butyrate (n = 7). Sleep, body temperature and motor activity were recorded for 24 h after each treatment. Data were analyzed by using ANOVA followed by t-test. Oral gavage administration of tributyrin, elicited an almost 50% increase in non-rapid-eye movement sleep (NREMS) in mice for 4 hours after the treatment. Similarly, intraportal injection of butyrate led to prompt and robust increases in NREMS in rats. In the first 6 hours after the butyrate injection, NREMS increased by 70%. Both the oral and intraportal administration of butyrate led to a significant drop in body temperature. Systemic subcutaneous or intraperitoneal injection of butyrate did not have any significant effect on sleep or body temperature. The results suggest that the sleep-inducing effects of butyrate are mediated by a sensory mechanism located in the liver and/or in the portal vein wall. Hepatoportal butyrate-sensitive mechanisms may play a role in sleep modulation by the intestinal microbiota.

Disclosures: **E. Szentirmai:** None. **N.S. Millican:** None. **A.R. Massie:** None. **L. Kapas:** None.

Poster

148. Somatic Influences on the Brain and Vice Versa

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 148.10/O10

Topic: F.08. Biological Rhythms and Sleep

Support: NIH R01HL122390

Title: Lipoteichoic acid, a cell wall component of the intestinal bacteria, induces sleep in mice

Authors: *A. R. MASSIE, N. S. MILLICAN, L. KAPAS, E. SZENTIRMAI;
Dept. of Biomed. Sci., Washington State Univ., Spokane, WA

Abstract: Accumulating evidence suggests that the intestinal microbiota is a source of sleep-inducing signals. These signals could be metabolites, such as butyrate, released from live bacteria or cell wall components of disintegrating bacteria. Among the cell wall components, lipopolysaccharide and fragments of peptidoglycans have been shown to have sleep-promoting activities. Lipoteichoic acid (LTA) is a main component of the cell wall of gram-positive bacteria, a ligand of the TLR2 receptor. We investigated if, similarly to other bacterial cell wall products, it also has sleep-promoting activities. Male C57BL/6 mice were instrumented with EEG and EMG electrodes and an intraabdominal transmitter to record sleep-wake activity, body

temperature and locomotion. Mice were injected intraperitoneally with saline at dark onset on the control day and with LTA on the experimental day and sleep, body temperature and motor activity were recorded for 24 h after each treatment. Injection of 100 µg LTA from *B. subtilis* induced a significant, 25.6 ± 8.3 min increase in non-rapid-eye movement sleep (NREMS) in the first three hours, which was followed by a short negative rebound. A higher dose, 250 µg, increased NREMS by 32.2 ± 8.1 min above baseline. These sleep effects were accompanied by a biphasic change in body temperature; after a short decrease, temperature was elevated above baseline. Injection of 250 µg LTA from *S. aureus* induced a 40.0 ± 8.3 min increase in NREMS. Body temperature showed a short hypothermic response without a subsequent febrile phase. After each treatment, sleep increases were also reflected in decreased motor activity. Peptidoglycan fragments from gram-positive and -negative bacteria as well as lipopolysaccharide from gram-negative bacteria have well-documented sleep-promoting activities. We demonstrated that another bacterial cell wall component, which is specific to gram-positive bacteria, also stimulates NREMS. It is possible that LTA, together with other products of the intestinal microbiota, plays a role in sleep signaling.

Disclosures: **A.R. Massie:** None. **N.S. Millican:** None. **L. Kapas:** None. **E. Szentirmai:** None.

Poster

148. Somatic Influences on the Brain and Vice Versa

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 148.11/O11

Topic: F.08. Biological Rhythms and Sleep

Support: NIH R01HL122390

Title: Sleep-promoting effects of nicotinic acid: The role of prostaglandins

Authors: *L. KAPAS, E. SZENTIRMAI;
Dept. of Biomed. Sci., Washington State Univ., Spokane, WA

Abstract: Introduction: The effects of nicotinic acid intersect with multiple pathways in sleep regulation. In addition to its complex actions on lipid metabolism, nicotinic acid also markedly stimulates the synthesis of prostaglandin D2, a potent endogenous sleep-promoting substance. It facilitates the alternative activation of macrophages, which are permissive for maintaining sleep after sleep loss and in cold environment. Butyrate, which is a ligand of the nicotinic acid receptors has pronounced sleep-promoting effects. Given the relationship between sleep and lipid metabolism, the complex actions of nicotinic acid on lipid metabolism and also the existence of multiple regulatory nodes where nicotinic acid may potentially interfere with sleep, we investigated the effects of systemic administration of nicotinic acid on sleep. Methods: Male C57BL/6 mice were instrumented with EEG and EMG electrodes and an intraabdominal

transmitter to record sleep-wake activity, body temperature and locomotion. We investigated effects of 1) intraperitoneal and oral administration of nicotinic acid, 2) indomethacin pretreatment on nicotinic acid-induced sleep, 3) monomethylfumarate, an agonist of the nicotinic acid receptor GPR109A and 4) nicotinamide, a nicotinic acid metabolite. Results: Intraperitoneal injection (100 and 200 mg/kg) or oral administration (1 g/kg) of nicotinic acid brought about marked, dose-dependent increases in NREMS. After 100 mg/kg nicotinic acid, NREMS was elevated by 41% above baseline for 4 h; 250 mg/kg nicotinic acid elicited robust and long-lasting increases in NREMS; NREMS was 120% above baseline in the 2-11 h time block (baseline: 179.0 ± 7.1 min, treatment: 394.6 ± 20.4 min, $p < 0.001$). The effects were abolished by indomethacin, an inhibitor of prostaglandin synthesis. Monomethylfumarate administration recapitulated the effects of nicotinic acid, but nicotinamide did not affect sleep. Conclusions: Nicotinic acid has a potent sleep-promoting activity in mice. The effects are mediated by the receptor GPR109A but are independent of the formation of nicotinamide. The production of endogenous prostaglandins is required for the manifestation of the sleep-promoting activity of nicotinic acid.

Disclosures: L. Kapas: None. **E. Szentirmai:** None.

Poster

148. Somatic Influences on the Brain and Vice Versa

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 148.12/O12

Topic: F.08. Biological Rhythms and Sleep

Support: NIH R01HL122390

Title: Lipopolysaccharide-induced sleep in rats: The role of hepatoportal sensory mechanisms

Authors: *N. S. MILLICAN, A. R. MASSIE, E. SZENTIRMAI, L. KAPAS;
Dept. of Biomed. Sci., Washington State Univ., Spokane, WA

Abstract: Introduction: Sleep circuits in the brain and the gut flora are linked through a dynamic bidirectional relationship. Circadian disruption and chronic sleep fragmentation promote intestinal dysbiosis, while depletion of intestinal microbiota induces significant reduction in sleep. This suggests that the gut flora is a source of sleep-inducing signals. Various components of bacterial cell walls, such as lipopolysaccharide (LPS), both translocate from the intestinal lumen and induce sleep when injected systemically. This suggests that fragments of disintegrating intestinal bacteria, once translocated into the portal circulation, could serve in sleep signaling. To test the effects of portally-circulating LPS on sleep, we injected LPS into the portal vein in rats. Methods: Male rats were implanted with a portal-vein catheter to receive injections, EEG and EMG electrodes to record sleep-wake activity, and an intraabdominal

transmitter to record body temperature and locomotion. We investigated effects of the portal-vein and subcutaneous injection of 20 µg/kg LPS on sleep-wake activity, body temperature, and locomotion. Results: Intraportal administration of LPS induced biphasic effects on NREMS. In the first 12 h after the treatment, NREMS increased 107 ± 13 min above baseline, which was followed by a negative rebound in the subsequent 12-h period. REMS was decreased by 12 ± 4 min in the first 12 h after treatment. EEG slow-wave activity was suppressed for 24 h. Body temperature showed an initial drop lasting for ~2-3 h, which was followed by fever. Locomotion was suppressed during the first 12-h period. Subcutaneous administration of the same dose of LPS did not have any effect on sleep or body temperature; locomotion was suppressed during the first 12-h period, though to a lesser extent than after portal-vein injections, and slightly increased over the second 12-h period. Conclusions: Rats are significantly more sensitive to the sleep- and fever-inducing effects of LPS after intraportal injection than after systemic administration. This suggests the existence of an LPS-sensitive sleep- and fever-inducing sensory mechanism in the hepatoportal region.

Disclosures: **N.S. Millican:** None. **A.R. Massie:** None. **E. Szentirmai:** None. **L. Kapas:** None.

Poster

148. Somatic Influences on the Brain and Vice Versa

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 148.13/O13

Topic: F.04. Stress and the Brain

Support: Divisional and BCHP Network Institutional Research Grant

Title: Gut dysbiosis attenuates the sympathoadrenal responses to acute hypoglycemia at birth and in young adult animals

Authors: ***B. B. NANKOVA**[1], F. HU[1], E. LAGAMMA[1,2];
[1]Pediatrics, New York Med. College, BCHP, Valhalla, NY; [2]Maria Fareri Children's Hosp. at Westchester Med. Ctr., Valhalla, NY

Abstract: The microbiome has co-evolved with their mammalian host to form a complex and dynamic interaction of genes and environment that play a pivotal role in various metabolic, nutritional, physiological, and immunological processes. The initial priming of the neonatal digestive system with optimal microbiota is hypothesized to shape future health and is influenced by several genetic and perinatal factors including nutrition during pregnancy, gestational age, mode of delivery, antibiotic therapy, and feeding practices. Perturbations of the normal microbial balance (gut dysbiosis) early in life have been linked to a wide range of diseases from gastrointestinal illnesses to metabolic, atopic and even neurodevelopmental conditions, yet the precise mechanism(s) remain elusive. Previously we showed that postnatal gut colonization

augments maturation and function of sympathoadrenal stress-responses to hypoglycemia thus providing a selective survival advantage of the newborn due to better adaptation to adverse conditions in the extra uterine environment (Giri et al., 2019). We hypothesize that altering gut flora in young adult animals would also alter the peripheral stress responses to insulin-induced hypoglycemia. Groups of adolescent mice were given a prolonged course of non-absorbable broad-spectrum antibiotics in the drinking water for two weeks. Daily fluid intake and body weights over the course of the experiment did not differ significantly between groups. Administration of the antibiotic mixture resulted in a nearly complete depletion of the gut microbiota, markedly enlarged ceca and in no detectable by-products of bacterial fermentation (sp. SCFA). Mice with reduced gut bacteria showed a significant improvement in fasting glycemia and intraperitoneal glucose tolerance (as compared to age-matched controls drinking plain water). Although no differences in plasma levels of corticosterone (lack of dysregulation of the HPA stress axis) or glucagon (intact parasympathetic signalling) were observed between the groups, animals with gut targeted depletion of gut microbiome had significantly lower basal and metabolic stress-induced urinary epinephrine levels. These results represent the first evidence that even in adolescent mice, depletion of the gut microbiota by a prolonged course of oral antibiotics affects the classical cholinergic-transsynaptic signal transduction pathways regulating the response to hypoglycemia regardless of postnatal age suggesting a mutable mechanism(s) exists throughout life. Unravelling these mechanisms could lead to new therapeutic possibilities through controlled manipulation of the gut microbiota.

Disclosures: **B.B. Nankova:** None. **F. Hu:** None. **E. LaGamma:** None.

Poster

148. Somatic Influences on the Brain and Vice Versa

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 148.14/O14

Topic: F.04. Stress and the Brain

Support: Ministry of Education, Singapore, RG144/17

Title: Alteration in the composition of gut microbiota following exposure to stressful or enriched environment

Authors: A. HEGDE[1], R. PURBOJATI[1], S. PETTERSSON[2], ***R. MITRA**[1];
[1]Nanyang Technological Univ., Singapore, Singapore; [2]Lee Kong Chian Sch. of Medicine, Nanyang Technological Univ., Singapore, Singapore

Abstract: Behavior is influenced by both external environment and internal physiological states. A large body of the prior work has established that the stressful or rewarding nature of environment is a critical factor influencing mental disorders. Chronic or traumatic stress creates a

predisposition to anxiety, depression and related disorders. In contrast, the variable period of exposure to environmental enrichment during early-life or adulthood protects against the same mental disorders. While these studies highlight the importance of the external environment, the effects of the body's internal milieu on the psychiatric conditions have been relatively less studied. In this context, we address if exposure to stress or environmental enrichment or both leads to a change in the gut microbiota of rodents. Our results show distinctive changes in the profile of gut microbiota in animals exposed to stress and enrichment in early life. Moreover, this finding parallels with stress-vulnerability and stress-resilience in these animals, as reflected in behaviours tested during adulthood.

Disclosures: **R. Mitra:** None. **S. Pettersson:** None. **A. Hegde:** None. **R. Purbojati:** None.

Poster

148. Somatic Influences on the Brain and Vice Versa

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 148.15/O15

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

Title: Deconstructing the infant pain experience

Authors: *E. P. DUFF[1], F. MOULTRIE[2], S. GOKSAN[4], L. BAXTER[2], S. P. FITZGIBBON[3], A. ABOS[5], T. D. WAGER[6], R. SLATER[2];
[2]Paediatrics, [3]Nuffield Dept. of Clin. Neurosciences, [1]Univ. of Oxford, Oxford, United Kingdom; [4]Inst. of Educ., Univ. Col. London, London, United Kingdom; [5]Univ. of Barcelona, Barcelona, Spain; [6]Psychology and Neurosci., Univ. of Colorado Boulder Dept. of Psychology and Neurosci., Boulder, CO

Abstract: To determine whether an infant is happy, distressed or in pain we must interpret their behaviour. We do so by relying on correspondences with these behaviours in individuals who are able to articulate their internal states. Neuroimaging presents an opportunity to improve our understanding of the infant experience by characterising concomitant brain activity. Previously, using FMRI, we found that pain in infants activates a similar set of brain regions to adults. Here we demonstrate how inferences can be extended by fingerprinting infant brain responses using validated multivariate neuroimaging signatures linked directly to adult states of pain and negative affect. This provides a powerful approach to infer the nature and development of experiences in infants and other vulnerable populations.
We acquired 3T FMRI data in two new born infant cohorts, and one adult cohort, during the application of non-tissue damaging punctate stimuli of different intensities to the foot. Infant FMRI data were processed using the DHCP functional and structural pipelines. Parameter maps were generated for each subject at each stimulus intensity. Cosine Similarity calculation of concordance of responses with six pain and negative affect signatures used the CANLAB

toolbox.
We found that infant responses to pain manifest similar activation of the Neural Pain Signature (Wager 2013) to adults, with robust intensity encoding suggesting graded intensities of pain. Infant responses did not show activation of the Stimulus Intensity Independent Signature (Woo 2016) as adults (Fig 1). The SIIPS network has been found to mediate psychological manipulations of expectations and perceived control, which are likely to develop after infancy. We also identified differences in signatures associated with stress, and no effect in other signatures.
This analysis of infant neural pain responses reveals a partially developed pain system. This work demonstrates how validated neural signatures derived from pain reports in healthy adults can be used to characterise responses in populations in the absence of verbal report.

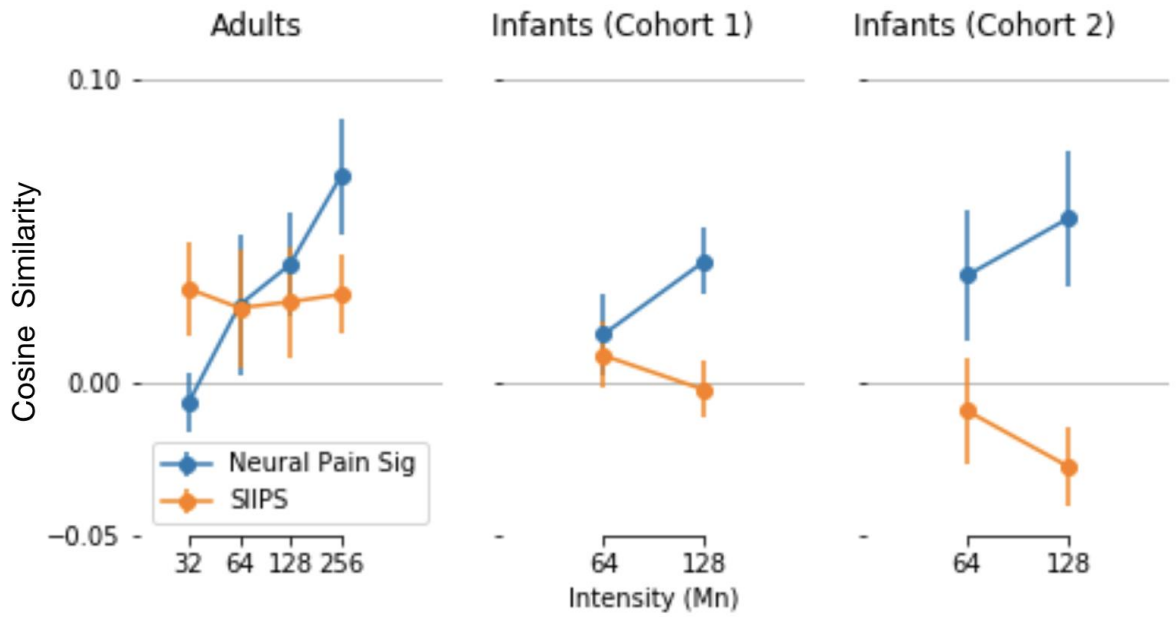

Fig 1. Concordance of NPS and SIIPS with Adult and Infant Pin Prick FMRI Responses

Disclosures: E.P. Duff: None. **F. Moultrie:** None. **S. Goksan:** None. **L. Baxter:** None. **T.D. Wager:** None. **S.P. Fitzgibbon:** None. **R. Slater:** None. **A. Abos:** None.

Poster

148. Somatic Influences on the Brain and Vice Versa

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 148.16/O16

Topic: F.04. Stress and the Brain

Support: P50-MH099910
MH 104184
MH 091258
MH 087597
MH 073030
MH 108286
ES 028202

Title: Using a humanized mouse model to dissect the mechanistic role of the maternal microbiome on offspring gut-immune-brain development

Authors: *E. JASAREVIC[1], C. HOWARD[1], P. KANE[1], T. L. BALE[2];
[1]Univ. of Maryland Sch. of Med., Baltimore, MD; [2]Pharmacol., Univ. of Maryland Baltimore, Baltimore, MD

Abstract: The maternal microbiome has emerged as a critical contributor to offspring immune system development and neurodevelopment. At birth, the maternal vagina serves as a source of microbiota that colonize the neonate gut, and these microbial communities stimulate immune cell populations that play a key role in health outcomes across the lifespan. Building on these clinical studies, we recently developed a method that utilizes C-section and oral gavage in mice to manipulate the composition of microbial communities that colonize the neonate gut and revealed an exciting causal role of the maternal microbiome in regulating offspring development. A significant translational advantage of this approach lies in the ability to transplant microbiota from human donors into C-section mice and conduct mechanistic studies that cannot be readily conducted in humans where confounding variables, including stress, diet, and antibiotic exposure are impossible to control. To develop a relevant humanized mouse model, we performed colonization experiments with two distinct human vaginal microbial communities that were transplanted into C-sectioned mice at embryonic day 18.5. The human vaginal microbial communities were selected based on their capacity to stimulate different components of the maternal immune system. Vaginal community state type I (CST I) is dominated by a single species, *Lactobacillus crispatus,* and exhibits high resistance to infection, while CST IV lacks *Lactobacillus* and contains a variety of species that are associated with increased risk for infection. As women harboring a CST IV show increased proinflammatory cytokine levels and heightened immune activation, we hypothesize that CST IV colonized mice will exhibit alterations in immune and brain development compared with CST I colonized mice. Specifically, we determined whether colonization by CST I and CST IV recapitulates microbiota composition in the postnatal day (PN) 2 gut of recipient male and female offspring by 16S rRNA marker gene sequencing. To examine the transcriptional response of the intestinal niche to colonization by CST I and CST IV, we are conducting RNA-sequencing on PN2 gut from male and female offspring. Given the ability of CST I and CST IV to recruit unique immune cell populations to the maternal vaginal mucosa, we will determine whether CST I and CST IV colonization in the neonate gut stimulate different immune cell populations in the periphery using a mass cytometry approach. Together, these studies identify a novel humanized mouse model to reveal new links

between the maternal microbiota, offspring immune and brain development, and risk for adverse offspring outcomes.

Disclosures: E. Jasarevic: None. **C. Howard:** None. **P. Kane:** None. **T.L. Bale:** None.

Poster

149. Neuropeptide Regulation: Feeding and Metabolism

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 149.01/O17

Topic: F.10. Food Intake and Energy Balance

Title: Role of spexin in regulation of leptin-mediated feeding behavior in the hypothalamus

Authors: *B. JEONG, B. LEE;
Univ. of Ulsan, Ulsan, Korea, Republic of

Abstract: Here, we report that leptin regulates feeding behavior via spexin, a novel neuropeptide. Spexin is coevolved with the galanin/kisspeptin family and has been implicated in satiety/food intake control, glucose, and lipids metabolism. We revealed that leptin induces spexin mRNA in the mouse medial basal hypothalamus (MBH). Especially, intracerebroventricular (icv) injection of leptin increased spexin mRNA levels in supraoptic nucleus and arcuate nucleus of the mouse hypothalamus. Chromatin immunoprecipitation assays revealed that leptin-activated signal transducer and activator of transcription 3 (STAT3) directly stimulated spexin transcription by binding to its binding domain in the 5'-flanking region of the spexin gene. Furthermore, spexin mRNA expression was increased by leptin but decreased by S3I-201, an inhibitor of STAT3, in the mouse MBH. Blockade of hypothalamic spexin synthesis by icv injection of antisense oligonucleotides resulted in decrease of animals' responses to leptin on food intake and body weight. These results suggest that spexin plays an important role in leptin signaling pathway on appetite control.

Disclosures: B. Jeong: None. **B. Lee:** None.

Poster

149. Neuropeptide Regulation: Feeding and Metabolism

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 149.02/O18

Topic: F.10. Food Intake and Energy Balance

Title: NPY2R and GLP-1R agonists activate neuronal circuits involved in energy homeostasis

Authors: *T. ZIMMERMANN[1], J. HECKSHER-SØRENSEN[2], A. OLDENBURGER[1], T. BAADER-PAGLER[1], A. NYGAARD MADSEN[2], U. ROOSTALU[2], L. C. BIEHL RUDKJÆR[2], R. AUGUSTIN[1];
[1]Cardiometabolic Dis. Reserach, Boehringer Ingelheim Pharma Gmbh & Co. KG, Biberach, Germany; [2]Gubra, Hørsholm, Denmark

Abstract: Obesity is a global major health problem, which results from an imbalance of energy homeostasis, with energy intake being a complex behavior controlled by endocrine, nutritional, thermal and metabolic signals. Of particular significance are the gut hormones glucagon-like peptide 1 (GLP-1) and neuropeptide Y (NPY). Both are released from the gastrointestinal tract in response to ingested food into the bloodstream, circulating to distant sites of action. They relay information regarding the nutritional status to the brain, thus regulating feeding and energy homeostasis. Here, we investigated the food intake and body weight-lowering effects of a long-acting NPY2R agonist alone or co-administered with the GLP-1R agonist semaglutide in diet-induced obese (DIO) mice upon subchronic dosing for 28 days. Semaglutide led to a significant decrease in food intake and reduced body weight by 19%, while the NPY2R agonist was associated with an acute food intake reduction translating into a decrease in body weight by 6% subchronically. However, the combined application of both compounds in DIO mice resulted in a synergistic reduction of food intake and body weight by 35%. In order to provide insights on the mechanism of this synergism, we investigated global activation of brain regions by c-Fos in DIO mice upon acute (4h; Cmax) administration of the single molecules and in their combination. We applied whole brain 3D brain imaging (iDISCO) and identified several regions being involved in feeding circuits. The GLP-1R agonist led to significant c-Fos upregulation, whereas the NPY2R agonist did not induce c-Fos expression. Interestingly, upon co-administration, c-Fos induction was significantly higher compared to semaglutide in the parastrial nucleus and the paraventricular nucleus of the hypothalamus. In summary, our data demonstrate that combining GLP-1R with NPY2R agonism results in a synergistic body weight-lowering efficacy compared to the single entities. This synergistic effect can be depicted by increased c-Fos expression in specific brain regions providing new mechanistic insights on both GLP-1R and NPY2R agonism and their individual and combined engagement of neuronal circuits.

Disclosures: **T. Zimmermann:** A. Employment/Salary (full or part-time):; Boehringer Ingelheim Pharma Gmbh & Co. KG. **J. Hecksher-Sørensen:** A. Employment/Salary (full or part-time):; Gubra. **A. Oldenburger:** A. Employment/Salary (full or part-time):; Boehringer Ingelheim Pharma Gmbh & Co. KG. **T. Baader-Pagler:** A. Employment/Salary (full or part-time):; Boehringer Ingelheim Pharma Gmbh & Co. KG. **A. Nygaard Madsen:** A. Employment/Salary (full or part-time):; Gubra. **U. Roostalu:** A. Employment/Salary (full or part-time):; Gubra. **L.C. Biehl Rudkjær:** A. Employment/Salary (full or part-time):; Gubra. **R. Augustin:** A. Employment/Salary (full or part-time):; Boehringer Ingelheim Pharma Gmbh & Co. KG.

Poster

149. Neuropeptide Regulation: Feeding and Metabolism

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 149.03/O19

Topic: F.10. Food Intake and Energy Balance

Support: Indian Institute of Science Education and Research Pune intramural funds, (AG)
Council of Scientific and Industrial Research (2015-2020), (AM)

Title: CART neuropeptide mediates anorexia in zebrafish by modulating the activity of a specific telencephalic region

Authors: *A. MADUSKAR[1], D. WAKHLOO[2], D. BODAS[1], T. KANIGANTI[1], N. SUBHEDAR[1], A. GHOSE[1];
[1]Indian Inst. of Sci. Educ. and Res., Pune, India; [2]Max-Planck-Institute for Exptl. Med., Göttingen, Germany

Abstract: Activity patterns of neuromodulators generate molecular representations of internal states and modify neuronal properties and circuit dynamics to effect homeostatic adjustments. In the context of feeding behaviour, interoceptory neurons in the brain sense peripheral and central signals of energy status of the body such as levels of glucose and presence of neurohormones, like leptin or ghrelin, and relay this information to downstream circuit nodes by releasing anorexigenic or orexigenic neuropeptides. Cocaine-and Amphetamine-Regulated-Transcript (CART) is an anorexigenic neuropeptide present in regions involved in regulating feeding behaviour (eg. arcuate neucleus in mammals) and energy homeostasis wherein the levels of this neuropeptide reflect the energy status of the animal i.e high CART levels in sated animals. Although administration of CART peptide has been shown to induce potent anorexia, the details of the downstream mechanisms employed by this neuropeptide still remain unclear. In the present study we used pharmacological intervention coupled with behavioural monitoring and neuronal activity imaging in adult zebrafish (*Danio rerio*, Indian wild-type strain) to better understand the molecular mechanisms involved in the initiation and maintenance of CART-mediated anorexia. We generated activity maps of the adult zebrafish brain in varying energy states and also under the influence of CART peptide. These studies found a strong correlation between high levels of the neural activity marker, phosphorylated extracellular signal-regulated kinase (pERK) in a region of the dorsal telencephalon and an associated anorexic behavioural output. We found that active N-methyl-D-aspartate (NMDA) receptor signalling is necessary for CART peptides' action, as the increase in pERK levels and reduction in feeding drive were both disrupted by blocking NMDA receptor signalling. We then tested if protein kinase A (PKA) is involved in mediating this effect. Indeed, we found that PKA activity was required for CART-induced upregulation of pERK in the dorsal telencephalic region and suppression of feeding

drive. Additionally, results of neuronal activity imaging in *ex vivo* whole brain preparations suggest that CART neuropeptide sensitizes the telencephalic neurons to glutamatergic inputs and this is correlated to CART-induced anorexia. Together these data suggest that CART action via PKA sensitizes NMDA receptors, perhaps via post-translational modification of receptor subunits, leading to a sustained ERK activation that maintains a neural representation of sated state.

Disclosures: **A. Maduskar:** None. **D. Wakhloo:** None. **D. Bodas:** None. **T. Kaniganti:** None. **N. Subhedar:** None. **A. Ghose:** None.

Poster

149. Neuropeptide Regulation: Feeding and Metabolism

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 149.04/O20

Topic: F.10. Food Intake and Energy Balance

Support: National Institute on Drug Abuse DA034748
Department of Veterans Affairs Medical Research Service BX001753
We thank the National Neurological Specimens Bank, Los Angeles

Title: Reduced number of hypocretin (orexin) labelled neurons in human obesity

Authors: *T. THANNICKAL[1,2], M. CORNFORD[3], J. M. SIEGEL[1,2];
[1]Dept Psychiat, Univ. California Los Angeles, North Hills, CA; [2]Neurobio. Res., VA Greater Los Angeles Healthcare Syst., North Hills, CA; [3]Pathology, UCLA, Los Angeles, CA

Abstract: Human narcolepsy is characterized by hypocretin (Hcrt) cell loss and an increased number of glial fibrillary acidic protein (GFAP) cells in the hypothalamus. There is a greatly increased prevalence of obesity in narcoleptic patients. Hcrt peptide knockout mice are also prone to obesity. The hypothalamus of 8 obese (4 male, 4 female, BMI 51.23 ± 5.2) and 5 control (3 female and 2 male, BMI 24.8 ± 0.9) human brains were used for pathological analysis. Immunohistochemical staining was done for Hcrt, melanin concentrating hormone cells (MCH) and GFAP. Cell number and size were analyzed with the MBF Bioscience Neurolucida program. We found a reduced number of hypocretin cells (range from 10% to 68%) which was correlated with BMI, which ranged from 35 to 70 ($r = -0.72$, $P = 0.001$). There was no change in Hcrt cell size ($t = 0.218$, df 12, $P = 0.8$). The number of MCH neurons, which are intermixed with Hcrt cells, did not differ between control and obese groups ($t = 0.351$, df = 6 and $P = 0.73$). The density of hypothalamic and thalamic GFAP cells was compared in obese and control. Hypothalamic, GFAP cell number was significantly ($t = 2.69$, df = 11, $P = 0.02$) increased (49.71±11.68%) in obese individuals and correlated with BMI ($r = 0.86$, $P= 0.006$). There was no change in GFAP number in the thalamus ($t = -0.695$, df = 11, $P = 0.50$). The presence of gliosis

in the hypocretin cell region is consistent with a degenerative process being the cause of Hcrt cell loss. This study shows a similarity between the pathology of narcolepsy and that of human obesity. Reduced Hcrt neuronal number is associated with obesity and sleep disorders. Hypocretin or hypocretin agonists may be useful in the treatment of obesity.

Disclosures: T. Thannickal: None. **M. Cornford:** None. **J.M. Siegel:** None.

Poster

149. Neuropeptide Regulation: Feeding and Metabolism

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 149.05/O21

Topic: F.10. Food Intake and Energy Balance

Support: International Brain Research Organisation

Title: Effect of food deprivation and mch ablation on nesfatin-1 distribution and release in the brain

Authors: *I. MARMOUZI[1], P. NGUYEN[1], L. ALHASSEN[2], A. ALACHKAR[3], O. CIVELLI[4]; [2]Dept. of Pharmacology, Pharmaceut. Sciences, and Developmental and Cell, [1]Univ. of California, Irvine, Irvine, CA; [3]Pharmacol., [4]Univ. of California Irvine, Irvine, CA

Abstract: Nesfatin-1 is an anorexigenic peptide encoded by nucleobindin2 (NUCB2) and expressed in paraventricular nucleus (PVN), zona incerta (ZI) and tuberal lateral hypothalamus (LHA), where it is notably co-expressed in 90% of melanin concentrating hormone (MCH) neurons. Nesfatin-1 is also detected in peripheral tissues, including the pancreas, duodenum and stomach, and can pass through the blood-brain barrier. Nesfatin-1 receptors have not yet been identified, however, it has been shown that this neuropeptide shares with MCH, a number of physiological functions such as arousal, exploration, food intake and ingestive behavior. Interestingly, Nesfatin-1's action on food seeking behavior is opposite to that of MCH, and is mediated by a neurocircuit that involves the central melanocortin-CRH-oxytocin system. Until now it is not known how Nesfatin-1 and MCH interact to modulate food intake, however, it is established that the neuronal activities of MCH in LHA are sensitive to Nesfatin-1 microinjections. To date no previous reports explain the functional differences between these two co-expressed peptides and how they interact within the brain. The goal of this experiment is to elucidate if MCH ablation can modulate Nesfatin-1 expression in different brain regions following either food intake or deprivation. For this purpose, we have applied 24h food deprivation to MCH conditional knockout neurons and MCH receptor knockout animals, and then compared early gene and neuropeptides expression using immunoreactivity and in situ hybridization. To eliminate MCH neurons in the conditional knockout model, we targeted the expression of the human diphtheria toxin receptor (DTR) to the gene for MCH (Pmch). The

injection of DT in heterozygous PmchDTR/+ mice resulted in the loss of 98% of MCH neurons. All animal groups were subject to locomotor activity testing 2h before perfusion or Snape freezing and brain tissues collection, and the sections were processed with immunohistochemistry and fluorescent in situ hybridization for Nesfatin-1, MCH, Oxytocin and c-Fos expression in different regions, including LHA, ZI, PVN, DVC, ARC and amygdala.

Disclosures: I. Marmouzi: None. **P. Nguyen:** None. **L. Alhassen:** None. **A. Alachkar:** None. **O. Civelli:** None.

Poster

149. Neuropeptide Regulation: Feeding and Metabolism

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 149.06/O22

Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant OD010996
NIH Grant NS099234

Title: Neuropeptidergic profile of hypothalamic neurons involved in the control of brown and white adipose tissue in normal rats and obese rats fed with high energy diet from early age

Authors: *G. CANO[1], S. L. HERNAN[1], A. G. RICHIE[1], H. ALLEN[1], A. STANZANI[1], D. TUPONE[2,3], A. F. SVED[1];
[1]Dept. of Neurosci., Univ. of Pittsburgh, Pittsburgh, PA; [2]Dept. of Neurolog. Surgery, Oregon Hlth. & Sci. Univ., Portland, OR; [3]Dept. of Biomed. and Neuromotor Sci., Univ. of Bologna, Bologna, Italy

Abstract: Brown adipose tissue (BAT) regulates heat production to maintain body temperature, whereas white adipose tissue (WAT) functions as an energy reserve. Complex brain circuitry controls BAT thermogenesis and WAT lipolysis via direct sympathetic innervation. Obesity is associated with dramatic changes in BAT and WAT body distribution and decreases BAT thermogenic activity. In a previous study, we used a diet-induced obesity (DIO) model in rats to examine the effect of early life obesity on the central circuitry controlling BAT and WAT activity. We found subtle changes in few brainstem areas. Here, we focus on the effect of early life DIO on hypothalamic nuclei that contain neuropeptides involved in the regulation of energy expenditure and thermogenesis, such as MCH, CART, POMC, and Urocortin. Preadolescent rats (28 days-old; n =24) were fed with high fat diet (HFD, 31.8% kcal from fat; 25.2% kcal from sucrose) for 8 weeks, whereas control rats were fed with chow (n=12). HFD and chow rats weighed 573 $\pm$ 17 g and 491 $\pm$ 15 g, respectively. Each rat was injected with a pseudorabies virus (PRV) that expresses RFP into inguinal WAT and a PRV expressing GFP into interscapular BAT. Rats were perfused at different survival times (96-124 hrs), and brains were processed. At

early survival, WAT and BAT infected neurons were found in brain regions involved in central sympathetic control. At longer survival, the infection progressed to hypothalamic areas involved in metabolic control such as the lateral hypothalamus (LH), Arcuate (Arc), and Edinger-Westphal (EW) nuclei. In EW, the % of WAT-infected neurons that were Urocortin-ir was decreased in HFD rats compared to chow rats (from 64.1 $\pm$ 3.8% to 47.3 $\pm$ 3.4%; $p<0.05$). Similarly, the % of WAT-infected neurons that were CART-ir was decreased in HFD rats (from 72.4 $\pm$ 2.5% to 49.9 $\pm$ 6.4%; $p<0.05$) in EW. No differences were observed in BAT-infected neurons in EW. Scarce BAT or WAT infected neurons were POMC-ir in Arc in both groups. BAT and WAT MCH-ir infected neurons in the LH in chow rats were 21.1 $\pm$ 1.5% and 29.6 $\pm$ 3.4%, respectively, and 23.5 $\pm$ 3.1% and 35.1 $\pm$ 2.1% in HFD rats. Though CART colocalizes with POMC in Arc, MCH in LH, and Urocortin in EW, infected CART-ir neurons were observed mainly in EW, where 12% were double-infected. Dense CART-ir fibers and terminals were observed in apposition to BAT and WAT infected neurons in numerous brain regions. These observations support a relevant role of EW CART-Urocortin neurons in the control of BAT and WAT activity. Our results suggest that early life HFD can induce subtle anatomic changes in neuropeptidergic neurons involved in the control of metabolism and energy expenditure that are anatomically linked to BAT and WAT.

Disclosures: G. Cano: None. **S.L. Hernan:** None. **A.G. Richie:** None. **H. Allen:** None. **A. Stanzani:** None. **D. Tupone:** None. **A.F. Sved:** None.

Poster

149. Neuropeptide Regulation: Feeding and Metabolism

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 149.07/O23

Topic: F.10. Food Intake and Energy Balance

Support: EMRPD1I0381
CMRPD1H0431
MOST 107-2320-B-182 -019 -MY3

Title: Deletion of neuropeptide FF type 2 receptor reduces the food intake in a mouse model

Authors: *Y.-T. LIN[1,2], S.-C. TSAI[3], T.-Y. LEE[3], H.-Y. LI[4], J.-C. CHEN[1,2,5];
[1]Grad. Inst. of Biomed. Sci., [2]Healthy Aging Res. Ctr., [3]Dept. of Biomed. Sci., Chang Gung Univ., Tao-Yuan, Taiwan; [4]Dept. of Natural Sci., Oregon Tech., Klamath Falls, OR; [5]Neurosci. Res. Ctr., Chang Gung Mem. Hosp., Tao-Yuan, Taiwan

Abstract: Neuropeptide FF (NPFF) belongs to a FMRF-NH_2 peptide family and is recognized as an opioid modulating peptide. NPFF involves in different physiological functions including the regulation of analgesic effect of opioids, and controls food consumption and cardiovascular

function through its interaction with two cognate receptors, NPFFR1 and NPFFR2. According to receptor binding affinity, NPFFR2 is suggested to be the physiological receptor for NPFF. Central control of food intake is mainly regulated in the hypothalamus that includes the arcuate nucleus, the nucleus of the solitary tract, paraventricular nucleus, ventromedial hypothalamic nucleus, lateral hypothalamic area and perifornical areas. NPFF has been reported to regulate feeding behavior, however, its downstream cellular mechanism remains unclear. In the current study, we explore the role NPFFR2 in food intake through NPFFR2 knockout mice. The NPFFR2 knockout mice were made by CRISPR-Cas9 strategy. The food intake was significantly reduced in NPFFR2 KO mice of an overnight feeding recording, or a continuously measurement of food intake within three hours after mice were starving for one night. We measured the anorexigenic and orexigenic peptides by real-time PCR in the hypothalamus of both WT and NPFFR2 KO mice. The NPFFR2 KO mice show down-regulated orexigenic peptides, agouti-related protein (AgRP) and neuropeptide Y (NPY), with no change in amount of anorexigenic peptides. We also measured the serum levels of insulin and leptin, their corresponding receptors in the hypothalamus. Overall, these findings indicate the deletion of NPFFR2 inhibits the downstream regulatory pathway of satiety signals in the hypothalamus.

Disclosures: **Y. Lin:** None. **S. Tsai:** None. **T. Lee:** None. **H. Li:** None. **J. Chen:** None.

Poster

149. Neuropeptide Regulation: Feeding and Metabolism

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 149.08/O24

Topic: F.10. Food Intake and Energy Balance

Support: AHA 16SDG26590000, UT Health Start-Up

Title: Chronically high systemic allopregnanolone improves parasympathetically-mediated glucose metabolism in females

Authors: *S. FEDORCHAK, M. LOPEZ, C. R. BOYCHUK;
Cell. and Integrative Physiol., Univ. of Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX

Abstract: It is a well-known fact that men and women develop differently during puberty. Some of the changes are clear, but others like metabolic changes are less understood due to the complexity of the systems. This study focused on a progesterone derivative synthesized in both sexes: allopregnanolone (ALLO). ALLO is able to cross the blood-brain barrier and is an endogenous modulator of $GABA_A$ receptors. In female mice, neurons in the dorsal motor nucleus of the vagus (DMV) show fluctuating $GABA_AR$ tonic inhibitory current associated with fluctuating ALLO levels during the estrous cycle. Since the DMV has efferent parasympathetic (PS) projections to peripheral organs important in glucose metabolism, this suggests a role for

ALLO in PS modulation of glucose metabolism. To test this, male and female CD-1 mice were subcutaneously implanted with a silastic capsule containing either vehicle (sesame oil+0.1% alcohol) or ALLO (0.1g/mL vehicle). Animal weight, blood glucose (BG) and food intake were monitored for 14 days post-operation. Metabolic testing was conducted between 5 and 10 days. Initial experiments were done in ovariectomized females (OVXF) to ablate endogenous ALLO fluctuations. Weight ($p=0.5726$), BG ($p=0.0736$) and food intake ($p=0.7134$) were not significantly affected by drug condition (ALLO or veh). Glucose tolerance testing (GTT, glucose 2g/kg) demonstrated that the OVXF given ALLO had significantly improved glucose handling at 15 minutes compared to vehicle (Sidak's; $p=0.0038$). Follow up experiments tested if peripheral PS signals were required for this response by pre-treatment with the muscarinic receptor antagonist, atropine methyl nitrate (AMN, 1mg/kg). AMN abolished the difference between ALLO and vehicle treated OVXF in GTT at the 15-minute point (Sidak's; $p=0.9983$). In an insulin tolerance test (insulin 0.5U/kg) ALLO did not affect glucose handling ($p=0.2808$). Pre-ITT AMN had no effect. To determine if the OVX was required for ALLO's effect on glucose metabolism, a cohort of intact females were run. In this group, again weight, blood glucose, and food intake were not different. Preliminary data suggests that ALLO-treated intact females maintain an improved glucose tolerance at 15 minutes after a bolus of glucose (Sidak's; $p=0.0572$). Interestingly, ALLO did not affect males in any metabolic parameter tested. Taken together, our results show that ALLO is a novel modulator of parasympathetically-mediated glucose metabolism with sex-specific effects on glucose sensitivity. Future studies will determine the target organ system(s) responsible for these responses and the role $GABA_A$ receptors in the DMV play in accomplishing these responses.

Disclosures: S. Fedorchak: None. **M. Lopez:** None. **C.R. Boychuk:** None.

Poster

149. Neuropeptide Regulation: Feeding and Metabolism

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 149.09/O25

Topic: F.10. Food Intake and Energy Balance

Title: Hunger, via the arcuate nucleus neuropeptide biglen, suppresses excitatory transmission in a nucleus accumbens circuit that opposes feeding behavior

Authors: *N. K. SMITH[1], J. PLOTKIN[1], B. A. GRUETER[2];
[2]Dept. of Anesthesiol., [1]Vanderbilt Univ., Nashville, TN

Abstract: In order to ensure survival, animals must enact dynamic behavioral patterns dependent on their most pertinent needs. When energy stores are low, hunger alters animal behavior through a number of central circuit nodes that control feeding behavior. The arcuate nucleus of the hypothalamus acts as a key energy status sensor, compiling energy state information via a

variety of sources. Arcuate nucleus neurons expressing Agouti-related peptide (AGRP) project across the brain and have been found to influence a growing number of behaviors via their canonical modulatory transmitters, AGRP and neuropeptide Y. However, despite the wide reach of these neurons, there is little work examining energy state dependent changes in basal forebrain structures. Here, we find that BigLEN, a recently identified arcuate nucleus AGRP neuron neuropeptide, is able to act within the nucleus accumbens to suppress excitatory transmission. Utilizing pharmacological tools, we link this action to the previously identified BigLEN receptor, GPR171. Antagonizing GPR171 in fasted animals results in a significant decrease in appetitive behaviors, including learned operant behaviors. These results support a role for a relatively novel arcuate nucleus peptide in the higher order decisions an animal makes to seek food. Additionally, they point to a key modulatory role of energy state within the forebrain in the control of motivated behaviors.

Disclosures: **N.K. Smith:** None. **J. Plotkin:** None. **B.A. Grueter:** None.

Poster

149. Neuropeptide Regulation: Feeding and Metabolism

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 149.10/O26

Topic: F.10. Food Intake and Energy Balance

Support: PHSDA024314

Title: Pituitary adenylate cyclase activating polypeptide excites proopiomelanocortin neurons in a sex- and diet-dependent manner: Implications for the regulation of energy homeostasis

Authors: *R. CHANG[1], J. HERNANDEZ[1], L. PEREZ[2], E. J. WAGNER[1];
[1]Col. of Osteo. Med. of the Pacific, [2]Grad. Col. of Biomed. Sci., Western Univ. of Hlth. Sci., Pomona, CA

Abstract: Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) is a neuropeptide expressed, among other places, in steroidogenic factor (SF) 1-containing neurons within the dorsomedial ventromedial nucleus (VMN) of the hypothalamus. SF-1 expressing neurons in the VMN of the hypothalamus have been shown to synapse directly with proopiomelanocortin (POMC) neurons in the arcuate nucleus (ARC). Thus, we tested hypothesis that PACAP excites POMC neurons via PAC1 receptor mediation and TRPC channel activation. Electrophysiological recordings were done in slices from both intact male and OVX (ovariectomized) female PACAP-Cre mice and eGFP-POMC mice. In recordings from POMC neurons in eGFP-POMC mice, PACAP induced a robust inward current and increase in conductance in voltage clamp, and a depolarization and increase in firing in current clamp. This effect was greater in estradiol-treated (100nM) slices from OVX females than those from males or vehicle-treated slices from OVX

females. These postsynaptic actions were abolished upon application of the PAC1 receptor antagonist PACAP6-38 (200nM) and TRPC channel blocker 2-APB (100μM). In optogenetic recordings from POMC neurons in PACAP-Cre mice, high-frequency photostimulation induced inward currents that were significantly enhanced by estradiol. When estradiol is coapplied with the estrogen receptor (ER) antagonist ICI 182,780 (1μM) or the ERα antagonist MPP dihydrochloride (3μM), the inward current was attenuated. Conversely, the G_q-coupled membrane ER ligand STX (10 nM) potentiated the inward current similar to that of estradiol. Importantly, the PACAP-induced excitation of POMC neurons was notably reduced in obese males exposed long-term to a high- fat diet (HFD). In vivo chemogenetic experiments in male PACAP-Cre mice demonstrated that cell type-specific stimulation with clozapine N oxide (CNO; 0.3 mg/kg; s.c.) produced a significant decrease in energy intake accompanied by an increase in O_2 consumption and metabolic heat production. These findings demonstrate that the PACAP-induced activation of PAC1 receptor and TRPC5 channels at VMN PACAP/ ARC POMC synapses is potentiated by estradiol in females, and attenuated under conditions of diet-induced obesity/insulin resistance. As such, they advance our understanding of how PACAP regulates the homeostatic energy balance circuitry under normal and pathophysiologic circumstances.

Disclosures: R. Chang: None. **J. Hernandez:** None. **L. Perez:** None. **E.J. Wagner:** None.

Poster

149. Neuropeptide Regulation: Feeding and Metabolism

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 149.11/O27

Topic: F.10. Food Intake and Energy Balance

Support: Merit Review 5I01 BX002465 by Department of Veterans Affairs

Title: Metabolic responses to BDNF antagonist ANA12 administered at different CNS sites in exercised animals

Authors: *C. WANG[1,3], V. MAVANJI[1], M. GRACE[1], C. KOTZ[1,4,2,3];
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Abstract: Background. Our preliminary studies found: 1) rats in running wheel exercise (RW) reduced food intake (FI) and weight gain (WG) vs. sedentary (Sed) rats; 2) mRNA for BDNF (brain-derived neurotrophic factor) in hypothalamus (HYP) was positively correlated with RW distance; 3) BDNF protein was significantly increased in paraventricular nucleus of HYP (PVN) of RW rats. We injected BDNF antagonist ANA12 (ANA) in different CNS sites to test if HYP BDNF contributes to exercise-induced reduction in FI and WG.
Methods. Three sets adult male SD rats were cannulated in PVN, lateral ventricle (LV) or third

ventricle (3V), and divided into four groups in each setting: Sed-vehicle (Sed-Veh), Sed-ANA (10 μg), RW-Veh, and RW-ANA. The rats were injected daily 2h before dark, and monitored for FI and WG.
Results. 1. PVN. RW-Veh rats reduced FI and WG vs. Sed-Veh, Sed-ANA increased FI and WG vs. Sed-Veh, but RW-ANA failed to antagonize RW (RW-Veh) reduced FI and WG. 2. LV. Both RW groups initially reduced FI vs. corresponding Sed rats, but at the end they ate little more vs. Sed rats. RW-ANA initially ate a little more than RW-Veh, and at the end both groups ate same amount. Both RW groups decreased WG vs. corresponding Sed rats, and both ANA12 groups had little more WG vs. corresponding Veh rats. In energy expenditure (EE), RW-Veh was at top in total EE and EE for activity and resting, followed by RW-ANA. 3. 3V. RW-Veh reduced FI and WG vs. Sed-Veh, RW-ANA increased FI and WG vs. RW-Veh, and Sed-ANA increased FI and WG vs. Sed-Veh.
Discussion. 1. An increase in FI and WG in Sed-ANA with PVN injection suggests blockade of PVN BDNF-TrkB activation in Sed rats. 2. No attenuation of exercise reduced FI and WG with PVN ANA12 (RW-ANA) suggests exercise may activate multiple sites which cannot be blocked at PVN alone. 3. The increased FI and WG in RW-ANA rats with 3V delivery suggests blockade in multiple sites, potentially around HYP. 4. Differing from findings in 3V ANA12, RW rats with LV ANA12 had a small increased FI and WG and little reduced EE (vs. RW-Veh), suggesting limited ANA12 diffusion in HYP. 5. The attenuation of exercise reduced FI and WG by antagonism of BDNF-TrkB via 3V delivery suggests hypothalamic BDNF contributes to the exercise induced appetite reduction.

Disclosures: C. Wang: None. **V. Mavanji:** None. **M. Grace:** None. **C. Kotz:** None.

Poster

149. Neuropeptide Regulation: Feeding and Metabolism

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 149.12/O28

Topic: F.10. Food Intake and Energy Balance

Support: CIHR Grant

Title: GHSR signaling in the DMH: Effects on energy balance under normal conditions and during chronic social defeat stress

Authors: *L. HYLAND[1], S. DESANTE[1], A. WISEMAN[1], S.-B. PARK[1], A. EDWARDS[1], Y. ABDELAZIZ[1], B. WOODSIDE[2], A. ABIZAID[1];
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Abstract: Ghrelin increases food intake and adiposity through its actions on the growth hormone secretagogue receptor (GHSR). The GHSR is highly expressed in the dorsomedial hypothalamic

nucleus (DMH), a region important for thermogenesis, food intake, circadian rhythms, and the generation of the stress response. Nevertheless, the effects of GHSR stimulation or blockade in this region remains to be determined. In experiment 1, we examined the effects of chronic ghrelin signalling stimulation or blockade in the DMH on metabolic parameters (i.e. food intake, body weight, energy expenditure and glucose clearance) in male C57BL/J6 mice using osmotic minipumps attached to unilateral cannulae aimed at the DMH and filled with saline, ghrelin, or a GHSR antagonist. Because ghrelin is elevated following chronic exposure to stress, we conducted a second experiment in which mice were exposed to chronic social defeat daily for a period of 10 days while receiving chronic intra DMH infusions of saline or a GHSR receptor antagonist and compared to non-stressed mice given the same intra-DMH treatments. We then compared metabolic outcomes as well as behavioral responses associated with chronic exposure to chronic social defeat including measures of social anxiety. Finally, in experiment 3, anaesthetized mice were given an acute intra-DMH infusion of either saline, ghrelin, or pre-treated with a GHSR antagonist followed by ghrelin, and were monitored for changes in core body temperature and blood glucose levels for 2 hours post-infusion. Our results show that chronic infusions of ghrelin into the DMH affects body weight and fat accumulation, while acute infusions affect blood glucose. Further, as expected, chronic stress increases food intake, but this increase was not attenuated by GHSR antagonist treatment.

Disclosures: L. Hyland: None. **S. DeSante:** None. **A. Wiseman:** None. **S. Park:** None. **A. Edwards:** None. **Y. Abdelaziz:** None. **B. Woodside:** None. **A. Abizaid:** None.

Poster

149. Neuropeptide Regulation: Feeding and Metabolism

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 149.13/O29

Topic: F.10. Food Intake and Energy Balance

Support: CIHR PJT-153173

Title: LPS suppresses melanin-concentrating hormone neurons via the COX2-PGE_2 pathway

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Abstract: Melanin-concentrating hormone (MCH) neurons are a lateral hypothalamic cell population critical in regulating energy homeostasis. In particular, these cells are known for their role in increasing food intake, decreasing energy expenditure, and promoting sleep. However, little is known about the role of MCH neurons during disease states when food intake and physical activity are commonly suppressed. To test whether the excitability of MCH neurons is altered during disease states, male Sprague Dawley rats were administered with bacterial

lipopolysaccharide (LPS, i.p.) to induce a well-characterized sickness behavior. We then performed *in vitro* whole-cell patch clamp on acute hypothalamic slices from these rats. We found that MCH neurons from LPS-treated rats were hyperpolarized and less excitable compared to those from untreated controls. As LPS is known to potently induce the expression of cyclooxygenase-2 (COX2), a prostanoid-synthesizing enzyme, in the hypothalamus, we wanted to determine whether COX2 was mediating this effect. Indeed, a COX2-specific inhibitor SC236 was sufficient to reverse the LPS-induced hyperpolarization. A similar inhibitory effect was seen by incubating naïve brain slices with LPS, which was abolished by co-incubation with SC236. Next, we sought to determine the downstream effector of COX2. A likely candidate is prostaglandin E2 (PGE2), an inflammatory prostanoid known to induce sickness. Interestingly, application of PGE2 in various concentrations to brain slices from naïve rats induced a bidirectional effect on the resting membrane potential. Specifically, lower concentrations (0.1-1 nM) induced a depolarization of MCH neurons, while higher concentrations (10-100 μM) induced a hyperpolarization. Using receptor subtype-specific antagonists, we found that the depolarizing and hyperpolarizing effects were mediated by the PGE2 EP2 receptor and EP3 receptor, respectively. Ionic mechanisms underlying these opposing effects on MCH neurons are currently under investigation. In conclusion, we determined that LPS causes a hyperpolarization of MCH neurons, which is due to endogenous PGE2 synthesis within the hypothalamus. This may contribute to anorexia resulting from high-grade inflammation, such as during infection. Additionally, the bidirectional response to low and high concentrations of PGE2 suggests that MCH neurons are dynamic and adaptive to conditions producing various levels of inflammation.

Disclosures: L. Fang: None. **M. Hirasawa:** None.

Poster

149. Neuropeptide Regulation: Feeding and Metabolism

Location: Hall A

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Program #/Poster #: 149.14/O30

Topic: F.10. Food Intake and Energy Balance

Support: NIDDK K08 DK118201
NCATS UL1 TR001102

Title: Selective deletion of the melanocortin-4-receptor in the prefrontal cortex alters feeding and executive function-like behavior

Authors: A. T. THOMPSON[1], A. KIM[2], Y. LI[1], E. R. DOUGLAS[1], S. SUBRAMANIAN[1], R. PATEL[1], K. RAMOS[1], V. BOLSHAKOV[1], B. B. LOWELL[2], K. J. RESSLER[1], *R. A. ROSS[1,2]; [1]McLean Hosp., Belmont, MA; [2]Endocrinol., Beth Israel Deaconess Med. Ctr., Boston, MA

Abstract: Background: Mutations of the melanocortin 4 receptor (MC4R) are strongly linked to obesity in humans, and globally removing MC4R in the mouse brain induces obesity. MC4R is strongly expressed in the hypothalamus, but deletion of MC4R within this region does not entirely account for the obesity induced by global knockout, which suggests MC4R in other regions influence this phenotype. Neurons of the arcuate hypothalamus produce the peptides that activate (α-MSH) and inhibit (AgRP) the MC4R. The MC4R is highly expressed in the medial prefrontal cortex (mPFC), which is implicated in human feeding behavior, obesity, and eating disorders. We hypothesized that manipulation of the MC4R in the mPFC ($mPFC^{MC4R}$) would affect feeding and executive function-like behavior.
Methods: We examined how pharmacologic manipulation of the MC4R affects neuronal dynamics in the mPFC using MC4R-2a-cre mice. For behavioral testing, we injected viral-mediated cre-recombinase into the IL-mPFC of male $MC4R^{lox/lox}$ mice to selectively delete $mPFC^{MC4R}$. Following this manipulation, we examined metabolic and behavioral changes including food intake, appetitive and aversive reversal learning, and novelty-suppressed feeding.
Results: MC4R agonism depolarized the membrane and increased action potential frequency of $mPFC^{MC4R}$ neurons. Selective deletion of $mPFC^{MC4R}$ increased food consumption and induced weight gain. This manipulation did not affect baseline exploratory behavior, but it increased the latency to feed in a novel context. Additionally, $mPFC^{MC4R}$ deletion impaired reversal learning by inducing perseverative behavior in a cognitive flexibility test without affecting initial learning.
Conclusions: Our data highlight a novel pathway from the arcuate nucleus of the hypothalamus to the medial prefrontal cortex that regulates food intake and other feeding behaviors. These findings contribute to our understanding of the mechanisms that govern feeding behavior, especially in the context of decision-making and cognitive rigidity, which are aberrant in individuals with eating disorders. We plan to investigate the downstream targets of $mPFC^{MC4R}$ cells to further integrate this pathway into the neural circuitry of feeding behavior as well as examine this circuitry in females.

Disclosures: A.T. Thompson: None. **A. Kim:** None. **Y. Li:** None. **E.R. Douglas:** None. **S. Subramanian:** None. **R. Patel:** None. **K. Ramos:** None. **V. Bolshakov:** None. **B.B. Lowell:** None. **K.J. Ressler:** None. **R.A. Ross:** None.

Poster

149. Neuropeptide Regulation: Feeding and Metabolism

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Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 149.15/O31

Topic: F.10. Food Intake and Energy Balance

Support: 5I01RX000441-04
IK2 BX003838-01A1

5R01DK100281-03
T32DK083250

Title: Effect of pharmacosynthetic activation of orexin on spontaneous physical activity and memory in aged mice

Authors: *P. E. BUNNEY[1], V. MAVANJI[2], P. J. ERICKSON[2], M. K. GRACE[2], C. M. KOTZ[3]; [1]Geriatric Res. Educ. and Clin. Ctr., [2]Minneapolis VA Hlth. Care Syst., Minneapolis, MN; [3]Integrative Biol. and Physiol., Univ. of Minnesota Twin Cities, Minneapolis, MN

Abstract: Background: Orexin synthesis decreases throughout the lifespan and is associated with reduced physical activity and impaired memory. We hypothesized that orexin neuron activation via Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) would increase spontaneous physical activity (SPA) and improve memory in aged mice. Methods: Mice were injected bilaterally with a Cre-dependent AAV vector containing an excitatory DREADD into the caudal lateral hypothalamus (orexin neuronal field). Following acclimation to metabolic behavior chambers for one week (Sable Promethion Caging System) and injections of saline across 4 days, mice were injected with 1, 3, or 5mg/kg clozapine n-oxide (CNO) or saline every other day. Changes in time spent moving were assessed within 6 h post-injection. After one week back in their home cages, animals were trained in object in context recognition memory measurement. Habituation to two contexts occurred on the first day. On sample phase 1, mice were placed in context 1 and were allowed to explore two different objects. Sample phase 2 was conducted 24 h later, and mice were placed in context 2 together with two identical objects (copies of one of the previously presented objects) and could explore the objects. Immediately after the end of sample phase 2, animals were injected with CNO (i.p, 5mg/kg). The memory test was performed 24 h later. Mice were reintroduced to context 2 and allowed to explore freely, with the presence of one copy of the previously presented object in context 2 together with a copy of one of the objects previously presented in context 1 but not presented in context 2. Results: At 9 months, all three doses of CNO increased SPA across 2, 4, and 6 hours post-injection in males ($p<0.01$). Nine-mo females showed increases in SPA at 3mg/kg at 6 hours and 5mg/kg at 4 and 6 hours ($p<0.01$). Both males and females showed enhanced exploration of the novel object in context relative to mice at 9mo ($p<0.01$). In 12mo females, SPA was increased with 1mg/kg CNO at 6 hours ($p<0.05$), 3mg/kg at 4 ($p < 0.05$) and 6 hours ($p<0.01$), and 5mg/kg at all timepoints ($p<0.01$). In contrast, in male mice that were 12mo, only 3 and 5mg/kg CNO increased SPA relative to saline ($p<0.01$). Neither males nor females showed increases in SPA at any dose and any timepoint at age 15mo. There were no significant effects of CNO administration on object in context memory in 12 and 15mo mice. These results suggest that as animals age, changes in the number of orexin neurons and/or activation of these neurons is such that their ability to be activated in order to increase SPA or enhance memory, is reduced.

Disclosures: **P.E. Bunney:** None. **V. Mavanji:** None. **P.J. Erickson:** None. **M.K. Grace:** None. **C.M. Kotz:** None.

Poster

149. Neuropeptide Regulation: Feeding and Metabolism

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 149.16/O32

Topic: F.10. Food Intake and Energy Balance

Support: 4295/2017CI UAEM

Title: Changes in serum concentrations of orexigenic and anorexigenic peptides related to sweetener consumption in overweight people

Authors: I. CONTRERAS, J. DOTOR, M. SANCHEZ, S. LOPEZ, *J. A. ESTRADA;
Lab. of Neurochemistry, Univ. Autonoma Del Estado De Mexico, Toluca, Mexico

Abstract: Obesity is public health issue around the word. In order to reduce its incidence, the use of non-nutritive sweeteners has been widely implemented; however, there is still much debate on whether or not these products help to reduce obesity. Our previous studies have demonstrated that non-nutritive sweeteners promote alterations in signaling pathways related to appetite in the CNS of mice, with sucralose and steviol glycosides showing contrary effects that are reflected in changes in feeding behavior in experimental animals. The objective of the present study was to evaluate the effect of two highly consumed non-nutritive sweeteners on the secretion of orexigenic and anorexigenic peptides in a small population of otherwise healthy, overweight people. 26 people with BMI >25.0Kg/m^2 were selected and randomly assigned into two experimental groups: sucralose and steviol glycosides. Before sweetener supplementation, participants underwent a one-week washout period in order to eliminate food and beverages with added sweeteners. 10 mL samples of venous blood were obtained along with anthropometric parameters. Participants were then supplemented with 4g of commercially available sucralose or steviol glycosides formulations per day for 6weeks. Participants maintained weekly food diaries to insure protocol compliance. After treatment, blood samples and anthropometric parameters were obtained again. Results show that the sucralose group increased weight and BMI more than the steviol glycosides group, apparently due to higher total calorie intake. Regarding oregixenic peptides, we observed that leptin concentrations were reduced in both groups; however, this reduction was enhanced in the sucralose group. GLP-2 presented a significant decrease in the steviol glycosides group while NPY presented no differences between groups. Altogether, our data suggests that short-term, frequent intake of non-nutritive sweeteners may alter the production of signals that control the hunger-satiety system in humans.

Disclosures: I. Contreras: None. **J. Dotor:** None. **M. Sanchez:** None. **S. Lopez:** None. **J.A. Estrada:** None.

Poster

149. Neuropeptide Regulation: Feeding and Metabolism

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Topic: F.10. Food Intake and Energy Balance

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NARSAD
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Commonwealth Government (Australia) Endeavour Research Fellowship

Title: Effects of silencing relaxin-3 production in nucleus incertus neurons on food intake, body weight, anxiety-like behaviour and limbic brain activity in female rats

Authors: *C. DE ÁVILA DAL'BO[1], S. CHOMETTON[3], S. MA[4], L. TORZ PEDERSEN[5], E. TIMOFEEVA[2], C. CIFANI[6], A. L. GUNDLACH[7];
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Abstract: The neuropeptide, relaxin-3 (RLN3) is part of the insulin-relaxin superfamily, is primarily expressed by neurons in the brainstem nucleus incertus (NI) and is implicated in motivated behaviours and stress responses. The present study was designed to investigate the effects of RLN3 mRNA knockdown in NI RLN3-positive neurons of female rats on body weight, food intake and anxiety-like behaviour. Female rats received a bilateral infusion into the NI of adeno-associated virus encoding microRNA against RLN3 (knockdown) or control. Three weeks after viral injections, food intake and body weight were measured every 24 h. At week 7, rats were subjected to the behavioural tests light/dark box (L/D), elevated plus maze (EPM) and large open-field (LOF) test followed. During week 8, rats were sacrificed, and intra-cardiac blood samples and brains were collected. The targeting and specificity of RLN3 knockdown in NI neurons was assessed using *in situ* hybridization. The *c-fos* mRNA expression was analysed in regions involved in stress and feeding. RLN3 mRNA expression in the NI of knockdown rats was significantly suppressed compared to control. Two-way ANOVA revealed significant difference on body weight. Bonferroni's multiple comparison tests revealed a decrease in body weight of RLN3 knockdown rats from week 2 which persisted until week 7. Food intake was decreased in knockdown group compared to control during week 4. In addition, RLN3 knockdown rats displayed significantly higher anxiety-like behaviour in the LOF, but not in the

EPM and L/D compared with virus control rats. After behavioural tests, corticosterone levels were not different between groups for all tests, however basal levels of corticosterone were significantly decreased in knockdown group compared to control. Moreover, *c-fos* mRNA expression significantly decreased in the paraventricular nucleus of the hypothalamus, bed nucleus of the stria terminalis, and in the lateral hypothalamus area in knockdown rats compared to control, at the basal level. Reduced RLN3 expression in NI neurons induced weight loss associated with an imbalance on food intake, elevated anxiety in environment with no escape, and disrupted the hypothalamic-pituitary adrenal axis in female rats.

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Poster

149. Neuropeptide Regulation: Feeding and Metabolism

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Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 149.18/O34

Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant GM109817
NIH Grant GM127251

Title: Distributions of immunoreactivities for hypocretin/orexin and neuronal nitric oxide synthase in the male rat hypothalamus: An analysis and representation in an atlas reference space

Authors: *V. I. NAVARRO, E. PERU, K. NEGISHI, M. ORTEGA, H. G. VIZCARRA, A. M. KHAN;
Biol. Sci., Univ. of Texas At El Paso, El Paso, TX

Abstract: Hypocretin/orexin (H/O)-expressing neurons of the lateral hypothalamic area (LHA) have been characterized as important mediators in food seeking behaviors, and their networks have been extensively characterized. Similarly, neuronal nitric oxide synthase (nNOS)-expressing neurons have been suggested to play with a role in feeding behavior and have also been described as being present within the LHA. However, studies have not detailed interactions between these neuronal populations, nor have they examined the greater extent of hypothalamic H/O-nNOS interactions within a systemized spatial framework indexed to a reference atlas. To address this issue, we employed multiple immunohistochemical approaches to generate detailed anatomical maps and cytoarchitectural analyses of H/O- and nNOS-expressing populations in hypothalamic regions. Dual-labeling high-sensitivity staining techniques, using diaminobenzidine (DAB) and nickel-enhanced DAB, enabled the visualization of H/O-immunoreactive (ir) neurons and dense networks of nNOS-ir axonal fibers. To further examine possible interactions, bright field imaging at x100 magnification was used in areas with higher

density of H/O-ir fibers and nNOS-ir cell bodies. An accompanying Nissl stain was performed in an adjacent tissue series to assign cytoarchitectonic boundaries, as delineated in *Brain Maps 4.0* (L. W. Swanson, 2018; *J Comp Neurol*). Consistent with previous studies, our results demonstrated dense labeling of nNOS-ir somata as well as H/O-ir fibers within the hypothalamic paraventricular nucleus. These fibers formed putative appositions with nNOS-ir neurons, suggesting possible interactions. Within the lateral hypothalamic area (LHA), extensive interactions between H/O-ir fibers and nNOS-ir cell bodies were observed, including in the LHAjp, LHAjd, LHAs and LHAd (see Swanson, 2018 for an explanation of these abbreviations). Though sparsely populated, zones of putative interactions between H/O-ir fibers and nNOS-ir cell bodies were also observed in the ventrolateral part of the ventromedial hypothalamic nucleus, where a distinct rostrocaudal gradient in density was observed. Collectively, these data indicate a potential interaction between two markers previously associated with feeding function and hint at an underlying shared system that can be investigated in the future. By contextualizing these data using a referenced systematic approach, we have identified distinct zones of putative interactions, and provide a high-resolution spatial framework for future functional assays such as single cell recordings and optogenetics.

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Poster

149. Neuropeptide Regulation: Feeding and Metabolism

Location: Hall A

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Topic: F.10. Food Intake and Energy Balance

Support: HHMI PERSIST Grant awarded to AMK
NIH Grant GM127251 awarded to AMK

Title: Generation of synthetic rat brain atlas plates at the level of the hypothalamus using multipoint warping

Authors: *J. G. PEREZ[1], O. FUENTES[1], A. M. KHAN[2];
[1]Computer Sci., [2]Biol. Sci., The Univ. of Texas at El Paso, El Paso, TX

Abstract: Neuroscientists that perform experiments using rat brains typically use canonical rat brain atlases to identify and localize neural system elements as well as identify potential connections in neuronal cell populations. These experiments involve cutting rat brain tissue into sections which are then stained and observed under a microscope. When attempting to map such experimental data to the canonical brain atlases, a challenge occurs when the experimental tissue falls in the gaps between atlas plates or contains regions from multiple atlas plates depending on

the plane of section at which the tissue was cut. Here, we present a method for generating synthetic images for those brain areas not represented on canonical atlas plates, focusing on those areas that contain the hypothalamus, a critical brain region important for normal organismal function that is a research focus of our laboratory. The method involves first taking a pair of Nissl-stained tissue section images from an atlas, resizing them to the same dimensions, and manually creating a set of correspondence points or control points which will be used for multipoint warping. Then, using this set of correspondence points, we generate intermediate plates between the input pair by using image morphing; more specifically, by distorting the images through inverse warping with the control points and then applying a cross-dissolve operation to combine the warped images. We demonstrate the effectiveness of this algorithm by using the Swanson atlas (*Brain Maps, 3rd/4th editions*; 2004; 2018), the Paxinos & Watson atlas (*The Rat Brain in Stereotaxic Coordinates, 7th edition*, 2014) and experimental tissue samples containing the hypothalamus to generate synthetic tissue plates. To evaluate our method, three images (named S1, S2, and S3) are taken in sequence from each of the three datasets and the algorithm is applied to S1 and S3 to generate a synthetic intermediate image. This synthetic image is compared visually to S2, a real intermediate image, by a mapping expert. The experimental results show that this method is accurate. Moreover, it provides the means to generate a dataset that can be used for our ongoing project to train a neural network pipeline to discern brain cytoarchitecture in three dimensions.

Disclosures: J.G. Perez: None. **O. Fuentes:** None. **A.M. Khan:** None.

Poster

149. Neuropeptide Regulation: Feeding and Metabolism

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Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 149.20/O36

Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant GM127251

Title: Using high-spatial resolution atlas-based mapping to examine the spatial relationships of hypocretin/orexin- and tyrosine hydroxylase-immunoreactive axonal fibers in the rat paraventricular thalamic nucleus

Authors: *M. A. PEVETO, A. F. SILVEYRA, B. E. PINALES, A. M. KHAN;
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Abstract: The paraventricular nucleus of the thalamus (PVT) is believed to serve as a relay center for mesolimbic circuitry components that govern a range of motivated behaviors including food and drug seeking. These behaviors can be influenced by different neurotransmitter and neuropeptide systems. Neuropeptide markers that have been shown to participate in regulating

these same functions include hypocretin/orexin (H/O), which is associated with arousal and feeding behaviors, and catecholamines, which could influence motivated behaviors (and which are identifiable by the presence of tyrosine hydroxylase (TH) in neural elements). Recent anatomical studies have demonstrated the presence of a dense population of H/O-immunoreactive (ir) fibers in the PVT as well as a subpopulation of TH-ir fibers; however, relatively scant literature exists that reports on the direct spatial relationships between H/O-ir and TH-ir projections in the PVT. Furthermore, few anatomical studies have provided data in a representation that offers high spatial-resolution throughout the extent of this brain structure using a standardized series of atlas templates. Here, we evaluated the distributions of H/O-ir axonal fibers in the rat PVT, as part of an ongoing effort to study the spatial relationships of H/O- and TH-ir axonal fibers in this gray matter region. Coronal rat brain sections (30 μm-thick) were collected and immunohistochemically stained for hypocretin 1 or 2/orexin A or B and TH. Nissl staining was performed on an adjacent series of tissue to aid in cytoarchitectural analysis. The plane of section was carefully determined for each tissue section using formal atlas mapping techniques and the locations of fluorescent markers were mapped onto a standardized rat brain atlas (L. W. Swanson, *Brain Maps 4.0*, *J Comp Neurol*, 2018). We found dense numbers of H/O-ir axonal fibers within the PVT, which were not confined to any specific portion of the nucleus but present in robust amounts throughout much of its extent. Rostrocaudally across distinct atlas levels, this expression appeared to be fairly uniform. TH-ir fibers were also observed to a lesser extent. These results provide an initial framework for further examination of fiber interactions between the two systems and for mapping of their distributions to standardized rat brain atlas templates.

Disclosures: **M.A. Peveto:** None. **A.F. Silveyra:** None. **B.E. Pinales:** None. **A.M. Khan:** None.

Poster

149. Neuropeptide Regulation: Feeding and Metabolism

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 149.21/O37

Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant GM109817 awarded to AMK
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NSERC Discovery Grant #06272 awarded to MJC
NSF DUE-1565063 (ACSScellence Program at UTEP)
HHMI PERSIST Education Grant awarded to AMK (Co-PI)

Title: Viral tracing of axonal projections from tyrosine hydroxylase-expressing neurons of the zona incerta in male mice

Authors: *E. MEJIA[1], M. S. PONCE[1], K. NEGISHI[1], A. HEBERT[2], M. J. CHEE[2], A. M. KHAN[1];
[1]Biol. Sci., Univ. of Texas at El Paso, El Paso, TX; [2]Neurosci., Carleton Univ., Ottawa, ON, Canada

Abstract: Neural circuits that signal one another through dopamine (DA) neurotransmission support a broad range of functions in organisms, including cognition, movement, and communicating the reward qualities of food or drugs of abuse. DA neurons in the mouse diencephalon are distributed across several adjacent gray matter regions, including the zona incerta (ZI), the dorsal part of the lateral hypothalamic area (LHA), and the dorsomedial (DMH), periventricular, and arcuate hypothalamic nuclei. This widespread distribution makes it challenging to delineate precise structural and functional characteristics specific to individual neuronal populations, a problem that is compounded by the presence of diverse neurotransmitter- and/or neuropeptide-encoding neuronal populations expressed alongside or within DA neurons in any given brain structure. The ZI is composed of heterogeneous, spatially overlapping neuronal populations that synthesize DA, GABA, and neuropeptides such as melanin concentrating hormone. Our recent work characterized the neurochemical identities of DA and GABAergic ZI neurons, but their efferent connections are unclear. Identifying these connections will direct us to their target sites that help carry out the functions of these neurons. Here, we used a gene-directed approach to selectively target DA-producing neurons in the ZI by delivering an AAV construct encoding mCherry, a red fluorescent reporter molecule, to a mouse expressing Cre recombinase in tyrosine hydroxylase-expressing neurons. dsRed-immunoreactive (ir) axonal fiber and cell distributions, which mark mCherry expression, were examined in these subjects using single-label immunoperoxidase histochemistry and mapped to the Allen Reference Atlas. In the striatum and pallidum, we observed dsRed-ir axons in the lateral septal nucleus and bed nuclei of the stria terminalis, respectively. The thalamus showed dsRed-ir axons in the nucleus of reuniens, and in the parataenial, paraventricular, central lateral, and parafascicular nuclei. In the hypothalamus, there were dsRed-ir axons throughout the median eminence, medial preoptic area, anterior hypothalamic area, LHA, tuberal nucleus, DMH, and posterior hypothalamic nucleus. Within the midbrain, dsRed-ir axons were found in the superior and inferior colliculi, midbrain reticular nucleus, nucleus of Darkschewitsch, and periaqueductal gray, which displayed the densest expression of dsRed-ir axons out of all regions examined. The maps produced from this effort can be used to guide functional studies of these neurons and to evaluate their role(s) as potential targets for experimental or therapeutic intervention.

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Poster

149. Neuropeptide Regulation: Feeding and Metabolism

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 149.22/O38

Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant GM127251 awarded to AMK
UTEP Doctoral Excellence Fellowship awarded to AA

Title: Computer vision-based tools to segment gray and white matter regions in experimental tissue sections and to analyze tracer injection sites mapped in digital atlas space: Use cases for the hypothalamus and ventral tegmental area for circuits related to feeding control

Authors: *A. ARNAL[1], O. FUENTES[2], A. M. KHAN[3];
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Abstract: We have scripted two computer vision-based programs that complete two tasks: (1) process and analyze raw images of experimental tissue sections stained for Nissl substance and segment gray and white matter regions from them; and (2) calculate the percent overlap of a mapped injection deposit area in relation to underlying brain regions within a standardized rat brain atlas (LW Swanson, 2018; *Brain Maps 4.0*, *J Comp Neurol*). First, we show that automating Task 1 can be achieved with Fully Convolutional Networks (FCN), a family of deep learning models trained end-to-end for pixel-wise classification of images. The proposed FCN was trained on a dataset augmented from nine Nissl-stained coronal images of a rat and parcellated using the Swanson atlas as a guide. For simplicity, the model is trained to segment the fornix, a distinct structure found in all levels of the available parcellations. We experiment with different resolutions, number of augmented samples, augmentation methods and network architectures. The Task 1 script can classify pixels based on texture with higher density classification ascribed to regions in and around the fornix. For Task 2, we illustrate the significance of parcellation or semantic segmentation with a quantitative analysis of brain regions and overlapping artifacts. Often, rigorous benchmark standards to document ground truth - such as atlas-based mapping of a tracer injection site in relation to the underlying cytoarchitecture - do not take into account the overlap of tracer injection deposits with the boundaries of the underlying brain regions. As a result, the neural connections defined by the deposit are usually inferred as being traced from a single assigned brain region to the exclusion of other regions within the tracer deposit footprint. Task 2 workflow involves the creation of two annotated images, one representing a given level or portion thereof of the Swanson atlas, and the other representing any given artifact superimposed on the atlas map. The tool allows areal calculations to be made of the fraction of deposit within a target site and the fraction that spills

over to other sites. We demonstrate the feasibility of our script using injection site deposits mapped for tracer deposits delivered into the ventral tegmental area. Collectively, these tools provide bench scientists interested in delineating cytoarchitecture from their experimental tissue sets - and to map their tracer deposits over such cytoarchitecture onto atlas maps - better ways to rigorously process and analyze their data.

Disclosures: A. Arnal: None. **O. Fuentes:** None. **A.M. Khan:** None.

Poster

149. Neuropeptide Regulation: Feeding and Metabolism

Location: Hall A

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Program #/Poster #: 149.23/O39

Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant GM109817
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HHMI PERSIST Education Grant

Title: Distributions of axons immunoreactive for α-melanocyte stimulating hormone and neurons immunoreactive for neuronal nitric oxide synthase in the hypothalamus of the adult male rat: An analysis of interactions and their representation in an atlas reference space

Authors: *E. PERU, V. I. NAVARRO, K. NEGISHI, R. GUZMAN, A. M. KHAN;
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Abstract: Nitric oxide (NO) in the brain has been demonstrated to exert an influence on hypothalamic functions including the control of food intake and in mechanisms underlying glucosensing for the induction of the counterregulatory response; however, the underlying mechanisms of these events remain unknown. To begin exploring the relationship among feeding and metabolic function and NO regulation, we examined the localization, distribution, and putative interactions of neuronal nitric oxide synthase (nNOS)-expressing neurons in relation to that of α-melanocyte stimulating hormone (αMSH)-expressing neurons which are known to be involved in energy balance and feeding control. Using fluorescence immunohistochemistry and confocal microscopy, we did not observe any colocalization of nNOS and αMSH in neuronal somata. This spatial separation allowed for the use of a combined diaminobenzidine/nickel-enhanced diaminobenzidine reaction to further distinguish the two populations. An adjacent tissue series was Nissl-stained for delineation of cytoarchitectonic boundaries and assignment of atlas plate levels in accordance with *Brain Maps: Structure of the Rat Brain, 4th Edition* (L. W. Swanson, 2018, *J Comp Neurol*). αMSH-immunoreactive (ir) axonal fibers were observed in regions which overlap with nNOS-ir perikarya. Under ×100 magnification, bright field analysis showed putative appositions between αMSH-ir fibers and nNOS-ir cell bodies in several lateral

hypothalamic area (LHA) subregions, including the LHAjd, LHAs, LHAjvv, LHAjvd, LHAjp and, to a lesser extent, the LHAa (see Swanson, 2018, for an explanation of abbreviations). Many putative axosomatic appositions were also observed throughout the extent of the paraventricular and ventromedial hypothalamic nuclei (PVH and VMHvl). The greatest aggregate abundance of αMSH-ir fibers and nNOS-ir somata in the same region was observed in the dorsomedial hypothalamic nucleus, ventral part (DMHv), wherein we also observed the dominant concentration of putative appositions. These findings capture, at high-spatial resolution, distinct hypothalamic regions of interaction between axonal fibers carrying αMSH signal and nNOS-expressing neurons. These findings should help to identify target regions for future studies of their functional interactions.

Disclosures: E. Peru: None. **V.I. Navarro:** None. **K. Negishi:** None. **R. Guzman:** None. **A.M. Khan:** None.

Poster

149. Neuropeptide Regulation: Feeding and Metabolism

Location: Hall A

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Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant GM109817
NIH Grant GM127251
HHMI PERSIST Grant

Title: The contribution of axonal projections from infralimbic area neuronal populations to the medial forebrain bundle: Analysis of morphology and interactions with hypocretin/orexin neurons in the rat

Authors: *K. NEGISHI, A. M. KHAN;
Biol. Sci., Univ. of Texas at El Paso, El Paso, TX

Abstract: Early anatomical studies have identified the medial forebrain bundle (mfb) as the most complex fiber system in mammals. Since then, relatively little work has focused on the fine structural details of the mfb, or the specific axonal trajectories of its fiber components. Fundamental knowledge of such details could help to refine the targeting of probes to test physiological function and to guide experimental design (e.g., when taking into account that optical stimulation could produce antidromic spiking). Our recent work has focused on connections between the medial prefrontal cortex and the diencephalon. The infralimbic area (ILA) appeared to be the primary source of medial prefrontal cortical projections to the mfb. Here, we visualized ILA axons using the *Phaseolus vulgaris* leucoagglutinin anterograde tracing method, together with cytoarchitecture analysis, to examine the fine morphology and spatial

features of mfb collaterals. ILA axons are already part of the mfb when they enter the rostral hypothalamus. Collaterals were not observed as the axons passed through the lateral preoptic area and the anterior region of the lateral hypothalamic area (LHA). Branching was first observed at the earliest appearance of the dorsal region (LHAd) of the LHA. From there, a group of axons formed dense terminals in the LHAd and suprafornical region, with axons continuing towards the midline thalamus. More caudally, ILA axon collaterals formed dense terminals in the posterior region of the LHA as they coursed dorsomedially towards the periaqueductal gray. Given the notable spatial overlap of ILA terminals and the known hypocretin/orexin (H/O) neuronal distributions, we next sought to explore the possibility of interactions. Double-labeled sections were prepared to identify putative ILA -> H/O neuronal appositions under x100 magnification. This approach identified potential interactions throughout the entire H/O field, markedly more than what has been reported in other studies. Our results indicate that the probability of interactions between ILA-originating projections and H/O-expressing neurons may be higher than that reported in the literature. More generally, our data underscore the need for more detailed studies of mfb components and their interactions with diverse neuronal populations.

Disclosures: **K. Negishi:** None. **A.M. Khan:** None.

Poster

150. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 150.01/O41

Topic: G.01. Appetitive and Aversive Learning

Support: NSERC Discovery Grant (MDI)
FRQNT Nouveaux Chercheurs (MDI)
Canada Research Chair program (MDI)

Title: A role for dopamine in aversive prediction error

Authors: V. OPARA[1], A. MAHMUD[2], ***B. P. LAY**[1], M.-P. COSSETTE[2], M. D. IORDANOVA[2];
[2]Psychology, [1]Concordia Univ., Montreal, QC, Canada

Abstract: Learning depends on prediction error, which is the mismatch between real and expected outcomes. This is best illustrated in the blocking paradigm in which learning about the association between a novel cue and an outcome is impaired in the presence of a good predictor for that outcome. This occurs because in the presence of the good predictor the outcome is expected and therefore the prediction error is minimal. Dopamine (DA) neuronal firing in the Ventral Tegmental Area (VTA) mimics prediction error in reward. Here, we sought to determine

whether VTA DA has any role in aversive prediction error. To address this, we employed an aversive blocking paradigm along with bidirectional optogenetic manipulations of VTA DA neurons. Optogenetically activating dopamine neurons in the VTA at the time of an expected shock in a blocking design further attenuated learning about the novel cue and shock. Conversely, optogenetically inhibiting VTA dopamine neurons at the same time point led to unblocking, that is robust learning about the association between the novel cue and the outcome. These results were taken as evidence for the role of VTA DA in aversive prediction-error such that activation and inhibition of VTA DA neurons reduces and increases, respectively, aversive prediction error. Additionally, we showed that this aversive prediction error signal is regulated by the VTA-nucleus accumbens (NAC) pathway. Stimulation of VTA DA terminals in the NAC at time of the expected shock in blocking augmented the blocking effect, much like VTA DA cell body stimulation. Our findings reveal that the VTA DA neurons and their targets (NAC) regulate aversive prediction-error. When considered collectively with similar research in the field of reward, our research points to a bidirectional valence-based prediction-error mechanism.

Disclosures: V. Opara: None. **A. Mahmud:** None. **B.P. Lay:** None. **M. Cossette:** None. **M.D. Iordanova:** None.

Poster

150. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 150.02/O42

Topic: G.01. Appetitive and Aversive Learning

Support: NIH/NIDA intramural research program (GS)
NSERC Discovery grant (MDI)
Canada Research Chair program (MDI)
NSERC Undergraduate Research Award (EM)

Title: Causal evidence supporting the proposal that dopamine transients function as a temporal difference prediction error

Authors: E. MAES[1], M. SHARPE[2], M. P. GARDNER[3], C. CHANG[4], G. SCHOENBAUM[5], ***M. D. IORDANOVA**[1];
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Abstract: Correlational and causal studies have shown that reward-evoked dopamine (DA) signals in the ventral tegmental area (VTA) function as reward prediction errors (RPE). A critical component to this signal, according to temporal-difference (TD) learning, is the backpropagation

of the error signal to the earliest possible predictor. That is, the DA response migrates back to the cue that predicts reward. It remains unknown, however, whether this cue-evoked DA response carries information regarding outcome expectation (prediction) or is itself a prediction-error. Here we sought to address this question using two classical behavioural paradigms, blocking and second-order conditioning. Our data show that optogenetic attenuation of dopamine signaling in the VTA at the start of a reward-predicting cue disrupts the acquisition of second-order conditioning without affecting blocking. That is, attenuating cue-evoked DA neuron activity prevented that cue from supporting further learning (second-order conditioning). While this result could be explained irrespective of whether we disrupted reward prediction or a reward prediction error signal, our blocking data are only consistent with the latter hypothesis. Identical optogenetic-shunting of DA neuron activity at time of the reward-predicting cue during the second phase of blocking did not disrupt the blocking effect, suggesting that the cue-evoked DA signal does not carry information about reward prediction. These results provide causal evidence that cue-evoked dopamine signals function as temporal-difference prediction errors.

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Poster

150. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

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Topic: G.08. Drugs of Abuse and Addiction

Support: Smith Family Award Program for Excellence in Biomedical Research
Israel Binational Science Foundation Start Up Grant 2015005
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Rhode Island Foundation Medical Research Fund 20144133
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NIDCD (5R01MH105368)

Title: Circuits that encode and predict alcohol associated preference

Authors: *K. M. SCAPLEN[1], M. TALAY[1], S. SALAMON[4], K. NUNEZ[2], A. G. WATERMAN[1], S. GANG[3], S. L. SONG[1], G. BARNEA[1], K. R. KAUN[1];
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[4]Pharmacol., Univ. of Cologne, Cologne, Germany

Abstract: Substance use disorders are chronic relapsing disorders often impelled by enduring memories and persistent cravings. Alcohol, as well as other addictive substances, remolds neural circuits important for memory to establish obstinate preference despite aversive consequences. How pertinent circuits are selected and shaped to result in these unchanging, inflexible memories is unclear. Using neurogenetic tools available in *Drosophila melanogaster* we define how circuits required for alcohol associated preference shift from population level dopaminergic activation to select dopamine neurons that predict behavioral choice. During memory expression, these dopamine neurons directly, and indirectly via the mushroom body (MB), modulate the activity of interconnected glutamatergic and cholinergic output neurons. Transsynaptic tracing of these output neurons revealed at least two regions of convergence: 1) a center of memory consolidation within the MB implicated in arousal, and 2) a structure outside the MB implicated in integration of naïve and learned responses. These findings provide a circuit framework through which dopamine neuron activation shifts from reward delivery to cue onset, and provides insight into the inflexible, maladaptive nature of alcohol associated memories.

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Poster

150. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 150.04/O44

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant R01DC015426

Title: Midbrain fMRI ensemble patterns encode the identity of sensory prediction errors in humans

Authors: *J. D. HOWARD[1], T. A. STALNAKER[2], G. SCHOENBAUM[3], T. KAHNT[1]; [1]Northwestern Univ., Chicago, IL; [2]Natl. Inst. on Drug Abuse, Intramural Res. Program, Baltimore, MD; [3]Natl. Inst. on Drug Abuse Intramural Res. Program, Baltimore, MD

Abstract: Prediction error signals encoded in the dopaminergic midbrain represent a fundamental mechanism by which reward predictions are updated during learning. Recent studies in rats and humans have demonstrated that, in addition to signaling violations in value expectations, these responses also signal violations in the sensory properties of expected outcomes. However, whether sensory prediction errors in the midbrain carry information about the identity of the sensory error itself, or whether they simply represent an unspecific error signal, remains unclear. To answer this question, we analyzed functional magnetic resonance

imaging (fMRI) data from a transreinforcer reversal learning task in which hungry human participants (N=23) intermittently experienced violations in value-matched food odor identity. These violations consisted of either experiencing a sweet odor while expecting a savory (SW→SV), or vice versa (SV→SW). There was no difference in the magnitude of average midbrain activity evoked by these two sensory prediction errors (t_{22} = 0.86, p = 0.40). However, using cross-validated linear support vector machines, we could decode the specific type of error (SW→SV vs. SV→SW) from multivoxel activity patterns in the midbrain (mean decoding accuracy = 60.1 ± 3.9% s.e.m., t_{22} = 2.61, p = 0.016, tested against 50% chance). Importantly, decoding of reward identity was possible on error trials, but not later in the block when a given odor identity was delivered as expected (t_{22} = 0.17, p = 0.87). This demonstrates that sensory prediction errors in the midbrain contain specific information about the type of error. A further implication is that this error signal itself may be sufficient to convey specificity to model-based representations in downstream areas such as the striatum and orbitofrontal cortex.

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Poster

150. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 150.05/P1

Topic: G.01. Appetitive and Aversive Learning

Title: Dopamine neuron ensembles encode the identity of sensory prediction errors in rats

Authors: *T. A. STALNAKER[1], Y. K. TAKAHASHI[1], J. D. HOWARD[2], S. J. GERSHMAN[3], T. KAHNT[2], G. SCHOENBAUM[1,4,5];
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Abstract: The firing of ventral tegmental area (VTA) dopamine neurons is widely believed to signal cached-value prediction errors. However, we have recently reported that classic error-signaling VTA dopamine neurons also transiently increase firing when the flavor of a liquid reward changes unexpectedly, even when its value remains unchanged. These results and similar findings in humans suggest that VTA dopamine neurons might signal errors in predicting information other than value. Yet this proposal is potentially problematic, because the signal does not seem to distinguish different sensory errors, either at the level of individual neurons or summed activity across populations. How then do downstream areas know what information is being signaled? Here we sought to test one possibility -- that such information is a property of

the pattern of firing across an ensemble of dopamine neurons. We trained rats on a variant of the odor-guided choice task used to demonstrate joint signaling of value and sensory prediction errors in our prior report. The delivery of one of two odors instructed left or right forced-choices between two fluid wells that delivered either one or three drops of discriminable but equally-preferred solutions of grape or tropical punch Kool Aid. To induce prediction errors, reward number and flavor were manipulated across a series of four transitions between trial blocks in each session. Transitions included reward omissions and unexpected deliveries in addition to two flavor transitions across which the number of drops remained constant. In one flavor transition, only one drop of the three changed flavor, leaving unchanged drops to provide a control condition to distinguish signaling of flavor errors from signaling of flavor itself. We recorded neural activity in VTA with drivable bundles of microelectrodes. Thirty dopamine neurons were identified using waveform characteristics as in previous papers. This population signaled classic reward prediction errors as well as sensory prediction errors at both flavor transitions. Although the population activity did not distinguish the direction of the flavor change, a decoding algorithm using a thirty-neuron pseudoensemble could decode flavor accurately (~80%), but only on trials immediately after flavor transitions and only on changed drops. This pattern indicates that the dopamine neuron ensemble was representing not the flavor itself, but flavor when it had been mispredicted. These results are consistent with the proposal that the VTA dopamine system signals a generalized prediction error, reflecting a failure to predict features of an unexpected event beyond and even orthogonal to value.

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Poster

150. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 150.06/P2

Topic: G.01. Appetitive and Aversive Learning

Support: Wellcome Trust Senior Investigator Award to TWR (104631/Z/14/Z)
The Behavioural and Clinical Neuroscience Institute, which was jointly funded by the Medical Research Council (MRC) and the Wellcome Trust.

Title: Dopamine D2 receptor-like stimulation blocks learning from negative feedback in the rat: A behavioral and computational study

Authors: *B. U. PHILLIPS[1,2], J. ALSIÖ[2], J. SALA-BAYO[2], S. R. O. NILSSON[4,2], L. LOPEZ-CRUZ[2], J. W. DALLEY[2,3], R. N. CARDINAL[2,3,5], A. C. MAR[4,2], T. W. ROBBINS[2];
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Univ. Med. Ctr., New York, NY; [5]Cambridgeshire & Peterborough NHS Fndn. Trust, NHS, Cambridge, United Kingdom

Abstract: To respond optimally in volatile environments, it is critical that organisms adapt behavior in response to both positive and negative feedback (wins and losses). This ability is disrupted in numerous neuropsychiatric and neurodegenerative conditions and is closely linked with the dopamine system, which is hypothesized to signal the discrepancy between expected and actual outcomes in the form of a reinforcement prediction error. However, the precise contribution of dopamine, including dopamine subreceptors, remains unclear. In this study we employed rodent reversal learning procedures that leverage probabilistic feedback to assess the contribution of positive and negative feedback to choice behavior. The effect of dopamine D2 receptor (D2R) agonism in balancing learning from wins and losses was evaluated on these tasks. Subsequently, computational reinforcement learning modelling was applied to choice data to estimate the value of latent performance variables. D2R agonism with quinpirole impaired optimal performance on both reversal learning tasks. Task parameters directed at assessing learning from wins and losses indicated that quinpirole (0.25mg/kg) completely abolished learning from negative feedback. Consistently, the reinforcement learning model that best described behavior revealed a dramatic reduction in the learning rate for losses in quinpirole-treated animals. Taken together, these results suggest that D2R stimulation impairs reversal learning in rats via blockade of learning from negative feedback.

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Poster

150. Appetitive and Incentive Learning and Memory I

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Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

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Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant DK111475

Title: Optogenetic stimulation of midbrain dopamine promotes decision-making towards actions associated with less effort and lower value reinforcers

Authors: *B. R. FRY[1], A. MCLOCKLIN[2], N. PENCE[2], R. SCHAEFER[2], J. BEATTY[3], C. L. COX[3], A. W. JOHNSON[1];
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Abstract: Dopaminergic signaling has long been implicated in the maintenance of effort and decision-making; however, much of the work investigating these phenomenon have used pharmacological manipulations that were limited in their ability to ascertain temporal and spatial specificity. To address these issues, we used mice expressing Cre-recombinase under the control of the tyrosine-hydroxylase—the rate limiting enzyme for dopamine synthesis—promoter (TH-Cre). TH-Cre mice received unilateral infusions of the Cre-dependent channelrhodopsin (ChR2) virus, AAV5-eF1a-DIO-hChR2(H134R), targeted at the ventral tegmental area (VTA). In addition, a ferrule tip was placed dorsal to this region, which permitted 473 nm wavelength laser stimulation of dopamine cells in awake behaving animals. Following recovery from surgery, mice were trained on an effortful decision-making task, which consisted of two levers being presented; one associated with a high value (20% sucrose solution) reward, and a second with a low value reward (5% sucrose solution). The amount of effort required to obtain the high reward progressively increased across trial blocks, from 1 lever response at the start of the test, to 40 responses by the end. Each session was divided into four blocks, within which two trial types occurred: (1) Forced trials, when animals were only presented with one of the two levers (i.e., no choice); (2) choice trials during which animals were free to choose between access to the high and low valued rewards. Once asymptotic performance was established, mice received two tethered non-stimulated sessions, and two sessions during which a 1s, 473 nm laser stimulation (5-ms pulses at 20Hz) preceded the extension of the levers during each choice trial. We found that optogenetic stimulation of dopamine cells within the VTA biased responding toward the least effortful and lower valued rewards; and did so without influencing the capacity of the mouse to retrieve previously acquired lever contingencies. Moreover, these optogenetic effects were not seen in TH-Cre mice treated with a control eYFP virus. These effects are consistent with the role of midbrain dopamine in encoding contingencies and facilitating decision-making between effortful actions.

Disclosures: B.R. Fry: None. **A. McLocklin:** None. **N. Pence:** None. **R. Schaefer:** None. **J. Beatty:** None. **C.L. Cox:** None. **A.W. Johnson:** None.

Poster

150. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 150.08/P4

Topic: G.01. Appetitive and Aversive Learning

Support: R01 DA 038599

Title: Optogenetic inhibiton of cue-elicted dopamine activity attenuates sign-tracking behavior to a Pavlovian food cue

Authors: *A. IGLESIAS[1], J. WONG[1], P. CAMPUS[2], K. DEISSEROTH[4], H. AKIL[1], S. B. FLAGEL[3];
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Abstract: Environmental cues can guide behavior in an adaptive manner, bringing one in close proximity to valuable resources. However, for some individuals, such cues attain inordinate control and can lead to maladaptive behavior. In rodents, individual differences in cue-motivated behaviors can be captured using a Pavlovian conditioned approach (PavCa) paradigm, wherein presentation of a discrete cue (conditioned stimulus, CS) is followed by delivery of a food reward (unconditioned stimulus, US). Following PavCa training, two distinct phenotypes emerge - goal-trackers (GT) and sign-trackers (ST). While both GTs and STs attribute predictive value to the reward cue, STs also attribute incentive value to the cue. The attribution of incentive motivational value, or incentive salience, transforms the cue into an attractive and desirable stimulus. For STs, both food- and drug-associated cues gain excessive incentive value and elicit maladaptive behaviors. The ST/GT model, therefore, can be utilized to elucidate the neurobiological mechanisms that encode adaptive or maladaptive cue-driven behaviors. STs and GTs rely on distinct neurobiological mechanisms. Notably, sign-tracking, but not goal-tracking, behavior is dopamine (DA)-dependent, and cue-elicited DA in the nucleus accumbens is thought to encode the incentive value of reward cues. Here we exploited the temporal resolution of optogenetics to determine if selective inhibition of cue-elicited DA would attenuate the propensity to sign-track. To do so, we utilized tyrosine hydroxylase (TH)-Cre Long Evans rats, which express cre-recombinase in DA neurons. An optogenetic viral construct containing halorhodopsin, a light-sensitive inhibitory channel, was infused and expressed in DA neurons within the ventral tegmental area. First, we assessed the tendency of TH-Cre Long Evans rats to sign-track (i.e. without optogenetic manipulation), and found that, out of a population of ~45 rats, ~85% are sign-trackers. We then assessed whether laser-induced inhibition paired with CS presentation would prevent the development of sign-tracking behavior. Indeed, pairing of the laser-light with CS presentation during the first 75 trials of CS-US (lever-food) presentations decreased the tendency to sign-track. Those with optogenetic inhibition of cue-elicited DA exhibited a bias towards goal-tracking, rather than sign-tracking behavior. When laser inhibition was terminated, these same rats then began to develop a sign-tracking response. These findings demonstrate that cue-elicited DA release is critical for incentive learning processes.

Disclosures: A. Iglesias: None. **J. Wong:** None. **P. Campus:** None. **K. Deisseroth:** None. **H. Akil:** None. **S.B. Flagel:** None.

Poster

150. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 150.09/P5

Topic: G.01. Appetitive and Aversive Learning

Support: NIDA 3 R01 DA 038599-04S2

Title: Investigating individual differences in cue-induced reinstatement of opioid-seeking behavior

Authors: *S. E. CHANG, M. M. CHOJECKI, L. D. KRUEGER, S. B. FLAGEL;
Mol. and Behavioral Neurosci. Inst., Univ. of Michigan, Ann Arbor, MI

Abstract: According to the Centers for Disease Control, ~ 130 people in the United States die every day due to overdosing on opioids. Understanding the factors that contribute to the development of opioid addiction and the likelihood of relapse are critical to ending this crisis. A number of behavioral traits are thought to increase susceptibility to addiction, many of which have been modeled in preclinical studies. Surprisingly few preclinical studies, however, have investigated individual differences in susceptibility to opioid use and relapse. We used outbred Sprague-Dawley rats to establish a self-administration paradigm for the synthetic opioid drug, remifentanil (REMI). REMI is a potent short-acting μ-receptor agonist, and ideal for examining drug-cue relationships. To control for the amount of drug-cue pairings and subsequently examine reinstatement of opioid-seeking behavior, we employed a "controlled intake" acquisition paradigm for intravenous REMI self-administration. Rats self-administered 5, 10, and 20 infusions (3.2 μg/kg), followed by 45 infusions (1.6 μg/kg). Acquisition occurred over ~2 weeks, with 3-4 daily sessions occurring for each infusion criterion. Rats were then exposed to a ~2-week abstinence period followed by extinction and a test for cue-induced reinstatement. Rats subsequently were exposed to additional extinction training and then tested for drug-induced reinstatement, with 3.2 and 6.4 μg/kg of REMI. The majority of rats met criteria for acquisition and readily self-administered REMI. Cue-induced reinstatement was observed, as relative to the extinction sessions, there was enhanced responding into the nose port that resulted in presentation of the discrete cue light previously paired with REMI delivery. Intravenous infusion of 3.2 μg/kg of REMI was not sufficient to elicit drug-seeking behavior, but administration of 6.4 μg/kg resulted in an increase in responding in the nose port previously associated with drug delivery. Upon examination of individual differences, we found that "sensation-seeking" rats, or those with increased locomotor response to novelty (i.e. high-responders), have a greater propensity for both cue- and drug-induced reinstatement of opioid-seeking behavior relative to low-responder rats. Ongoing studies are investigating whether other traits, such as an increased propensity for sign-tracking behavior, predict vulnerability to opioid abuse and relapse. The self-administration paradigm established here will open up many avenues of future research, allowing us to investigate the neural mechanisms that contribute to individual differences in cue-induced opioid-seeking behavior.

Disclosures: S.E. Chang: None. **M.M. Chojecki:** None. **L.D. Krueger:** None. **S.B. Flagel:** None.

Poster

150. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 150.10/P6

Topic: G.01. Appetitive and Aversive Learning

Support: NIDA R01-DA-038599
NIDA R21-DA-045146
NSF GRFP

Title: Investigating the role of corticosterone receptor signaling on dopamine-dependent Pavlovian incentive learning

Authors: *S. A. LOPEZ[1], Y. KIM[2], R. T. KENNEDY[2], S. B. FLAGEL[3];
[1]Neurosci. Grad. Program, [2]Chem., [3]Mol. and Behavioral Neurosci. Institute, Psychiatry, Neurosci. Grad. Program, Univ. of Michigan, Ann Arbor, MI

Abstract: Through associative learning, cues in the environment become predictors of biologically relevant stimuli (e.g. food). When such cues, however, are attributed with incentive value, they can gain inordinate control and elicit aberrant behavior. For example, when individuals with addiction or post-traumatic stress disorder encounter cues that were previously associated with reward or trauma, they often relapse or demonstrate hyperarousal in response to these cues. Using an animal model that captures individual variation in the propensity to attribute incentive value to reward cues, we are able to examine the neurobiological processes that contribute to such cue-driven psychopathologies. Rats that undergo Pavlovian training, consisting of discrete cue presentation followed by delivery of a food reward, will often develop either a sign- or goal-tracking conditioned response. While both sign-trackers (ST) and goal-trackers (GT) attribute predictive value to the cue, only for ST is the cue attributed with incentive value and transformed into a “motivational magnet”. It has been shown that different brain circuits are engaged in response to the cue in ST vs. GT, and that dopamine (DA) is necessary for incentive, but not predictive learning processes. Interestingly, DA has long been known to interact with corticosterone (CORT), the primary regulator of the stress response in rats, to mediate reward-motivated behaviors. When “stress” levels of CORT enter the brain they act upon glucocorticoid receptors (GR) and increase DA overflow within the nucleus accumbens (NAc), a critical node of incentive value attribution. Yet, little research has been done to directly investigate the interaction between these two molecules in the context of ST and GT. Here we assessed the effect of CORT (i.e. GR agonist) on the attribution of incentive value to reward cues and NAc DA transmission during Pavlovian learning. To do so, 3 mg/kg CORT or vehicle were administered systemically prior to Pavlovian conditioning sessions and DA samples were obtained via *in vivo* microdialysis within the NAc shell of male and female rats during the first

and last day of Pavlovian conditioned approach training. CORT administration resulted in an increase in the acquisition of sign-tracking behavior, and enhanced the incentive motivational value of a discrete food-cue. In support of these behavioral effects, we hypothesize that DA in the NAc shell will also increase in response to CORT administration. These data highlight a role for CORT in DA-dependent learning processes that are relevant to cue-driven psychopathologies.

Disclosures: **S.A. Lopez:** None. **Y. Kim:** None. **R.T. Kennedy:** None. **S.B. Flagel:** None.

Poster

150. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 150.11/P7

Topic: G.01. Appetitive and Aversive Learning

Support: NIH grant R01-DK103808
NIH grant R01-DK111475

Title: Optogenetic stimulation of melanin concentrating hormone and orexin differentially influence cue potentiated feeding

Authors: N. RUSSELL[1], L. RAYCRAFT[1], R. BUGESCU[1], G. M. LEINNINGER[2], *A. W. JOHNSON[1];
[2]Dept. of Physiol., [1]Michigan State Univ., East Lansing, MI

Abstract: Within the lateral hypothalamus (LH), both Melanin Concentrating Hormone (MCH) and orexin (ORX) have been described as orexigenic neuropeptides. At the same time, the actions of these feeding signals appear in many ways to contrast with one another, including with respect to the regulation of sleep and in glucose sensing. Furthermore, ORX cells are able to directly modulate the activity of MCH cells via GABAergic inhibition. To examine potential opposing roles played by these two distinct populations of LH neurons, we used two separate mouse lines where Cre-recombinase was under the control of the Pmch gene (Tg-MCH-Cre) or ORX promoter (ORX-IRES-Cre). Mice received bilateral injections of a Cre-dependent channelrhodopsin (ChR2) virus, AAV5-eF1a-DIO-hChR2(H134R) into the LH along with implantation of ferrule tips. Following recovery from surgery, mice were trained for cue-potentiated feeding (CPF) in which under conditions of mild food deprivation, mice learned to associate one auditory conditioned stimulus with delivery of sucrose (CS+), whereas a second cue was unpaired (CS-). Mice were tested under ad-libitum feeding conditions, where the degree to which each stimulus would evoke CPF was examined. Tests were conducted separately for CS+ and CS-, with four trials of each stimulus presented. Within these tests, laser stimulation (473 nm, 5 ms pulses, 20 Hz) was timed to coincide with stimulus presentation on half of the

trials, whereas for the remaining trials no optogenetic stimulation occurred. During stimulated trials Tg-MCH-Cre mice displayed enhanced CPF, whereas stimulation attenuated feeding in the task in ORX-IRES-Cre mice. Collectively, our results suggest that within the LH ORX cells exert direct control over MCH cells, promoting the rapid transition between motivated food-related behaviors.

Disclosures: **N. Russell:** None. **L. Raycraft:** None. **R. Bugescu:** None. **G.M. Leinninger:** None. **A.W. Johnson:** None.

Poster

150. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 150.12/P8

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant DK111475

Title: Estrus cycle interactions with melanin concentrating hormone on peak interval responding in rats

Authors: *L. M. RAYCRAFT[1], E. NOBLE[2], S. E. KANOSKI[3], A. W. JOHNSON[1]; [1]Psychology, Michigan State Univ., East Lansing, MI; [2]Biol. Sci., [3]USC, Los Angeles, CA

Abstract: Feeding behaviors have traditionally been studied within a context of circadian timing; however, interval timing (i.e., timing in the milliseconds to minutes range) may play a central role, as this distinct form of timing is critical for learning and decision-making. In this manner, interval timing may inform decisions to engage in food-related behaviors, such as food-seeking or consumption. Notably, no studies have examined signals controlling appetite regulation on interval timing. In this study, we examined the role of the lateral hypothalamic (LH) feeding peptide Melanin concentrating hormone (MCH) in an interval timing task. Male and female Sprague Dawley rats received targeted LH injections of a Designer Receptor Exclusively Activated by Designer Drugs (DREADD) packaged within an adeno-associated virus driven by the MCH promoter, pMCH. Placement of an intracerebroventricular cannula enabled central delivery of the synthetic ligand, clozapine-N-oxide (CNO), and activation of LH MCH cells during the interval timing task. In the peak interval task, rats first learned to respond on a lever for sucrose reward following the passage of a 20 s target criterion. Next, intermixed probe trials were introduced: in these trials, the lever extended but no sucrose was provided, regardless of lever responding. Probe trials allowed measurement of maximum lever responding across a trial, and enabled assessment of the subject's interval timing function. Under control conditions, a normal distribution of lever responding that peaked near the 20 s criterion duration indicated that all rats showed intact temporal performance. Interestingly, stimulation of LH MCH

neurons selectively modified the right-hand side of the timing function in females, whereas it had no effect on the timing function in males. In females, this effect of prolonged peak rate responding was specific to estrus cycle stage. Moreover, CNO activation of MCH neurons restored responding in diestrus females to the behavioral phenotype observed both during proestrus/estrus and to that observed in males. Finally, analysis of estrus cycle stage and task performance under vehicle conditions revealed that diestrus females show a leftward shift in the timing function. Collectively, these findings suggest that MCH may affect performance in interval timing tasks in a sex-specific manner—in females, this depends on estrus cycle stage. Furthermore, estrogen itself may be an important modulator of interval timing.

Disclosures: L.M. Raycraft: None. **E. Noble:** None. **S.E. Kanoski:** None. **A.W. Johnson:** None.

Poster

150. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 150.13/P9

Topic: G.01. Appetitive and Aversive Learning

Support: Grants 2013CB530902 to Y.-D.Z
81571125 to Y.-D.Z
81571088 to Y.S

Title: Anterior paraventricular thalamus to ventromedial nucleus of the hypothalamus projection modulates compulsive sucrose seeking induced by high-fat diet

Authors: *Z. YUAN[1], X. MA[2], J. CHENG[3], Y.-D. ZHOU[3], Y. SHEN[3];
[1]Zhejiang Univ., Hangzhou, China; [2]Zhejiang Univ., Zhejiang, China; [3]Zhejiang Univ. Sch. of Med., Zhejiang, China

Abstract: High-fat diet (HFD) leads to compulsive eating. The paraventricular thalamic nucleus (PVT) plays a key role in reward and fear circuits and modulates feeding behavior, suggesting that PVT may be associated with HFD-induced feeding behavioral change. Here we report that HFD-induced compulsive sucrose seeking caused a high level of c-Fos expression in PVT. Injection of palmitate fatty acids in PVT also enhanced compulsive sucrose seeking in mice fed with regular chow. Photostimulating of PVT induces compulsive sucrose seeking in normal diet mice and is primarily achieved by the projection of PVT to Ventromedial Nucleus of the Hypothalamus (VMH). Photoinhibition and chemical inhibition of PVT both down-regulate the compulsive sucrose seeking of HFD mice. Thus, we provide direct evidence that PVT modulates compulsive sucrose seeking in HFD mice.

Disclosures: **Z. Yuan:** None. **X. Ma:** None. **J. Cheng:** None. **Y. Zhou:** None. **Y. Shen:** None.

Poster

150. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 150.14/P10

Topic: G.01. Appetitive and Aversive Learning

Support: R01 DA 038599

Title: Assessing the effects of selective inhibition of neuronal projections from the lateral hypothalamus to the paraventricular nucleus of the thalamus on Pavlovian conditioned approach behavior

Authors: A. JOHNSON[1], A. IGLESIAS[2], P. CAMPUS[3], *S. B. FLAGEL[1];
[2]The Mol. & Behavioral Neurosci. Inst., [3]Dept. of Psychiatry, [1]Univ. of Michigan, Ann Arbor, MI

Abstract: The survival of an organism is often dependent on their ability to properly respond to cues in the environment. Associative learning processes contribute to an individual's response to such cues. For some, reward-paired cues are attributed with incentive motivational value (incentive salience), and can gain excessive control, leading to maladaptive behavior. We are able to investigate the neural mechanisms underlying incentive learning using a model where some animals attribute predictive value to cues (goal-trackers, GT), and others attribute predictive and incentive value to reward cues (sign-trackers, ST). These phenotypes emerge through Pavlovian conditioned approach (PavCA), in which a discrete cue (a lever) is paired with a food reward. Prior work has implicated the paraventricular nucleus of the thalamus (PVT) as a neural hub responsible for mediating sign- and goal-tracking behavior. We postulate that top-down cortical projections to the PVT encode the predictive value of cues, whereas bottom-up subcortical processes relay the incentive value of cues. A subcortical node for this incentive value encoding is the lateral hypothalamus (LH), which sends dense orexinergic projections to the PVT. Orexin has a known role in motivation, and administration of orexin receptor antagonists into the PVT attenuates the incentive value of food-paired cues in STs. To assess its role in encoding the incentive value of reward cues, we utilized a dual-vector approach to selectively express an inhibitory (G_i) DREADD (Designer Receptors Exclusively Activated by Designer Drugs) in the LH-PVT pathway. Following PavCa, rats developed a conditioned response and received either vehicle or clozapine-N-oxide (CNO; 5 mg/kg) to activate the DREADD. Selective inhibition of the LH-PVT pathway decreased lever-directed behaviors for STs, without affecting the behavior of GTs. STs with CNO showed a reduction in the number of lever contacts and an increased latency to approach the lever. Further, they responded less for presentation of the lever-cue on a test of conditioned reinforcement. These results indicate that

inhibition of the bottom-up LH-PVT pathway decreases the incentive value of reward-paired cues, highlighting a role for this pathway in aberrant cue-driven behaviors.

Disclosures: A. Johnson: None. **A. Iglesias:** None. **P. Campus:** None. **S.B. Flagel:** None.

Poster

150. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 150.15/P11

Topic: G.01. Appetitive and Aversive Learning

Support: NIH, NIDDK Grant R01DK085721

Title: DREADD silencing of neurons in the paraventricular thalamus interferes with context-mediated renewal of food seeking in a sex-dependent manner

Authors: *A. M. K. MADDEN, G. D. PETROVICH;
Psychology, Boston Col., Chestnut Hill, MA

Abstract: Modern environments (contexts) are filled with food cues that can stimulate feeding even under physiological satiety. These food cues remain a critical factor for relapse to maladaptive eating behaviors because food-cue associations can be resistant to extinction. Our group previously reported sex differences in an animal model of context-mediated renewal (reinstatement) of responding to food cues after extinction and sex specific Fos induction within the paraventricular thalamus (PVT). That work implies that PVT activity could underlie the observed individual differences in context-mediated renewal of responding to food cues. To test whether PVT activity is critical for context-mediated renewal, we used an ABA behavioral paradigm and designer receptors exclusively activated by designer drugs (DREADDs) to silence PVT activity during tests for renewal. We injected the PVT of young adult male and female Long-Evans rats with either pAAV5-hSyn-hM4D(Gi)-mCherry or pAAV5-hSyn-eGFP. After full recovery from surgery and food deprivation to 90% body weight, we trained the rats across 5 acquisition sessions (8 trials per session) of tone (CS) followed by palatable food (US) in a distinct context, followed by 2 extinction sessions of tone-only presentations in a different distinct context. Conditioned responding was measured by appetitive behavior directed towards the food receptacle (foodcup behavior) and CS-specific responding was determined by elevation above baseline responding (10 seconds pre-CS). All subjects exhibited CS-specific responding that increased across acquisition training, and appropriate extinction (Of note, three females did not show extinction and were removed from the final dataset). Following extinction, we administered subthreshold doses of clozapine (Clz; 0.1mg/kg; i.p.) or vehicle and measured elevation in each context over two days, counterbalanced for test order across groups. This created six groups, three per sex, with dual controls for DREADDs expression and Clz exposure:

eGFP+Clz, hM4Di+Veh, and hM4Di+Clz. Renewal was inferred from higher elevation responding in the acquisition compared to extinction context. Pre-histological analysis of elevation difference suggests that dampening PVT activity interferes with context-mediated renewal in males, but not in females (mean±SEM; males: GFP+Clz=18.8±6.5 hM4Di+Veh=8.6±7.6, hM4Di+Clz=3.7±5.5; females: GFP+Clz=21.5±3.6, hM4Di+Veh=15.2±13.0, hM4Di+Clz=12.5±4.6). These preliminary results suggest that the PVT functions differently within the circuitry underlying context-mediated renewal in males and females.

Disclosures: A.M.K. Madden: None. **G.D. Petrovich:** None.

Poster

150. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 150.16/P12

Topic: G.01. Appetitive and Aversive Learning

Support: NIDDK grant R01DK085721

Title: Fos expression in central amygdala following novel food and context exposure

Authors: *E. G. GREINER[1], G. D. PETROVICH[2];
[2]Psychology, [1]Boston Col., Chestnut Hill, MA

Abstract: Novel foods and novel environments impact consumption, but there is very limited research into how the two interact, and whether there are sex differences. Our prior work showed consumption differences between male and female rats when presented with a novel food in a novel context, with females showing sustained, suppressed consumption throughout testing. Therefore, we sought to determine whether there are neural activation differences in males and females following novelty exposure. Following 20hr acute food deprivation, male and female Long Evans rats (n=8 per group) were tested for consumption in either their home cage or in a novel context and were given either a familiar (rat chow) or novel (Test Diet pellets; TD) food. Rats were perfused 90 minutes after the start of testing and their brains tissues were immunohistochemically processed for Fos protein detection. Due to the role of the central amygdala (CEA) in mediating consumption we focused our initial analysis on its subregions. Consumption patterns across different groups matched patterns in our previous study, with home cage tested groups given familiar food consuming more than novel context tested groups who received novel food ($F(7,56)=6.523$, $p<0.001$). Preliminary analysis (n=4 per group) of Fos induction found significantly greater number of Fos-positive neurons within the CEA in rats given TD in home cage than all other groups ($F(3,76)=8.081$, $p<0.001$), with the least number of Fos-positive neurons in the group that was given rat chow in a novel context. The medial portion

of the CEA alone had a similar pattern, with significantly higher number of Fos-positive neurons in the home cage tested TD group than the novel context tested rat chow group ($p<0.001$). However, activation patterns in the capsular region of the CEA (CEAc) were distinct and there was greater Fos induction in the novel context tested rats who received TD compared to rats who experienced both familiar food and familiar environment ($p<0.001$). These results suggest that a novel food in a familiar environment leads to an overall increase of Fos induction in the CEA. The alternative pattern in the CEAc indicates that the different subregions of the CEA may mediate distinct aspects of novelty processing depending on the stimulus type.

Disclosures: E.G. Greiner: None. **G.D. Petrovich:** None.

Poster

150. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 150.17/P13

Topic: G.01. Appetitive and Aversive Learning

Support: NIH, NIDDK Grant R01DK085721

Title: Investigation of the prelimbic cortex neuronal ensembles plasticity during context-mediated renewal of responding to food cues

Authors: *D. S. LAFFERTY, G. D. PETROVICH;
Psychology, Boston Col., Chestnut Hill, MA

Abstract: Learned environmental cues strongly influence the drive to seek food and other rewards, and can persist even after extinction. Context-mediated renewal is a behavioral paradigm that models persistent drive for food. In this preparation, cue-food association is acquired in one context, extinction of the conditioned responding occurs in a different context, and the renewal of responding is induced by the acquisition context. Prior work utilizing DREADDs methodology has demonstrated that the ventromedial prefrontal cortex, including the prelimbic cortex (PL) subregion, is critical for context-mediated renewal of responding to food cues. Our goal was to determine if the PL is a critical site of plasticity and, specifically, whether the activation of the same PL neuronal ensemble recruited during cue-food acquisition is later required during renewal. This was accomplished with the chemogenetic Daun02 inactivation method, which selectively incapacitates recently activated neurons. First, male transgenic *Fos-lacZ* rats were surgically implanted with bilateral cannula targeting PL. Only males were used in this experiment because prior work demonstrated that females do not show consistent renewal of responding in this preparation. After recovery, rats were food restricted and underwent Pavlovian conditioning in which they were presented with a tone cue (conditioned stimulus, CS) followed by delivery of palatable food pellets (unconditioned stimulus, US). Acquisition of the CS-US

association occurred in a distinct context that varied in olfactory, visual, and tactile features from the context used for extinction training. There were 5 acquisition training sessions (each with 8 CS-US pairings), with Daun02 (or vehicle for control) infusions into the PL after the final acquisition session. This was followed by 2 extinction sessions (each with 8 CS-only presentations) and, finally, testing for renewal of responding with CS-only presentations in each context, on separate days counterbalanced for order. Renewal of responding was assessed by comparing conditioned responding (approach to the foodcup) during the CS in the acquisition vs. extinction contexts. After their final renewal test session, rats were perfused for collection of brain tissue to verify cannula placement and effectiveness of Daun02 induction, as well as analysis of *c-fos* gene protein (Fos) expression. Our prediction is that Daun02 infusions into the PL will impair renewal due to impaired recall of the cue-food memory upon testing in the acquisition context. Preliminary results suggest a potential effect of Daun02 in the ventral portion of the PL, pending final histology and Fos analyses.

Disclosures: D.S. Lafferty: None. **G.D. Petrovich:** None.

Poster

150. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 150.18/P14

Topic: G.01. Appetitive and Aversive Learning

Support: NIH grant DA035443
NIH grant MH106972
NIH grant DA038942
NIH grant DA024635

Title: Contribution of dorsal striatal direct and indirect pathway projections to goal-directed and habit learning

Authors: *M. MALVAEZ, A. LIANG, M. D. MURPHY, K. M. WASSUM;
UCLA, Los Angeles, CA

Abstract: Optimal behavior relies on a balance between two distinct strategies; one goal-directed in which the relationship between actions and their consequences is considered, and one habitual, which allows routine tasks to be conducted without forethought of their consequences. The balance between these systems allows adaptive and efficient behavior, but disruption of this balance can lead to symptoms characteristic of several psychiatric and neurological diseases. Goal-directed and habit learning and their behavioral control are known to rely on the anatomically distinct dorsomedial (DMS) and dorsolateral (DLS) striatum, respectively. But very little is known about the subregion-specific contribution of the two major subtypes of medium

spiny striatal projection neurons: the direct (dMSNs; characterized by D1 receptor expression) and indirect (iMSNs; characterized by A2A2 receptor expression) pathway projections. Using chemogenetics and DRD1-cre and A2A-cre driver mice, we selectively inactivated dMSNs or iMSNs in either the DLS or DMS during instrumental lever press-->reward training. Following training, devaluation via sensory-specific satiety of the food outcome was used to probe goal-directed versus habitual control of instrumental behavior. The data suggest dMSN activity contributes to both goal-directed and habit learning in a sub-region specific manner. In the DMS, chemogenetic inactivation of dMSNs during instrumental training caused insensitivity to devaluation following limited training, suggesting DMS dMSN activity is necessary for action-outcome learning. Conversely in the DLS, dMSN inactivation during instrumental training caused sensitivity to devaluation even after overtraining, indicating DLS dMSN activity is necessary for habit formation. Ongoing experiments are assessing the contribution of dorsal striatal iMSNS in goal-directed and habit learning.

Disclosures: **M. Malvaez:** None. **A. Liang:** None. **M.D. Murphy:** None. **K.M. Wassum:** None.

Poster

150. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 150.19/P15

Topic: G.01. Appetitive and Aversive Learning

Support: JSPS KAKENHI Grant Number JP19K14473

Title: Transient inactivation of ventral hippocampus promotes learning of the odor-safe association in rats

Authors: *K. SHINOHARA, Y. YASOSHIMA;
Osaka Univ., Suita-Shi, Japan

Abstract: Animals often reduce consumption of a novel food or drink, which is known as food neophobia. In the absence of negative post-ingestive consequences, consumption increases with exposure (attenuation of neophobia). Several reports have indicated that the medial temporal lobe structures are involved differentially in the occurrence and attenuation of neophobia to a taste stimulus (e.g., sodium saccharin solution). The neural mechanisms of the neophobia to a food-related odor stimulus remain unknown, although olfaction is a sensory modality which has an essential role in a formation of food preference. Here, we investigated the involvement of ventral hippocampus (VH) and basolateral nucleus of amygdala (BLA) in the olfactory neophobia. Male adult Wistar rats put on an 18-hour water deprivation were able to access an almond odor solution (0.15% benzaldehyde) for 20 min per day, which was conducted for four

consecutive days. Before the first odor exposure, they received a microinjection of a gamma aminobutyric acid type A agonist muscimol (50 ng/0.25 μl) or saline into the bilateral VH or BLA. The present result showed that control rats avoided consuming a novel bezaldehyde at the first odor exposure, but that consumption gradually increased over repeated exposures, suggesting that control animals showed salient occurrence of olfactory neophobia. Rats treated with muscimol infused into VH also kept off a novel bezaldehyde. However, they showed significantly higher consumption at the second test compared to that observed in control rats, suggesting that VH inactivation promotes the attenuation of olfactory neophobia. On the other hand, rats treated with muscimol infused into BLA showed the occurrence and attenuation of olfactory neophobia similar to that of control rats. These results indicate that VH acts as the regulation of the attenuation of olfactory neophobia and that alterations in function of VH may contribute to promotion of the odor-safe associative learning.

Disclosures: K. Shinohara: None. **Y. Yasoshima:** None.

Poster

150. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 150.20/P16

Topic: G.01. Appetitive and Aversive Learning

Title: Lateral habenula lesions impair absolute but not relative reward comparisons

Authors: *M. R. PAPINI[1], S. E. CONRAD[2], R. DONAIRE[3], S. GUARINO[1], C. TORRES[3]; [2]Psychology, [1]Texas Christian Univ., Fort Worth, TX; [3]Univ. of Jaen, Jaen, Spain

Abstract: Lateral habenula (LHb) activity is hypothesized to increase nonreward processing, an effect consistent with a role in either negative emotion or the regulation of choice between signals for rewards of different value. We studied the effects of quinolinic acid LHb lesions in two reward-loss situations. One involved negative emotion (consummatory successive negative contrast, cSNC) and the other involved negative emotion plus free-choice tests with levers signaling downshifted vs. unshifted rewards (autoshaping successive negative contrast, aSNC). In the cSNC task, animals received either 32% or 4% sucrose for ten 5-min sessions (preshift). On sessions 11-14 (postshift), both groups received 4% sucrose. In the aSNC task, sessions 1-10 (preshift) involved 6 single-lever trials per session, 3 with one lever signaling 12 pellets and 3 with the other lever signaling 2 pellets. On sessions 11-14 (postshift), one lever was downshifted from 12 to 2 pellets, while the other lever remained unshifted. On sessions 7 and 9 (preshift), animals received a single free-choice trial with levers signaling different reward magnitudes. On sessions 11 and 13 (postshift), animals received free-choice trials with one lever signaling the downshifted reward and the other signaling the unshifted reward. Histological results showed that cell counts in the medial and lateral LHb areas were lower in LHb animals than in shams.

Behavioral results in the cSNC task showed similar rejection of the solution after a 32-to-4% sucrose downshift in LHb and controls. Behavioral results in the free-choice tests of the aSNC task yielded a preference for the large-reward lever in preshift tests and a switch to the unshifted, small-reward lever after reward downshift in controls. LHb animals were indifferent during free-choice preshift tests, but they exhibited a normal switch to the unshifted, small-reward lever after reward downshift on the alternative lever. These results suggest that the LHb is more involved in choice among options signaling different absolute reward values than in negative emotion. They also highlight a counterintuitive dissociation between reward magnitude and reward downshift: Failure in an absolute-reward task does not necessarily imply failure in a relative-reward task.

Disclosures: M.R. Papini: None. **S.E. Conrad:** None. **R. Donaire:** None. **S. Guarino:** None. **C. Torres:** None.

Poster

150. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 150.21/P17

Topic: G.01. Appetitive and Aversive Learning

Support: Technical assistant Alejandro Rangel-Hernández
Shaun Harris for the English revision
Supported by PAPIIT 201018

Title: Rat licking microstructure analysis: Effect of long-term sucrose consumption on taste preferences

Authors: *H. VILLARREAL-VÁZQUEZ[1], E. BOLAÑOS-AQUINO[2], G. VERA-RIVERA[3], M. I. MIRANDA[3];
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Abstract: Sweet tastants have a positive hedonic value because of their association with high-caloric food and their preference forms part of an innate behavioral repertoire. Sweet food ingestion arouses brain learning and reward circuitry through fast sensory inputs and slow post-ingestive consequences that regulate following food intake and seeking behavior. Previous work in rodent models has shown that the ingestion of novel sweet tastants leads to an appetitive response, which is measured as an increase in their intake; long-term sucrose consumption produces changes in appetitive re-learning that could trigger an escalating consumption due to the inability to learn new negative consequences related to the same taste. The purpose of this

research was to assess, in female and male adult Wistar rats, the effects of long-term sucrose consumption (16 days) on compulsive-like sugar consumption behavior (e.g., Increase/acceleration of the lick rate), using a liking microstructure analysis during a two-bottle preference test. Accordingly, four groups of rats were subjected to 16 days *ad libitum* consumption of 10 % sugar water solution (MALE and FEMALE SUGAR-groups) or tap water (MALE and FEMALE WATER-groups) as the only *ad libitum* liquid. During 14 days, body weight and liquid consumed were measured every day. During days 15 to 18, rats were habituated to drink in the licking chamber and then rats were water deprived 18 hrs. A total of four preference tests in the licking chamber were carried out over a 30 min session, every 24 hrs: Sucrose vs. water (days 19 and 20); sucrose vs. fructose 8 % (day 21) and sucrose vs. saccharine 0.1 % (day 22). The results showed that during the first two preferences all groups preferred sugar over water, but the rate of licking is different between SUGAR and WATER groups, also showing a tendency towards differences between sexes. Furthermore, sucrose was preferred over fructose and saccharin in WATER groups, but similar preference was observed between all sweeteners in SUGAR groups. FEMALE WATER-group showed a preference tendency of sucrose over water, fructose or saccharine. Overall details of microanalysis will be discussed further. The results demonstrate changes in sweeteners preference after long-term sugar consumption with a sex bias tendency.

Disclosures: H. Villarreal-Vázquez: None. **E. Bolaños-Aquino:** None. **G. Vera-Rivera:** None. **M.I. Miranda:** None.

Poster

150. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 150.22/P18

Topic: G.01. Appetitive and Aversive Learning

Support: NIH RO1DA 040965
NIH P30GM103398

Title: Exercise reduces incubation of craving for food high in fat

Authors: *G. E. KIRKPATRICK[1], P. M. DINGESS[3], T. E. BROWN[2];
[1]Biomed. Sci., [2]Sch. of Pharm., Univ. of Wyoming, Laramie, WY; [3]Biol. Sci., Univ. of Alaska, Anchorage, AK

Abstract: We have previously shown that cue-induced craving for food high in fat increases over time (incubation of craving). However, it is unknown whether incubation of high-fat food craving can be reduced. To address this question, male Sprague Dawley rats underwent self-administration training for a high-fat (HF) pellet, which was associated with the presentation of a

stimulus complex (light+tone) for 10 d. One-day after training rats were tested for lever responding in the absence of the HF pellet but in the presence of the cues. During the 30 d abstinence period rats were separated into one of two groups, exercise or no exercise and tested again on day 30, identically to the 1 d test. The exercise group ran on an inclined treadmill 10 min/day during the abstinence period where the speed progressively increased over time. Consistent with our previous work, we found that rats without exercise demonstrated significant incubation of craving (1d: 24.71±4.19; 30d: 56.86±10.95; data are mean ± SEM). However, rats exposed to exercise during the abstinence period had an attenuation in lever responding at 30 d (1d: 18.67±4.72; 30d: 34.67±5.74). These results indicate that exercise could reduce increased craving during abstinence, which may reduce overeating and the development of obesity.

Disclosures: G.E. Kirkpatrick: None. **P.M. Dingess:** None. **T.E. Brown:** None.

Poster

150. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 150.23/P19

Topic: G.01. Appetitive and Aversive Learning

Support: NSF Grant DGE-1313911
NIH Grant MH112337

Title: Physical exercise ameliorates increases in sign-tracking behavior in rats exposed to high levels of kynurenic acid during adolescence

Authors: *S. B. MILLER, M. C. EDDY, N. E. DEANGELI, D. J. BUCCI;
Psychological and Brain Sci., Dartmouth Col., Hanover, NH

Abstract: Kynurenic acid (KYNA) is an end-product of tryptophan metabolism that is synthesized by astrocytes and acts as an endogenous antagonist of alpha-7 acetylcholine receptors and NMDA glutamate receptors. KYNA concentration is increased in the brains of persons with schizophrenia, and experimentally-induced increases in KYNA during development have been shown to produce cognitive and behavioral changes in rats that are reminiscent of impairments in schizophrenia. Pertinent to the present study, treating rats with l-kynurenine (L-KYN; the precursor of KYNA) to increase KYNA levels during adolescence increases sign-tracking behavior, which is thought to reflect heightened sensitivity to the value of reward-related cues and is associated with the propensity to engage in drug use. Recent studies suggest that physical exercise can reduce KYNA levels in the brain. Thus, the present study consisted of two experiments that tested whether exercise could reverse the increase in sign-tracking behavior in rats treated with L-KYN. In Experiment 1, male rats were treated with vehicle or L-KYN starting on postnatal day (PND) 27 and continuing throughout adolescence. Half of the rats in

each group also had 24-hour access to a running wheel beginning on PND 27. Beginning on PND 35, rats were trained in an autoshaping procedure (wheels were locked 2 hours before each session). Each daily session consisted of 50 trials (25 CS+ trials and 25 CS- trials) during which one response lever was inserted into the conditioning chamber for 10 sec and followed immediately by delivery of food reward (the CS+ lever), or another lever was inserted and nonreinforced (CS- lever). Even though food delivery was not contingent upon pressing the lever, rats in all groups exhibited a significant amount of sign tracking behavior (CS+ lever pressing) as observed previously. Consistent with prior research, exposure to L-KYN increased sign-tracking behavior in the non-exercise group compared to the vehicle-treated non-exercise group. However, wheel running normalized the level of sign-tracking behavior in the L-KYN exercise group such that it was comparable to control levels. In Experiment 2, rats were treated similarly except that behavioral training was conducted during adulthood instead of adolescence. Exposure to KYNA during adolescence increased sign-tracking during adulthood, as shown previously. However, in contrast to Experiment 1, exercise during adolescence did not counteract the effects of KYNA exposure. Together, the findings indicate that physical exercise can ameliorate behavioral changes while KYNA levels are high, but cannot reverse behavioral changes observed during adulthood.

Disclosures: **S.B. Miller:** None. **M.C. Eddy:** None. **N.E. DeAngeli:** None. **D.J. Bucci:** None.

Poster

151. Fear and Aversive Learning and Memory: Acquisition

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 151.01/P20

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant R15MH104836

Title: Carriers of the met allele of the bdnf val66met polymorphism develop weaker fear memories in a fear-potentiated startle paradigm

Authors: *J. N. WEISER, M. R. RIGGENBACH, B. E. MOSLEY, J. J. HIPSKIND, L. E. WIREMAN, K. L. HESS, T. J. DUFFY, J. K. HANDEL, M. G. KASCHALK, K. E. RENEAU, S. J. HELWIG, P. R. ZOLADZ;
Psychology, Sociology, & Criminal Justice, Ohio Northern Univ., Ada, OH

Abstract: The val66met polymorphism is a common single nucleotide polymorphism in the prodomain of the *BDNF* gene that converts the amino acid valine (val) to methionine (met) at codon 66. The polymorphism has been associated with compromised brain-derived neurotrophic factor (BDNF) signaling, impaired synaptic plasticity, impaired learning, and increased susceptibility for multiple psychological disorders. Rodents and humans expressing the met

allele exhibit heightened anxiety-like behavior, an attentional bias for and greater amygdala responses to emotional stimuli, and altered fear conditioning processes. However, research examining the impact of this polymorphism on fear learning has been inconsistent. Thus, we examined the influence of the val66met polymorphism on fear conditioning, extinction, and extinction memory. One hundred and twenty healthy participants completed differential fear conditioning in a fear-potentiated startle paradigm, followed by extinction and extinction memory sessions 24 and 48 hr later, respectively. Participants were genotyped for the val66met polymorphism and divided into met allele carriers and non-carriers. Results revealed a statistical trend suggestive of diminished fear acquisition in met carriers. Most importantly, met carriers exhibited a significantly weaker fear memory than non-carriers 24 hr later, an effect that was particularly evident in female participants and persisted to extinction memory testing. These results are consistent with previous work demonstrating that the met allele is associated with impaired amygdala-dependent fear learning and extend such findings by demonstrating a sex-dependent component to such effects.

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Poster

151. Fear and Aversive Learning and Memory: Acquisition

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 151.02/P21

Topic: G.01. Appetitive and Aversive Learning

Title: Fear conditioning responses and regulatory role of intracerebral NMDA antagonist D-AP5

Authors: *A. MORA-GALLEGOS, J. FORNAGUERA, D. QUESADA-YAMASAKI; Univ. of Costa Rica, San Pedro, Costa Rica

Abstract: Associative fear learning is important to respond to cues that predict fear as a form of survival. In disorders like PTSD, an association is given between relevant but also irrelevant cues present in the trauma. This association is a form of generalization of cues as a consequence of an impaired associative fear learning. This kind of learning is studied in animals as fear conditioning and involves behavioral and brain measures as freezing and the participation of NMDA receptors in different brain regions like ventral hippocampus (VH). The involvement of the VH in the response to different cues in the acquisition of fear learning through fear conditioning has not been fully described. Our main objective was to evaluate the role of an NMDA receptor antagonist (D-AP5) after unilateral injection in VH in the acquisition of fear conditioning. In our first experiment we evaluate the behavioral effects of the injection of the antagonist or vehicle (saline solution 0.9%) at three different time points (5, 10 or 15 min) before

training on fear conditioning. Once the time of injection was chosen, in a second experiment we evaluate the effect of the injection of the antagonist in the same brain area at three different doses (5, 7.5 and 10 μg/μl). Our fear conditioning protocol was performed on three consecutive days as follows: Habituation, training and test. Training day protocol consists of 3 minutes of acclimation followed by 5 tone + foot shock pairings. Test day was identical as training but without foot shock to evaluate levels of freezing to the context and to the tone. Our results showed that rats with D-AP5 injection in VH (10 μg/μl) 5 minutes before training differed significantly from vehicle in their levels of freezing when context and tone were evaluated (test day) but no differences were observed during training day. On test day, rats with D-AP5 increased their levels of freezing when the cue (tone) appears, but didn´t showed freezing to the context while vehicle rats showed freezing to both cue and context. These data suggested that different mechanisms are involved in the processing of differential cues related to associative fear learning in ventral hippocampus, however, the study of brain areas like basolateral amygdala and dorsal hippocampus are targets of study in order to describe a robust explanation. These results suggest that differential targets to treat disorders like posttraumatic stress disorder (PTSD) are needed to develop more specific strategies in relation to cues involved in generalization and extinction of fear-related symptoms.

Disclosures: **A. Mora-Gallegos:** None. **J. Fornaguera:** None. **D. Quesada-Yamasaki:** None.

Poster

151. Fear and Aversive Learning and Memory: Acquisition

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 151.03/P22

Topic: G.01. Appetitive and Aversive Learning

Support: Pueblo County
Colorado State University-Pueblo Foundation
Colorado State University-Pueblo Institute of Cannabis Research
Dr. Hasan, Department of Health and Human Services

Title: HU-211-sensitive, cognitive learning and memory processes modulate GluN2B surface expression in the mouse brain

Authors: J. J. VIGIL, S. D. KOCH, A. L. UHERNIK, ***J. P. SMITH**;
Biol., Colorado State University-Pueblo, Pueblo, CO

Abstract: Disorders of learning and memory are often life-long, debilitating, and progressive disorders. Effective treatments for these disorders and their symptoms are lacking due to limited knowledge of the cellular and molecular mechanisms of learning and memory in general. It is well established that the ability to learn and remember depends greatly on a neuron's ability to

regulate its expression of surface receptors. Insertion and removal of the membrane bound GluN2B NMDA receptor subunit has been strongly implicated in the processes of learning and memory, as well as apoptosis, cell survival, and excitotoxicity, while dysfunction of its expression has been implicated in several learning and memory disorders. This study evaluates the effect of a cognitive learning task on the surface expression of the GluN2B subunit in the mouse brain and seeks to determine whether the cannabinoid, HU-211, a putative GluN2B inhibitor, can affect learning and memory. Using a quantitative immunohistochemical approach, it was determined that trace fear conditioning enhanced the surface expression of the GluN2B subunit in select brain regions, and that HU-211 inhibited the acquisition of a trace fear memory. Overall, this suggests that HU-211 may be an effective tool to evaluate the role of GluN2B and its surface trafficking in cognitive learning and memory processes.

Disclosures: **J.J. Vigil:** None. **S.D. Koch:** None. **A.L. Uhernik:** None. **J.P. Smith:** None.

Poster

151. Fear and Aversive Learning and Memory: Acquisition

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 151.04/P23

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant 1P20GM103653

Title: Using high resolution near infrared imaging to measure fear-learning induced changes in AMPA/NMDA ratios throughout the fear circuit

Authors: *B. SHULTZ, N. MOHAMMADMIRZAEI, A. FARKASK, B. COLLINS, D. KNOX;
Psychological and Brain Sci., Univ. of Delaware, Newark, DE

Abstract: Changes in AMPA/NMDA ratios, the two major ionotropic glutamatergic receptors, have been proposed as a neurobiological signature of learning and memory. An increase in AMPA receptors and/or function, with unchanged NMDA receptors and/or function, in the synapse underlies LTP, as well as learning and memory. The increase in AMPA receptor density/function in the synapse is brought on by enhanced shuttling of AMPA receptor subunits into the synapse, intracellular modification of the amino tail of the AMPA receptor, and enhanced translational synthesis of AMPA receptors in the dendrites of neurons. Changes in AMPA/NMDA ratios are typically measured using a combination of pharmacological and patch clamp electrophysiological techniques. The combination of these techniques can be difficult to implement in a lab and changes in AMPA/NMDA ratios can only be examined in a single brain region. High resolution (21 μm) scanning of brain tissue in combination with immunohistochemistry and near infrared imaging may be used to measure changes in

AMPA/NMDA ratios driven by enhanced translation of the AMPA receptor. Emission spectra within the near infrared electromagnetic spectrum has low autofluorescence in brain tissue and can be used as a semi quantitative measure of multiple proteins in a single brain region when combined with the correct high resolution scan. Thus, high resolution near infrared immunohistochemistry might be used to examine changes in AMPA/NMDA ratios throughout the fear circuit after fear learning and memory. To examine this possibility we conducted three experiments. In experiment 1 we subjected rats to fear conditioning (pairing tone- CSs with a footshock UCS), tone-CS presentation (CS-only), or left rats in their housing colony. In experiment 2 we performed a similar procedure except rats were euthanized immediately after fear conditioning of CS-only presentations. Finally, in experiment 3 animals we administered anisomycin, to inhibit synthesis of novel proteins, prior to fear conditioning. While the study is ongoing, preliminary results suggest that high resolution near infrared immunohistochemistry can detect enhanced expression of AMPA/NMDA receptor subunits the mPFC, hippocampus, and amygdala brought on by fear learning and memory. Immunohistochemistry is a relatively simple technique that is widely utilized in many labs and near infrared imaging is becoming more available to laboratories. The preliminary results suggest that AMPA/NMDA ratios can be examined in multiple brain regions in the same animal in different types of learning and memory paradigms by combining high resolution near infrared imaging with immunohistochemistry.

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Poster

151. Fear and Aversive Learning and Memory: Acquisition

Location: Hall A

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Program #/Poster #: 151.05/P24

Topic: G.01. Appetitive and Aversive Learning

Support: Australian Research Council Discovery Grant to NMH and RFW (DP170103952)
Australian Government Research Training Fellowship to MWS

Title: The conditions under which consolidation of conditioned fear requires *de novo* protein synthesis in the basolateral amygdala complex

Authors: *M. J. WILLIAMS-SPOONER[1], R. F. WESTBROOK[1], N. M. HOLMES[2]; [1]Sch. of Psychology, Univ. of New South Wales, Sydney, Australia; [2]Sch. of Psychology, Randwick, Australia

Abstract: The present series of experiments examined the conditions under which consolidation of conditioned fear requires *de novo* protein synthesis in the basolateral amygdala complex (BLA). The experiments used a conditioning protocol in which rats were exposed to pairings of a

serial S2-S1 compound (a tone and light, counterbalanced) and shock, and then tested for freezing (index of fear) to S2. Experiments 1 and 2 demonstrated that, if S1 had been previously paired with shock in a prior stage of training, consolidation of fear to S2 was unaffected by BLA infusions of the protein synthesis inhibitor, cycloheximide, but was disrupted by BLA infusions of the DNA methyltransferase inhibitor, 5-AZA. In contrast, Experiments 3-5 showed that consolidation of fear to S2 was disrupted by BLA infusions of cycloheximide if S1 had not been pre-trained (Experiment 3), had been previously presented in an explicitly unpaired relation to shock (Experiment 4), or had been appetitively pre-trained through prior pairings with sucrose (Experiment 5). Taken together, these results show that the prior training of S1 critically determines the protein synthesis requirement for consolidation of fear S2 in our serial-order conditioning protocol. When S2 is conditioned in compound with an S1 that had not been previously fear-conditioned, consolidation of fear to S2 requires *de novo* protein synthesis in the BLA. When S2 is conditioned in compound with an already-fear-conditioned S1, consolidation of fear to S2 requires DNA methylation, but not *de novo* protein synthesis, in the BLA.
This work is supported by an Australian Research Council Discovery Grant to NMH and RFW (DP170103952), and an Australian Government Research Training Fellowship to MWS.

Disclosures: M.J. Williams-Spooner: None. **R.F. Westbrook:** None. **N.M. Holmes:** None.

Poster

151. Fear and Aversive Learning and Memory: Acquisition

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 151.06/P25

Topic: G.01. Appetitive and Aversive Learning

Support: Australian Research Council Discovery Project Grant to NMH and RFW (DP170103952)
Australian Government Research Training Fellowship to OAQ

Title: The experience of danger changes the way the brain consolidates innocuous information: The roles of the perirhinal cortex and basolateral amygdala

Authors: *O. QURESHI, R. F. WESTBROOK, N. M. HOLMES;
Sch. of Psychology, Univ. of New South Wales, Sydney, Australia

Abstract: Rats exposed to pairings of two innocuous stimuli, S2 and S1 (light and tone, counterbalanced) and then to pairings of S1 and foot shock exhibit defensive responses (freezing) when tested with S2. Our previous work has shown that the perirhinal cortex (PRh), not the basolateral amygdala (BLA), codes the S2-S1 association when the pairings occur in a safe context, whereas the BLA, not the PRh, codes the association in a dangerous context. The present work shows that the BLA is also required for consolidation of the S2-S1 association

when the pairings occur in a dangerous context (Experiment 1). Additionally, neural activity (Experiment 2) and more specifically, NMDAr activation (Experiment 3) in the BLA is required for consolidation when the context is rendered dangerous immediately after the pairings in a safe context. Thus, danger before or after learning about innocuous information alters the neural substrates underlying consolidation of that information: specifically, it shifts the substrates from the PRh to the BLA.

Disclosures: O. Qureshi: None. **R.F. Westbrook:** None. **N.M. Holmes:** None.

Poster

151. Fear and Aversive Learning and Memory: Acquisition

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 151.07/P26

Topic: G.01. Appetitive and Aversive Learning

Support: Pueblo County
Colorado State University-Pueblo Foundation
Colorado State University-Pueblo Institute of Cannabis Research
Dr. Hasan, Department of Health and Humann Services

Title: Fear memory extinction is enhanced by cannabidiol when given during acquisition in female mice

Authors: *A. L. UHERNIK, Z. T. MONTOYA, C. R. TURNER, J. P. SMITH;
Biol., Colorado State University-Pueblo, Pueblo, CO

Abstract: Cannabidiol (CBD) is reported to have therapeutic potential for psychiatric conditions that affect learning and memory, including anxiety and post-traumatic stress disorders. Pre-clinical contextual fear-learning and memory experiments in rodents have commonly been used to test this hypothesis, with recent work showing a memory-enhancing effect of CBD when administered just prior to extinction training in male mice. While this suggests a potential efficacy for CBD in a clinical setting where the aim is to extinguish previously acquired fear-memories, however in our previous published work, we show that learning and memory is modulated by cannabidiol when administered during trace fear-conditioning in C57Bl/6 male mice increasing resistance to extinction. Given that PTSD is more prevalent in women compared to men, we repeated our previous experiments using female C57Bl/6 mice as the subject, we asked the question “Does CBD also enhance the acquisition of fear memory when administered prior to acquisition in female mice?” Interestingly, we saw an enhancement of the level and rate of auditory-cued extinction following a single dose of CBD prior to acquisition and reduced generalized fear. This raises questions about the difference between males and females in the

acquisition of a fear memory indicating a need for more research to answer these before using a potential treatment for learning and memory disorders.

Disclosures: A.L. Uhernik: None. **Z.T. Montoya:** None. **C.R. Turner:** None. **J.P. Smith:** None.

Poster

151. Fear and Aversive Learning and Memory: Acquisition

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 151.08/P27

Topic: G.01. Appetitive and Aversive Learning

Support: PAPIIT 201018
Technical assistant Gabriela Vera-Rivera
Technical assistant Shaun Harris

Title: Characterization of conditioned taste aversion without liquid deprivation: Effects on learning and aversive memory extinction

Authors: *A. ALCALÁ-RAMÍREZ, A. RANGEL-HERNÁNDEZ, M. I. MIRANDA; Neurobiología Conductual y Cognitiva, Inst. de Neurobiología, UNAM, Univ. Nacional Autónoma de México, Queretaro, Mexico

Abstract: Water restriction protocols are common in animal research; many studies use water deprivation as a motivated component during behavior or learning task performance. Conditioned taste aversion (CTA) is a robust associative learning of novel taste (conditioned stimulus) with visceral malaise (unconditioned stimulus). CTA is a very useful experimental model for the neurobiological study of taste recognition memory; however, the procedure includes a significant water deprivation over several days. Liquid deprivation during CTA allows readable measures of associative aversive memory and the subsequent extinction aversive memory rate after first retrieval. During all CTA phases experimental animals are water-deprived; consequently, thirst could be an important motivational component that modulates the strength of conditioning as well as the extinction process. However, few studies have analyzed the deprivation effect on CTA. Therefore, the goal of this research was to characterize a CTA protocol without water privation, by evaluating the strength of taste aversive learning and the memory extinction rate compared to a traditional CTA protocol. Male and female nine-week-old Wistar rats were used. Half of the rats had *ad libitum* access to water throughout the experiment; a control water-deprived group was simultaneously trained to CTA protocol under water deprivation schedule. For *ad libitum* schedule group, during the first 5 days, water basal consumption was measured every hour (11:00 am-4:00 pm) throughout the dark phase. In CTA acquisition, saccharin solution (0.1%) was presented during 1 h at the middle of measurements

(1:00-2:00 pm) *to ad libitum* group. Similarly, 24 h later, at the same time saccharin was presented to evaluate CTA retrieval, as well as during the next three extinction following days. After normalized consumption with respect to CTA acquisition, results showed a significant and stronger CTA in rats with *ad libitum* schedule compared to regular CTA liquid deprivation. Furthermore, water-deprived rats showed greater saccharin consumption than *ad libitum* rats during aversive memory extinction, indicating a slower aversive memory extinction rate in non-deprived rats. These findings demonstrate that water deprivation (e.g., thirst) induces weaker aversive taste association and accelerates memory extinction in comparison to identical CTA procedure conditions, except for water restriction. Overall this data indicates an important modulating effect of liquid satiety during taste learning.

Disclosures: A. Alcalá-Ramírez: None. **A. Rangel-Hernández:** None. **M.I. Miranda:** None.

Poster

151. Fear and Aversive Learning and Memory: Acquisition

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 151.09/P28

Topic: G.01. Appetitive and Aversive Learning

Support: MOST 107-2410-H-006-054
MOST 106-2410-H-006-037

Title: Conditioned taste aversion can be acquired under dexmedetomidine-induced anesthesia in rats

Authors: *C.-H. CHENG, H.-Y. HSIAO, D.-Y. CHEN;
Dept Psychology, Natl. Cheng Kung Univ., Tainan, Taiwan

Abstract: It has been shown that brain and cognitive function may be affected by anesthesia, but certain type of learning and memory can still be performed even under general anesthesia. For example, we have demonstrated that rats could learn a modified inhibitory avoidance task when they were anesthetized by dexmedetomidine, and the amygdala and hippocampus also play important roles in this learning. In the present study, we planned to explore whether if another typical memory task, conditioned taste aversion (CTA), could be learned under anesthesia. It is expected that anesthetized rats may still be able to associate gustatory stimulus with visceral illness, then showed significant taste aversion when they are awake. However, single pairing under anesthesia may be not enough to establish a strong memory. A series of experiments were conducted to determine the number of pairings is required for significant CTA, and evaluate the effect if the illness is given 6 hours later. Water-deprived male Sprague-Dawley rats (250-350 g) were anesthetized by dexmedetomidine (60 μg/kg, s.c.). Thirty minutes later, 1% saccharin solution was injected directly to the tongue of anesthetized rats by pump for 10 min

(speed=15µl/min). Nicotine (1 mg/kg, s.c.) was injected to induce illness in the CTA group, and the control group received saline. After 20 minutes of pairing, rat's tongue was rinsed by water to remove the taste of saccharin, and then atipamezole (0.6 mg/kg, s.c.) was injected to reverse anesthesia. Rats were given 30-min access to water when they returned to home cage. Their CTA were assessed by one-bottle and two-bottle tests in the following days. In Experiment 1, single pairing did not show significant CTA (one-bottle test: $t(14)= 1.094$, $p>.05$; two-bottle test: $t(14)= 0.881$, $p>.05$). In Experiment 2, two-pairings showed significant CTA (one-bottle test: $t(14)= 2.982$, $p<.05$; two-bottle test: $t(14)= 3.471$, $p<.01$). Furthermore, Experiment 3 examined whether if nicotine given 6 hours after saccharin could still induce CTA. The result indicated that rats showed marginal CTA in one-bottle test ($t(10)= 1.838$, $.05 < p < .10$), and very significant CTA in the two-bottle test ($t(10)= 4.923$, $p<.001$). In summary, anesthetized rats could learn CTA after two pairings, and even if the interval was increased up to 6 hours. Further experiments are required to explore the roles of the amygdala or other brain in CTA learning under anesthesia.

Disclosures: C. Cheng: None. **H. Hsiao:** None. **D. Chen:** None.

Poster

151. Fear and Aversive Learning and Memory: Acquisition

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 151.10/P29

Topic: G.01. Appetitive and Aversive Learning

Support: ARC DP160100004

Title: The role of basolateral amygdala parvalbumin neurons in the blocking of Pavlovian fear

Authors: *J. YAU, G. MCNALLY;
Univ. of New South Wales, Sydney, Australia

Abstract: Principal (PN) basolateral amygdala (BLA) neurons are essential for the acquisition, extinction and expression of simple forms of Pavlovian conditioning. During these simple forms of learning, PN activity is tightly regulated by parvalbumin-expressing (PV) BLA interneurons. However, both fear learning and the activity of PNs, are influenced prediction error: the discrepancy between the actual and expected outcomes of a conditioning trial. Whether, when, and how activity of BLA PV neurons contribute to fear prediction errors remains poorly understood. Here, we used PV cre rats to address this. We used fibre photometry to record activity of BLA PV neurons during the associative blocking of learned fear. We show that the US-related activity of these neurons varies with prediction error. Then we used optogenetics to study the causal role of BLA PV neurons in gating fear learning in response to prediction errors and we found that optogenetic inhibition of PV+ neurons around moments of an expected shock

restored prediction error and prevented the associative blocking of Pavlovian fear. Our findings suggest that prediction error acts to regulate fear learning via BLA PV neuronal gating of PN activity.

Disclosures: J. Yau: None. **G. McNally:** None.

Poster

151. Fear and Aversive Learning and Memory: Acquisition

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 151.11/P30

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant DA011806
NIH Grant MH061933

Title: GABA neurons in the prelimbic cortex regulate locomotion and associative learning

Authors: *E. MARRON, M. TIPPS, T. ROSE, B. VO, K. WICKMAN;
Pharmacol., Univ. of Minnesota, Minneapolis, MN

Abstract: A balance between excitation and inhibition in the nervous system is critical for its correct function. Specifically, in the prefrontal cortex, dysregulation of cell excitability is been linked to a number of disorders such as anxiety, schizophrenia and drug addiction. The activity of principal cells is regulated by inputs from projecting neurons but also by local GABAergic interneurons. In the prefrontal cortex GABAergic interneurons form extensive nets and connections with principal cells thus modulating their activity through the release of the inhibitory neurotransmitter GABA. In addition long range GABA terminals can also produce GABA release in the prefrontal cortex. In this work, we used a chemogenetic approach to explore the role of GABA neurons in controlling specific behaviors that have been linked to prefrontal function, specifically the prelimbic sub-region; we hypothesized that GABA neurons of the PrLC, play a role in regulating principal cell excitability and thus controlling learning/behavior. To study the role of PrLC GABA neurons we used a well-established associative learning paradigm, fear conditioning. To selectively target GABA neurons of the PrLC area we stereotaxically injected a Cre dependent AAV expressing an inhibitory DREADD (hM4Di) into the PrLC area of GADcre mice. To complement this approach, we also used a mDlx driven hM4Di to selectively target GABA neurons. Activation of hM4Di with CNO during the adquisition/consolidation phase of the fear conditioning protocol with CNO produced a significant decrease in freezing to both context and cue, suggesting a deficit in learning. Importantly, a similar manipulation in which we introduce an excitatory DREADD (hM3Dq) into principal cells of the PrLC reproduced the same phenotype. A deeper understanding on the

relationship between excitation/inhibition in the prefrontal cortex and the role of could open new avenues for the treatment of disorders such as stress, schizophrenia and drug addiction.

Disclosures: E. Marron: None. **M. Tipps:** None. **T. Rose:** None. **B. Vo:** None. **K. Wickman:** None.

Poster

151. Fear and Aversive Learning and Memory: Acquisition

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 151.12/P31

Topic: G.01. Appetitive and Aversive Learning

Support: Miami University Department of Psychology

Title: Enhanced emotional memory formation and alcohol consumption in mice exposed to early life stress during infancy

Authors: *K. M. SCHUH, E. A. SNEDDON, J. J. QUINN, A. K. RADKE;
Psychology, Miami Univ., Oxford, OH

Abstract: Early life trauma can have negative cognitive and emotional effects, leading to pathologies including depression, anxiety, post-traumatic stress disorder (PTSD), and problems with drugs and alcohol. In the current study, we investigated the effects of early life stress (ELS) by employing an infant stress-enhanced fear learning (SEFL) protocol in mice. Male and female C57BL/6J mice were exposed to ELS on postnatal day (PND) 17, when each mouse received 0 or 15 footshocks (1 mA) over a 1-h period followed by maternal separation for 3 h after the conditioning session. Mice were undisturbed until adulthood (PND 60-90) when they received contextual fear conditioning in a novel context (0 or 1 mA footshock delivered 180 seconds into the session). Fear memory retrieval and extinction were tested by placing mice back in the chamber for 8 min every 24 h for 3 days. Results revealed enhanced fear learning, measured as freezing, in both male and female adult mice that underwent ELS. Increased freezing was not seen in any treatment group in the original ELS context, suggesting that animals did not remember the ELS-paired context. Two weeks later, anxiety-like behavior was tested in an open field maze over a 30-min session. Animals exposed to ELS were more prone to anxious-like behavior, spending significantly more time against the maze walls (thigmotaxis). A subset of the mice were next restricted to 85% of their free-feeding weight and trained to respond for a food reward (14 mg food pellet) on a fixed ratio 1 schedule of reinforcement. Mice were trained to discriminate between an active nose poke (100% probability of reward) and an inactive nose poke (0%). Next, the contingencies of the active and inactive nose pokes were reversed and, after performance reached a pre-set criterion, reversed again. The results revealed no difference between treatment groups, suggesting that ELS did not affect flexible choice learning. In a final

experiment, adult mice exposed to 15 footshocks on PND 17 demonstrated increased alcohol consumption in a two-bottle choice drinking in the dark (DID) paradigm (15% ethanol vs. H2O). Together, the results of our studies demonstrate that infant footshock enhances fear learning and alcohol drinking in adult mice, replicating studies done in rats, without affecting appetitive learning or behavioral flexibility. This model may be useful in studying comorbid PTSD and alcohol use disorder (AUD) and in understanding the neurobiological mechanisms that contribute to ELS effects on vulnerability for anxiety and alcohol dependence.

Disclosures: K.M. Schuh: None. **E.A. Sneddon:** None. **J.J. Quinn:** None. **A.K. Radke:** None.

Poster

151. Fear and Aversive Learning and Memory: Acquisition

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 151.13/P32

Topic: G.01. Appetitive and Aversive Learning

Title: VTA kappa opioid receptor stimulation paired with CRF elicits conditioned place aversion, but kappa stimulation alone does not

Authors: *J. M. R. BASTACKY[1], D. P. DUNN[2], M. C. HALPERIN[1], H. S. KILLEN[1], E. B. MARGOLIS[3], P. J. CURRIE[4];
[1]Neurosci., [2]Reed Col., Portland, OR; [3]Neurol., UCSF, San Francisco, CA; [4]Dept. Psychology, Reed Col., Portland, OR

Abstract: Recent slice electrophysiology findings have demonstrated that kappa opioid receptor (KOR) agonism in VTA dopamine neurons, which is typically inhibitory, becomes excitatory in the presence of the stress response hormone CRF. Previous work has suggested that kappa opioid receptors and stimulation by their endogenous ligand dynorphin are necessary for the aversive component of stress. In this work we examined the effects of central injections of the KOR-specific agonist U69593 (U69) with or without CRF in the same solution on conditioned place preference in rats. Conditioned place preference or aversion was assessed by the difference of time spent in the drug-conditioned chamber before vs after four drug pairings over eight days. U69 alone elicited no effect, but U69 with CRF produced a conditioned place aversion effect. This suggests the necessity of CRF for VTA KOR's aversive properties. In light of electrophysiological evidence, it is possible that the excitation of a subset of VTA dopamine neurons was triggered by the combined drug administration and led to its aversion-inducing effects.

Disclosures: J.M.R. Bastacky: None. **D.P. Dunn:** None. **H.S. Killen:** None. **E.B. Margolis:** None. **P.J. Currie:** None.

Poster

151. Fear and Aversive Learning and Memory: Acquisition

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 151.14/P33

Topic: G.01. Appetitive and Aversive Learning

Support: ARC DE170100392
NHMRC APP1086855

Title: The impact of chronic fluoxetine treatment in adolescence or adulthood on context fear learning and perineuronal nets

Authors: D. CHAN, ***K. D. BAKER**, R. RICHARDSON;
UNSW Sydney, Sydney, Australia

Abstract: Selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine (Prozac), are commonly prescribed pharmacotherapies for anxiety disorders. Preclinical research suggests that fluoxetine may have the potential to reduce the heightened fear seen in anxiety disorders. These studies have shown that fluoxetine markedly alters fear regulation in adult non-human animals; this adjunct impairs the acquisition of context fear conditioning, enhances the rate of extinction of learned fear to cues and reduces relapse of extinguished fear. These effects are associated with altered expression of extracellular matrix structures called perineuronal nets (PNNs) in the amygdala and hippocampus, two brain regions which regulate fear. PNNs are essential for neurodevelopment, they preferentially surround mature parvalbumin (PV) neurons, and they regulate plasticity in the adult brain. However, very little is known about whether fluoxetine has similar behavioural and neural effects in adolescents as in adults, despite estimates that 8-18% of adolescents meet full diagnostic criteria for an anxiety disorder. In the current study, we investigated the effect of fluoxetine exposure during adolescence or adulthood on context fear learning and PNNs in the basolateral amygdala (BLA) and the CA1 subregion of the hippocampus in rats. Adolescent (from postnatal day [P] 30) or adult (P60+) male rats received fluoxetine (~10mg/kg/day) in their drinking water, or water only, for two weeks before context fear conditioning or neural analyses. The behavioral results indicated that fluoxetine differentially affected the adolescents and adults; relative to controls, this adjunct reduced context conditioning in the adults, replicating past research, but not in the adolescents. In contrast, fluoxetine had similar effects on PNNs across age. Adults had a higher number of PV neurons surrounded by a PNN in the BLA and CA1, but fluoxetine increased the number of these neurons at both ages. Although adults also had a higher number of PNNs around non-PV cells than adolescents in the BLA (but not CA1), fluoxetine was without effect on these PNNs. Contrary to previous reports, fluoxetine did not shift the percentage of PNNs toward non-PV cells in either the BLA or CA1 in the adults, and a similar result was found in the adolescents.

These findings demonstrate that fluoxetine differentially affects fear learning in adolescent and adult animals, but this adjunct may not have age-specific effects on PNNs.

Disclosures: D. Chan: None. **K.D. Baker:** None. **R. Richardson:** None.

Poster

151. Fear and Aversive Learning and Memory: Acquisition

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 151.15/P34

Topic: G.01. Appetitive and Aversive Learning

Support: PSC-CUNY Traditional B Award
NIH Grant G12MD007599

Title: Serotonin input to the dorsal BNST contributes to sex differences in fear learning

Authors: *R. RAVENELLE[1], H. YOON[2], E. LIKHTIK[3,1], N. S. BURGHARDT[2,1];
[1]The Grad. Center, CUNY, New York, NY; [2]Psychology, [3]Biol., Hunter College, CUNY, New York, NY

Abstract: Post-traumatic stress disorder (PTSD) is a stressor and trauma-related disorder that is characterized by intense fearful memory formation. Interestingly, women are twice as likely as men to develop PTSD, indicating that there are sex differences in the circuits underlying this disorder. Given that serotonin dysfunction has been implicated in PTSD and serotonin modulates fear learning, we have begun investigating whether serotonin neurotransmission plays a role in this sex difference. Using auditory fear conditioning as a model of fear learning, we first show that enhancing extracellular levels of serotonin throughout the brain with the selective serotonin reuptake inhibitor (SSRI) citalopram differentially affects fear learning in male and female C57Bl/6J mice. Mice were given a single injection of citalopram or saline (i.p.) 60 minutes before fear conditioning, which involved 5 presentations of a tone (2kHz, 85dB, 30s) that co-terminated with a footshock (0.7mA, 2s). During recall testing the next day, the higher dose of citalopram (20mg/kg) enhanced fear memory in both sexes, whereas the lower dose (10mg/kg) only enhanced fear memory in females, indicating that females are more sensitive to increases in serotonin than males. We next tested whether selectively enhancing serotonin in the bed nucleus of the stria terminalis (BNST), a region with known sexual dimorphism, is sufficient to induce a sex difference in fear learning. Serotonergic inputs to the dorsal BNST (dBNST) were targeted using the TpH2-ChR2-EYFP BAC mouse line in which channelrhodopsin (ChR2) is expressed exclusively on serotonin neurons. During auditory fear conditioning, serotonin was stimulated in the dBNST with blue light (473nm, 10mW, 5ms pulses, 20Hz) during each tone presentation and fear memory was tested the next day in the absence of light. Stimulation of serotonin in the dBNST during training led to stronger fear memory the next day in females but not males,

further confirming enhanced sensitivity to serotonin in females. Based on previous work indicating that the 5-HT_{2C} receptor in the BNST plays a role in fear learning, we are currently testing whether optogenetic stimulation of serotonin in the dBNST during fear conditioning activates more 5-HT_{2C} receptor containing cells in females than males. Identification of the mechanism by which serotonin in the BNST increases fear learning in females may provide much needed insight into why women are at a higher risk of developing PTSD than men, potentially leading to novel treatment options for trauma exposed women.

Disclosures: **R. Ravenelle:** None. **H. Yoon:** None. **E. Likhtik:** None. **N.S. Burghardt:** None.

Poster

151. Fear and Aversive Learning and Memory: Acquisition

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 151.16/P35

Topic: G.01. Appetitive and Aversive Learning

Title: Oxytocin signaling modulates the switch from passive to active defensive responses in fear memory circuits

Authors: *R. STOOP[1], R. TRIANA DEL RIO[2], D. SCHEGGIA[3], C. HEGOBURU[4], C. LEROUX[2], T. YIN[5], E. VAN DEN BURG[2];
[1]Psychiatry, Ctr. For Psychiatric Neuroscience, Univ. Lausanne, Lausanne, Switzerland; [2]CNP, Lausanne, Switzerland; [3]Ctr. for Psychiatric Neurosci., CHUV, Lausanne Univ. Hosp., Prilly, Switzerland; [4]CHUV Psychiatrie Neurosci. Ctr., Lausanne Prilly, Switzerland; [5]CIBM, Lausanne, Switzerland

Abstract: In rodents, different defensive behaviors can be expressed when facing danger: passive responses (freezing) or active responses such as escape from the threat (active avoidance). In a previous translational study we had shown how in humans bilateral damage of the basolateral amygdala (BLA) affects active avoidance (AA) to imminent threat by modeling this damage in rats with chemogenetical inhibition of the corresponding BLA region during the expression of AA. Its expression required BLA activation of oxytocin sensitive neurons in the central amygdala (CeA, Terburg et al., Cell, 2018). In the current project, we addressed the question to what extent activation of this pathway is also required during the acquisition of the AA learning. Interestingly, we had found two groups of rats that showed opposite defensive behaviors: high avoiders (HA) successfully acquired AA of the imminent threat in the Threat-and-Escape Test (TET), whereas low avoiders (LA) did not. To assess whether the BLA-CeA projection was important also during acquisition we first developed criteria to categorize the two populations of rats. We observed that the success to learn avoidance of the imminent threat is correlated with low levels of anxiety, higher social interest and group housing. We also correlated the two behavioral populations with a resting state fMRI phenotype that defines

differences in the BOLD activation and functional connectivity of amygdala with the prelimbic cortex. Based on these criteria we selected candidate HA (cHA) rats before TET training. Pharmacological inhibition with oxytocin receptor (OTR) antagonist in the CeA or chemogenetic inhibition in an OTR Cre+ line of cHA rats shows that the activation of oxytocin sensitive neurons in the CeA is necessary to decrease freezing levels and allow AA learning in the TET task. Similarly, chemogenetic activation of CeA OTR+ neurons rescued AA in cHA whose BLA was down-regulated chemogenetically, reflecting the relevance of OTR signaling for switching between freezing and escape behavior. These data support the necessity of oxytocin signaling in the CeA and PL to modulate the plasticity of a circuit during acquisition of avoidance of imminent threats.

Disclosures: R. Stoop: None. **R. Triana Del Rio:** None. **D. Scheggia:** None. **C. Hegoburu:** None. **C. Leroux:** None. **T. Yin:** None. **E. van den Burg:** None.

Poster

151. Fear and Aversive Learning and Memory: Acquisition

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 151.17/P36

Topic: G.01. Appetitive and Aversive Learning

Support: ERC Grant StG678832
Floshield

Title: Testing the theory of epigenetic priming for memory formation

Authors: *A. BURNS[1], P. ARGUELLO-PASCUALLI[1], M. FARINELLI-SCHARLY[2], S. HUGUES[2], J. GRAFF[1];
[1]UPGRAEFF, Brain and Mind Institute, EPFL, Lausanne, Switzerland; [2]E-PHY-SCIENCE, Valbonne, France

Abstract: Over the past decade, the study of epigenetics and the role that chromatin topology plays on gene transcription and downstream pathways that regulate learning and memory has gained considerable attention. Several studies have shown that neurodegenerative disorders, such as Alzheimer's disease, are characterized by disease-relevant changes in epigenetic signals, and that some of the memory-related deficits can be ameliorated by increasing histone acetylation levels via an HDAC-inhibitor (HDACi) treatment. Based on the evidence gathered thus far, it has been suggested that epigenetic signals can target specific genes in order to 'prime' them for transcription (Gräff and Tsai, 2013, Nat Rev Neurosci). While this theory has gained experimental support in cancer and developmental biology, it still remains to be tested in the context of learning and memory. In this project, we test the concept of epigenetic priming by determining the targets and effects of HDAC inhibition during contextual fear conditioning on an

epigenetic, transcriptional and electrophysiological level. We have found that long-term potentiation (LTP) is increased in the hippocampus after combined HDACi treatment and fear conditioning, but not by either treatment alone. Conversely, no such effect was observed in the striatum, a brain region that is not a direct target of fear conditioning, despite showing similarly reduced HDAC activity. Additionally, we have used bulk and single cell sequencing methods to determine how memory related genes and pathways are differentially targeted in the hippocampus. These results will shed light on the mechanisms of HDACi-mediated epigenetic priming and may pave the way for potential new therapies against memory-related disorders.

Disclosures: A. Burns: None. **P. Arguello-Pascualli:** None. **M. Farinelli-Scharly:** A. Employment/Salary (full or part-time):; E-phys Science. **S. Hugues:** A. Employment/Salary (full or part-time):; E-Phys Science. **J. Graff:** None.

Poster

151. Fear and Aversive Learning and Memory: Acquisition

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 151.18/P37

Topic: G.01. Appetitive and Aversive Learning

Support: ERC Grant StG 678832

Title: An epigenetic contribution to neuronal competition during memory formation

Authors: *G. SANTONI, J. GRAFF;
UPGRAEFF, Brain and Mind Institute, EPFL, Lausanne, Switzerland

Abstract: Over the past years, multiple lines of evidence have shown that memories appear to be stored in a small set of neurons scattered throughout the brain, so-called engram cells. During encoding there are a large number of activated cells that are suitable to build the memory trace, but only a few of them will persist and truly define a stable engram, or memory trace. Whether the allocation of a neuronal cell into a memory engram occurs randomly or through a specific mechanism has been at the forefront of modern research in this field. Based on these studies, we now know that the neuronal competition to participate in the memory engram is not random. In fact, many studies have revealed that it is a defined electrophysiological signature that will allocate specific neurons to the memory trace. However, the molecular mechanisms - i.e. the transcriptional and epigenomic programs and their dynamic response to environmental stimuli - that determine the allocation of a given neuron into the memory trace remain to be determined. Consequently, the goal of this project is to determine whether and to what extent epigenetic mechanisms prime neurons to be allocated to a given memory and the underlying epigenomic and transcriptomic mechanisms thereof. We are focusing on histone acetylation as an epigenetic mechanism since several studies shown that histone acetylation, catalysed by histone

acetyltransferases (HATs) or HDAC inhibitors (HDACi), generally promotes cognitive performance, whereas histone deacetylation, catalysed by histone deacetylases (HDACs), tends to negatively regulate memory performance.

Disclosures: G. Santoni: None. **J. Graff:** None.

Poster

152. Human Motivation and Emotion I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 152.01/P38

Topic: G.03. Emotion

Support: CONICET
FONCYT-PICT (2017-1818, 2017-1820)
CONICYT/FONDECYT Regular (1170010)
FONDAP (15150012)
Global Brain Health Initiative
INECO Foundation
Inter-American Development Bank (IDB)

Title: Multidimensional markers of a novel sync based measure of interoception

Authors: *S. FITTIPALDI[1,2], S. ABREVAYA[1,2], L. DE LA FUENTE[1,2], A. BIRBA[1,2], P. SALAMONE[1,2], M. HILDEBRANDT[3], S. ALARCO MARTI[1], M. MARTORELL[1,2], A. YORIS[1,2], A. GARCIA[1,2,4], L. SEDENO[1,2], A. IBANEZ[1,2,4,5];
[1]Lab. of Exptl. Psychology and Neurosci. (LPEN), Inst. of Cognitive and Translational Neurosci. (INCyT), Buenos Aires, Argentina; [2]Natl. Scientific and Tech. Res. Council (CONICET), Buenos Aires, Argentina; [3]Chair for Clin. Psychology and Behavioral Neurosci., Dresden, Germany; [4]Fac. of Education, Natl. Univ. of Cuyo (UNCuyo), Mendoza, Argentina; [5]Ctr. for Social and Cognitive Neurosci. (CSCN), Sch. of Psychology, Univ. Adolfo Ibanez, Santiago, Chile

Abstract: Introduction: Interoception (i.e., the sensing of inner-body signals) has proven relevant for constraining socio-emotional processing models and advancing clinically applicable tools for neuropsychiatric conditions. Research on this area is typically based on heartbeat detection (HBD) tasks, in which subjects track their own heartbeats to yield a measure of interoceptive accuracy (IA). However, the lack of standard methods to calculate IA, alongside major caveats in the most typical measurement approaches, has led to inconsistencies and controversies in the field. Against this background, the present study aimed to validate a newly developed IA measure that overcomes several recognized limitations of other metrics. Specifically, our measure taps on the subjects' ability to dynamically adjust their HBD

performance in synchrony with cardiac frequency changes, thus offering a more sensitive approach to the phenomenon.
Methods: We obtained electrocardiographic and high-density electroencephalographic recordings from 114 healthy volunteers (aged between 17 and 84) as they performed a validated HBD task in which they were instructed to press a key following their heartbeats. IA was calculated as the absolute difference between cardiac frequency (mean R-R) and inter-response intervals. A subsample of participants also took part in a resting-state fMRI session (n = 72) and a social cognition assessment (n = 50). We analyzed the associations of IA index with canonical markers of interoception, including modulations of the heart-evoked potential (HEP), functional connectivity (FC) patterns, and socio-cognitive performance. Additionally, results were compared with those obtained through the use of typical interoceptive indexes (Schandry and d').
Results: IA was correlated with HEP amplitude in a window of 300-400 ms after the R-wave peak over fronto-central topographies. Also, IA was associated with FC among interoceptive hubs, including the insular, anterior cingulate, and postcentral cortices. Finally, IA was correlated with the capacity to recognize negative emotions. Furthermore, all these associations were stronger and more specific than for Schandry and d' indexes.
Discussion: Our synchrony-based IA measure was related to multidimensional markers of interoception, highlighting its validity. The development and dissemination of this and other robust interoceptive metrics is crucial to achieve consensus and foster progress in the field.

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Poster

152. Human Motivation and Emotion I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 152.02/P39

Topic: G.03. Emotion

Title: Intersubject correlation analysis of EEG rhythm during listening to music

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Abstract: Previous studies have suggested the relationship between brain activity and emotion during listening to music. BoSC system (Boundary Surface Control system) is a three dimensional sound field reproduction system based on the boundary surface control principle. We investigated the neural mechanism of comfortable and uncomfortable emotion evoked during listening to music reproduced by the BoSC system. We utilized intersubject correlation (ISC) analysis of brain activity measured by electroencephalogram (g.USBamp, g.tec Medical

Engineering GMbH, Austria). The sampling frequency was 512Hz. Twelve healthy male participants (aged 21.8 ± 0.55) took part in this study. The participant listened to two music pieces in two experimental conditions. One of the musical stimuli was a classical orchestra piece, which is considered to be comfortable ("Symphony No.4 Italia Mov.1", Music A), and another was an avant-garde musical piece, which is considered to be not comfortable ("Acousmonium", Music B). Each musical stimulus was played by using two front loudspeakers (2-ch condition) or all loudspeakers (96-ch condition) of the BoSC system. The participants answered a short questionnaire asking for their subjective evaluation of the musical stimulus. The participants rated Music A as significantly more comfortable than Music B in both conditions ($p<.05$). The EEG data were transformed to time-frequency domain by using Wavelet transform, and then submitted to ISC analysis. ISC was calculated by utilizing general linear model (GLM) for each frequency band. In delta frequency, the ISC analysis showed significant ISCs in Music A at the frontal and posterior electrodes ($p <.05$), and in Music B at the midline electrodes ($p<.05$). Significant ISC differences were in the 96-ch condition at the frontal electrodes (Music A > Music B, $p<.05$) and in both conditions at the midline electrodes (Music A < Music B, $p<.05$). We suggest that the delta activation in the frontal region is related to comfortable emotions, while the delta activation in the midline region is related to uncomfortable emotions evoked by listening to music.

Disclosures: Y. Inagaki: None. **S. Shimada:** None.

Poster

152. Human Motivation and Emotion I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 152.03/P40

Topic: G.03. Emotion

Support: NSERC Grant, RGPIN-2017-05832
CIHR Grant, MOP-130516
NSERC CGS-M
NSERC CGS-D
Department of Medicine, McGill University
Graduate Mobility Award, Gouvernement du Québec
CRBLM Student Stipend

Title: Isolating the neural correlates of processing socio-emotional threat and ambiguity across modalities with influence of individual differences: An fMRI-adaptation study

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(BRAMS), Montreal, QC, Canada; [5]The Ctr. for Res. on Brain, Language and Music (CRBLM), Montreal, QC, Canada; [6]Univ. Nacional de Quilmes, Buenos Aires, Argentina

Abstract: The neuroimaging literature of socio-emotional processing has largely focused on the visual domain, particularly facial expressions. The current project aims to bridge the gap in our understanding of auditory emotional processing and to assess how the amygdala responds to each of these two modalities. To do so, we measured behavioural and neural correlates of graded perceptions of threat, namely, anger (direct threat), fear (indirect threat) and, most interestingly, subject-specific perceptions of ambiguous threat, expressed through non-linguistic vocalizations and faces. Healthy subjects (N=29) were first presented with morphed stimuli of each modality, created along a fear-anger continuum while performing a two-alternative forced-choice task (fear/anger). The conditions were presented in a counterbalanced, pseudo-randomized order; as to analyze anticipated behavioural and neural adaptation effects using fmri-adaptation (fmri-a). This technique allowed for investigation into the functional preference of discrete neuronal populations in processing emotional information. Subject-specific psychometric curves were built, and the morph level corresponding to maximal perceived ambiguity calculated. Visual and auditory subject-specific stimuli representing clear anger, clear fear and ambiguity were then presented while subjects underwent fMRI with a fast (TR=529ms), high-resolution (2 mm^3 isotropic) multiband sequence. Behavioural results showed a bias towards auditory anger and visual fear, as well as greater task-difficulty for ambiguous stimuli of either modality. Adaptation effects were observed within-modality, where subjects responded to ambiguous stimuli as more fearful when anger was presented prior, and vice versa for anger. Imaging results revealed increased activity in the saliency and central executive networks and deactivation within the default mode network for ambiguous stimuli. Additionally, amygdala activity reflected biases observed in the behavioural findings, with stronger activity for auditory anger and visual fear conditions. The fMRI-a analysis revealed adaptation in the saliency network in response to ambiguous emotion perception, regardless of modality, as well as adaptation in frontal regions in response to anger. Results provide a first look into the neural perception of emotional ambiguity and subject-specific biases of threat perception across modalities. They also provide new insights into amygdala function in tasks requiring simultaneous recruitment of emotional and cognitive processes, as well as novel findings of behavioural and neural adaptation in response to ambiguous emotion.

Disclosures: J.C. Whitehead: None. **I. Spoiusas:** None. **J.L. Armony:** None.

Poster

152. Human Motivation and Emotion I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 152.04/P41

Topic: G.03. Emotion

Support: Deanship of Research, Jordan University for Science and Technology 41/2013

Title: Headache in patients with musculoskeletal pain: A link to vitamin D and calcium

Authors: *K. K. ABDUL-RAZZAK[1], A. ALHUSBAN[1], M. AL-FARRAS[2];
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Abstract: Headache is a nonspecific compliant that might present in the setting of a more serious disease or as a separate problem. Anxiety and depression are common among patients with headache. Chronic pain and Psychological symptoms and are closely related and often coexist. Musculoskeletal pain, fatigue and psychological symptoms are symptoms accompany vitamin D deficiency.

The aim of this study was to investigate the association between vitamin D deficiency, anxiety, depression and headache in patients with non-specific musculoskeletal pain.

A cross-sectional study was conducted involving 215 participants (83.7% females and 16.3% males) with MSP. Participants answered questions regarding their demographics, headache and psychological symptoms The Hospital Anxiety and Depression Scale (HADS) was used to assess psychological symptoms. Vitamin D, parathyroid hormone and plasma calcium levels were measured.

Pearson correlation and Chi-square (X^2) test were performed to find the correlation and the association respectively between variable of interest. Paired t-test was used to compare the differences between two groups. Findings with p value <0.05 were considered to be statistically significant.

The results of this study revealed that self-reported headache, vitamin D deficiency, anxiety and depression were common among outpatients with MSP (82.8% , 68.37%, 60.46%, respectively). Headache more common in patients with lower intake of daily calcium. Patients with headache were more likely to report higher levels of anxiety. HADS-Anxiety was negatively correlated with total daily intake of calcium, participants' age and positively correlated with HADS-Depression.

There was a strong association between headache and neck pain. Logistic regression analysis for predictors of headache showed that female gender and higher HADS-anxiety scores (11-21) are significantly associated with headache, OR=2.298 (1.021- 5.171), p=0.044, and OR =2.606(1.158- 5.866), p=0.021, respectively. Relative to baseline, all measured outcome parameters significantly improved after vitamin D supplementation with increased intake of dairy products.

In conclusion, headache is prevalent among patients with MSP. It is associated with increased anxiety levels. The predictors of headache were female gender and clinical anxiety score. Supplementation with vitamin D improved MSP and associated disorders.

Disclosures: K.K. Abdul-Razzak: None. **A. Alhusban:** None. **M. Al-Farras:** None.

Poster

152. Human Motivation and Emotion I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 152.05/P42

Topic: G.03. Emotion

Title: Personality traits, emotionality, and striatal dopamine D_2 receptors in substance use disorder and obesity

Authors: *V. RAMIREZ[1,2], C. E. WIERS[4], G.-J. WANG[3], N. D. VOLKOW[5];
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Abstract: Self-constraint and emotionality are implicated in drug abuse and obesity, which may be due to an impaired dopaminergic system. We studied differences in personality traits between abusers of different drugs, obese, and healthy controls, and their association with dopamine D_2 receptor availability (D2R). Using the Multidimensional Personality Questionnaire (MPQ), we characterized Positive Emotionality (PEM), Negative Emotionality (NEM), and Constraint (CON) and compared obese individuals (OB), alcohol abusers (AA), marijuana abusers (MA), and cocaine abusers (CA) with healthy controls (HC). In CA and HC, we measured associations between MPQ traits with D2R in the caudate, putamen, and ventral striatum, using PET and [^{11}C]raclopride. CASES (i.e., OB, AA, MA, and CA combined) showed lower PEM (p = .025) and CON (p = .003) and higher NEM (p < .0001) compared to HC. A MANCOVA model of ALL Groups (i.e., OB, AA, MA, CA, and HC combined) showed a group effect only on NEM (p < .0001) and CON (p = .002). Specifically, NEM was higher in AA (p < .0001), CA (p < .0001), and MA (p < .0005), but not in OB (p > .05) relative to HC. Only CA showed lower CON (p = .009) and PEM (p= .023) than HC. NEM and CON were negatively correlated for ALL Groups (r= .26, p < .0001) and for OB (r= .45, p = .002) and CA (r= .22, p = .02). Consistently, NEM indirectly affected CON (Sobel t = -3.516, S.E. = 0.533, p < .0005) and viceversa (t = 2.192, SE = 0.310, p = .028). D2R was lower in CA (p < .0001) compared to HC in all striatal areas, but it did not correlate with MPQ traits. The consistent increases of NEM in drug abuse groups highlights its crucial role in addiction phenotype but not in that of obesity. The lack of linkage between D2R and MPQ traits indicate that negative emotions seen in drug abuse may not be rooted in dopaminergic receptors systems. Future studies should explore if NEM directs vulnerability factors for addiction or obesity.

Disclosures: V. Ramirez: None. **C.E. Wiers:** None. **G. Wang:** None. **N.D. Volkow:** None.

Poster

152. Human Motivation and Emotion I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 152.06/Q1

Topic: G.03. Emotion

Title: Relationship between scholarship and self-esteem levels in obese adult patients

Authors: *N. V. VEGA-CABRERA[1], M. BAUTISTA-ÁVILA[2], L. E. VEGA-CABRERA[3], O. A. JARAMILLO-MORALES[4];
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Abstract: Background. Obesity can have physical or psychological consequences, within these last, we have emotional distress, loss of self-esteem and high levels of anxiety and depression .However, it is not well established whether education level participates in the detriment of self-esteem in obese adult patients . Objective. Determine the level of self-esteem in obese adult patients attending the outpatient of General Hospital Area Family Medicine Unit N° 8 in the Mexico city. Materials and methods. Cross-sectional descriptive study. Inclusion criteria: age greater than 18 years, Mexican Institute of Social Security patients, regardless of gender or scholarship . Sample: 234 patients. CI: 90%. Instrument: Coopersmith Self-Esteem Inventory (for adults). Dependent variable: levels of self-esteem and independent variable: obesity. Results. A ratio of 79.9% was obtained women and 20.1% men. With respect to self-esteem, a frequency of 7.3% low self-esteem, 14.5% average low self-esteem, high average 59.8% and 18.4% self-esteem high esteem in obese patients was found. Furthermore, it was shown that levels of self-esteem may increase by the degree of studies.Conclusion. The results show the first evidence indicating positive levels of self-esteem in obese adults attending the outpatient HGZ / UMF No. 8 mainly related to educational characteristics of each individual.

Disclosures: N.V. Vega-Cabrera: None. **M. Bautista-Ávila:** None. **L.E. Vega-Cabrera:** None. **O.A. Jaramillo-Morales:** None.

Poster

152. Human Motivation and Emotion I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 152.07/Q2

Topic: G.03. Emotion

Support: MOST 105-2410-H-007027-MY2

Title: Effective connectivity of reward network in high and low sense of humor

Authors: Y.-C. CHAN[1], W.-C. HSU[2], Y.-C. CHEN[1], P. LI[3];
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Abstract: Introduction: Dynamic causal modeling (DCM) analysis allows inferences about effective connectivity between neural systems and, in the present study, about differences related to the two groups and two reward processing (anticipation and outcome) during the humor processing. Studies within the newly emerging field of affective neuroscience have examined the neural mechanisms underlying motivation and emotion during humor processing (Chan et al., 2018). The feeling of amusement has been shown to be associated with the *mesolimbic reward system* (MRS), including the amygdala, midbrain, NAc, OFC, and vmPFC (Chan et al., 2018). The MRS is related to dopamine release and it appears that the midbrain, particularly the ventral tegmental area (VTA) and substantia nigra (SN), is involved with dopamine neurotransmission during humor appreciation (Mobbs et al., 2003), while the amygdala plays a core role in the feeling of pleasure experienced during the receipt of social rewards (Rademacher et al., 2010). Participants: Fourteen right-handed participants with a 'high sense of humor' (HSH) and 14 participants with a 'low sense of humor' (LSH), matched for age and education, were recruited for this study. All experimental protocols performed were approved by the Research Ethics Committee. Task and stimuli: The reward processing, reward task and humor stimuli were based on Chan et al.'s (2018). fMRI data acquisition: Imaging was performed using a 3 T Siemens scanner. Each of the four functional runs consisted of 280 volumes. Thirty-six interleaved slices (no gap) were acquired per volume Structural scans were a T1-weighted spoiled grass. Imaging analysis: The fMRI data preprocessing and analysis were performed by SPM 12. In order to reveal the underlying effective connectivity between groups, DCM (Friston et al., 2003) and Parametric empirical Bayes (PEB) (Friston et al., 2016) was employed. Results: A significance threshold of posterior probability (Pp) > 0.95 was set. HSH showed greater connectivity from midbrain to vmPFC than LSH during reward anticipation phase, while HSH showed greater connectivity from amygdala to midbrain than LSH during reward outcome phase. Discussion: The present study demonstrated that the effective connectivity from amygdala to midbrain was

significant in HSH. The findings might represent that effective connectivity from amygdala to midbrain was sensitive to the affective amusement of humor appreciation. Previous humor studies showed that amygdala and midbrain activated while appreciating humorous stimuli (Chan et al., 2018).

Disclosures: **Y. Chan:** None. **W. Hsu:** None. **Y. Chen:** None.

Poster

152. Human Motivation and Emotion I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 152.08/Q3

Topic: G.03. Emotion

Support: MSCA-IF-EF-ST Grant 795994

Title: Reproducibility of the emotion regulation network using fMRI at ultra-high magnetic field

Authors: *S. BERBOTH[1], C. WINDISCHBERGER[2], C. MORAWETZ[3];
[1]Charité Universitätsmedizin Berlin, Berlin, Germany; [2]Med. Univ. Vienna, Vienna, Austria; [3]Med. Univ. Vienna, Wien, Austria

Abstract: Introduction. In recent years, a myriad of neuroimaging studies has investigated the neural basis of emotion regulation (ER), and substantial progress has been made toward building neurally plausible models of ER. However, the stability of the ER network (i.e. the reproducibility of the activated regions) remains unknown. In this study, we aimed to characterize the test-retest reliability of activation during an ER fMRI task.
Methods. 22 participants (18 female, age: M=22.6±3.2yrs) performed a well-established ER task (see Fig. 1A for details) during three scanning sessions separated by one week. We acquired four runs/session and 80 trials/session using the CMRR multiband EPI sequence (TR=1.4s; TE=23ms; 78 slices; voxel size=1.5x1.5x1.2mm^3) at ultra-high field (7 Tesla).
Results. A repeated-measures ANOVA of the emotional state ratings indicated a significant main effect of ER condition (F(1,21)=44.05, p<0.001), no significant main effect of session and a significant interaction effect between ER condition and session (F(1.53,32.16)=16.25, p<0.001). Participants felt significantly less negative during Decrease compared to Look (t(21)=6.64, p<0.001). At the neuronal level, contrasting Decrease versus Look across the three sessions revealed increased activity in bilateral ventrolateral and dorsolateral prefrontal cortex (VLPFC and DLPFC), parietal and temporal regions, and the SMA (Fig. 1B, indicated in purple). This ER network was consistently activated during all three sessions (Fig. 1B). Region-of-interest analyses of the PFC regions revealed a significant main effect of ER condition (VLPFC: left: F(1,21)=8.41, p=.009; right: F(1,21)=11.82, p=.002; DLPFC: left: F(1,21)=8.46, p=.008; right: F(1,21)=17.23, p<0.001), but no significant effect of session and no significant

interaction effect between ER condition and session.

Conclusions. Our findings provide evidence for highly replicable and robust ER networks across multiple scanning sessions that include bilateral PFC regions, which might represent promising candidate biomarkers.

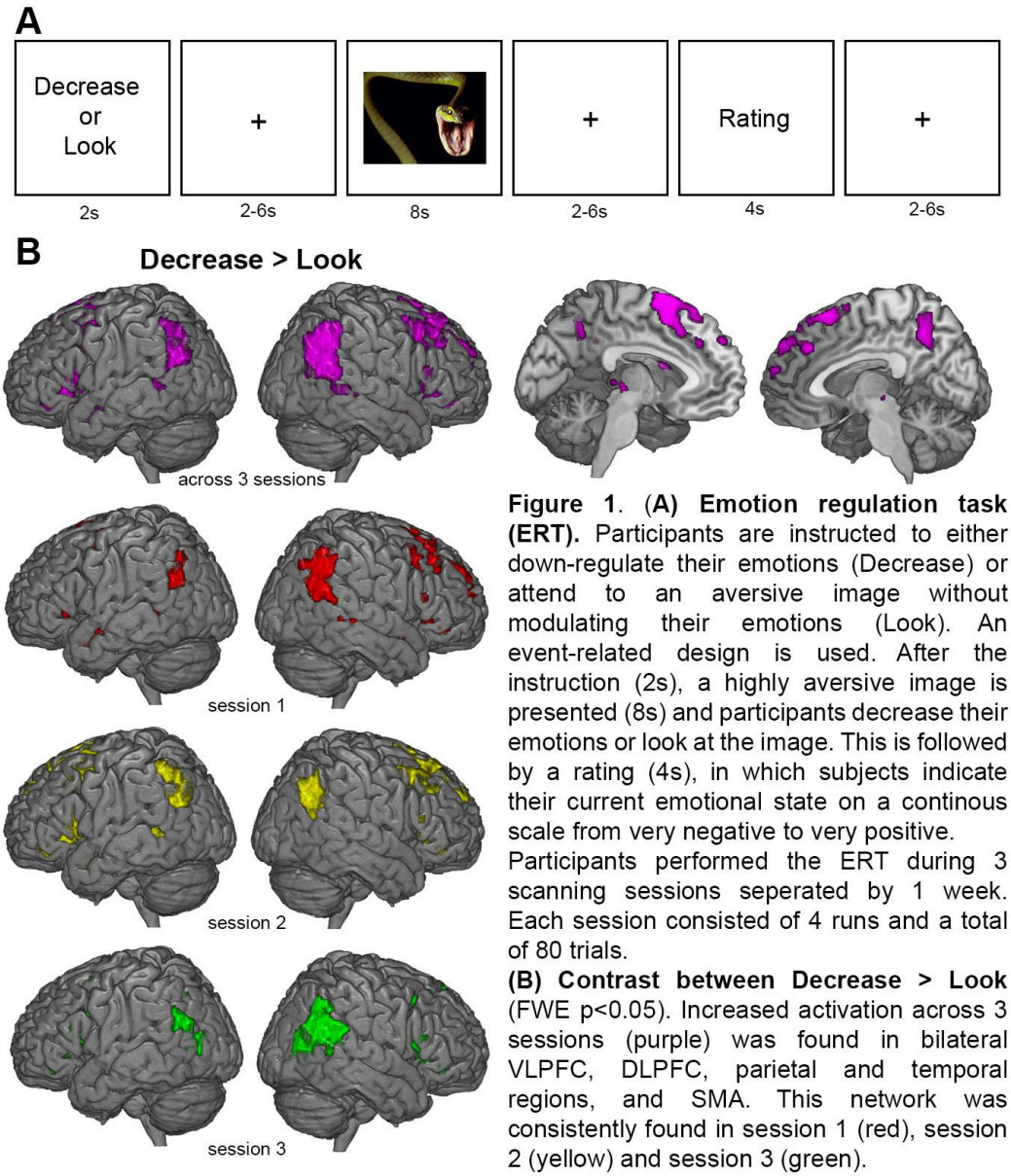

Figure 1. (**A) Emotion regulation task (ERT).** Participants are instructed to either down-regulate their emotions (Decrease) or attend to an aversive image without modulating their emotions (Look). An event-related design is used. After the instruction (2s), a highly aversive image is presented (8s) and participants decrease their emotions or look at the image. This is followed by a rating (4s), in which subjects indicate their current emotional state on a continous scale from very negative to very positive. Participants performed the ERT during 3 scanning sessions seperated by 1 week. Each session consisted of 4 runs and a total of 80 trials.
(B) Contrast between Decrease > Look (FWE $p<0.05$). Increased activation across 3 sessions (purple) was found in bilateral VLPFC, DLPFC, parietal and temporal regions, and SMA. This network was consistently found in session 1 (red), session 2 (yellow) and session 3 (green).

Disclosures: S. Berboth: None. **C. Windischberger:** None. **C. Morawetz:** None.

Poster

152. Human Motivation and Emotion I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 152.09/Q4

Topic: G.03. Emotion

Support: FEDER Funds/Spanish Ministery of Science, Innovation and Universities -- National Agency PSI2015-69664-P
ICREA Academia
BES-2013-067440

Title: Reward-related oscillatory activity associated with gossip information

Authors: *J. MARCO-PALLARES, H. ALICART;
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Abstract: Information is critical for survival and different studies have shown that it might recruit areas of the reward network. Among different types of information, gossip is one of the most engaging ones as shown by its widespread presence in different societies and cultures. However, the neural mechanisms underlying gossip processing are not well understood. The goal of the present study was to uncover the neural oscillatory correlates of the anticipation and processing of novel information (as an intrinsic cognitive reward) depending on the degree of elicited curiosity and the content of the information, including social information and gossip. 24 healthy volunteers participated in this EEG experiment. The task consisted of 150 questions and answers divided into three different conditions: trivia-like questions, personal-gossip information about celebrities and personal-neutral information about the same celebrities. Behavioral results showed that, although it was rated with intermediate values of curiosity and satisfaction compared to the other two conditions, gossip was the information which was better remembered in a surprise test performed one week after the EEG study. In addition, electrophysiological results revealed an increase in the P300 amplitude and the beta oscillatory activity of gossip information compared to personal and trivia-like information. These results demonstrate that gossip information presents a high arousal and salience, which is accompanied by a rewarding effect evidenced by the increase of beta oscillatory power and the recruitment of areas of the brain reward network as shown in previous fMRI studies. This combined activation would be in the bases of the differential processing, encoding and recall of gossips, as well as in its engaging and motivational nature.

Disclosures: **J. Marco-Pallares:** A. Employment/Salary (full or part-time):; University of Barcelona. **H. Alicart:** None.

Poster

152. Human Motivation and Emotion I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 152.10/DP09/Q5

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: G.03. Emotion

Support: Center of Innovation Program
JSPS KAKENHI 16H05958
JSPS KAKENHI 16K13507

Title: Emotional tactile stimulation device for non-invasive neuroimaging

Authors: *N. KANAYAMA[1], M. HARA[2], J. WATANABE[3], R. KITADA[4], M. SAKAMOTO[5], S. YAMAWAKI[6];
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Abstract: *Background* The physical contact with objects can cause some emotional feelings, which may play an important role in recognizing them and maintaining our own homeostasis. As compared to vision, less neuroimaging studies have been conducted. One of the issues with neuroimaging studies on emotion induced by touch is lack of common experimental setup and stimulus sets that can be used across different studies; while stimulus presentation systems for vision and audition are available. To conduct a neuroscience study about emotional touch, researchers need to produce customized device for stimulus presentation in touch. In order to address this issue, we developed an MRI-compatible tactile stimulator that can be flexibly used to stimulate the human skin. This device can present a piece of material by producing lateral motion of the material on the human skin. The purpose of the present study is two folds: (1) to confirm that this stimulator can present stimuli with negative and positive valence and (2) compatibility with functional neuroimaging (e.g., safety in MRI scanner, low level noise on EEG and MRI, etc.).
Methods and results We have measured the emotional response using visual analogue scale when participants get touch to various materials controlled by this device, compared to the emotional stimulus sets in visual and auditory modalities. For tactile stimulus, we have used 8 different materials (Satin, fur, Poron sponge, urethane, human skin gel, GelGems, plastic turf, and bristle) to induce emotional response. For visual and auditory stimulus sets, 6 pictures and sounds for positive, neutral, and negative emotion (in total 18 pictures) were selected from IAPS

and IADS. We adopted two types of pressure (intense/weak) and three stimulation speeds (1 cm/s, 3 cm/s, 30 cm/s) for tactile stimulations. We obtained positive and negative emotional responses by stroking palm and back of hand, which were comparable with the emotional stimulus sets in the visual and auditory sensory modalities. Also the level of noise caused by the device drive was confirmed to negligible to conduct EEG (< 10dB at 50 Hz) and fMRI (loss rate of tSNR < 5 %) experiments.
Conclusion These results demonstrated that the developed device could be used for cognitive-affective neuroscience research using together with EEG recording and fMRI scan. Hence, the device should help in standardizing affective tactile stimulation for and raising the level of researches in psychology and cognitive neuroscience.

Disclosures: **N. Kanayama:** None. **M. Hara:** None. **J. Watanabe:** None. **R. Kitada:** None. **M. Sakamoto:** None. **S. Yamawaki:** None.

Poster

152. Human Motivation and Emotion I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 152.11/Q6

Topic: G.03. Emotion

Title: Predicting emotion regulation success from distributed patterns of event-related potentials during anticipation and implementation of different regulation strategies

Authors: E. SCHUBERT[1], J. AGATHOS[1], M. BRYDEVALL[1], D. FEUERRIEGEL[1], P. KOVAL[1], C. MORAWETZ[2], ***S. BODE**[1];
[1]Melbourne Sch. of Psychological Sci., The Univ. of Melbourne, Melbourne, Australia; [2]Med. Univ. Vienna, Vienna, Austria

Abstract: The ability to control one's emotions, termed emotion regulation (ER), is vital for everyday functioning. It is important to understand the factors influencing ER success, including processes relating to the anticipation of ER strategy use (i.e. prior to active regulation) and implementation (i.e. during active regulation) of ER strategies. The present study investigated whether brain activity during the anticipation and implementation of two widely-studied ER strategies - distraction and reappraisal - was related to regulation success. Brain activity was recorded using electroencephalography (EEG) while participants (N = 27; 20 female; 7 male) were presented with negative images from the Nencki Affective Picture System (NAPS) that were selected to evoke either disgust or sadness. Preceding each trial, participants were cued either to passively view an image, or to use distraction or reappraisal to decrease their emotional responses to the images. ER success scores were calculated from subsequent self-reported disgust and sadness ratings. Multivariate support vector regression was used to predict ER success scores from small moving analysis time-windows of spatiotemporal patterns of event-

related potentials recorded during the anticipation stage (before image presentation) and implementation stage (during image presentation) for each ER strategy separately. ER success for reappraisal could be predicted from patterns of brain activity during anticipation, while ER success for distraction could be predicted during implementation. The results were highly similar for sadness and disgust. These findings suggest that anticipatory cognitive processes form a key determinant of reappraisal success but may not be similarly important for distraction. This may reflect the fact that reappraisal is a more cognitively demanding ER strategy than distraction, requiring enhanced preparation of mental resources.

Disclosures: S. Bode: None. **E. Schubert:** None. **J. Agathos:** None. **M. Brydevall:** None. **D. Feuerriegel:** None. **P. Koval:** None. **C. Morawetz:** None.

Poster

152. Human Motivation and Emotion I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 152.12/Q7

Topic: G.03. Emotion

Support: KAKENHI, No.17K01826

Title: Relationship between empathy and conformity based on behavior measurement and brain function measurement

Authors: *M. ASAKA[1], Y. HOSOKAWA[2], A. OMURA[3], H. SATO[3], H. KAWAGUCHI[1]; [1]Dept. of Life Sci., [2]Grad. Sch. of Life Sci., Toyo Univ., Itakura, Gunma, Japan; [3]Dept. of Biosci. & Engin., Shibaura Inst. of Technol., Saitama, Saitama, Japan

Abstract: In recent years, there is a growing interest in the conforming behavior on SNS, yet no clear explanation has been offered as to how individual differences in empathy affects the degree of conformity to opinions of others in the anonymous. The objective of this study was to examine the relationship between conformity and empathy through a conformity experiment that employed an SNS-like setting. Our subjects, 28 student volunteers, participated in an Instagram-like conformity task while they wore a fNIRS device. We measured the rate at which each subject conformed to the evaluation by others and how the subject's cerebral blood flow changed during the task. In addition, each subject was asked to complete an empathy scale questionnaire. In the conformity task, we observed whether or not the subjects "like" a graphic image when it was displayed together with a number of "likes" by others on a computer screen. The images used in this task were those that had actually been posted on Instagram; and those with less than 50 likes were classified as "low-quality" and those more than 10,000 likes as "high-quality". The number of "likes" to be displayed was randomly selected from the range of 0 to 50 likes (condition of "low evaluation by others") or from the range of 10,000 to 99,999 likes (condition

of “high evaluation by others”) regardless of the image quality. Thus, we had 4 different conditions based on the “quality” and the “evaluation by others”. The number of times a subject conformed to the evaluation by others was divided by the total number of trials to obtain the subject’s conformity rate. Also, 52 fNIRS channels were positioned from the frontal to temporal lobes with the frontal pole (Fpz) as the reference position. The questionnaire scores revealed the individual differences in orientation: self-oriented empathy by seeing oneself in the particular situation and others-oriented empathy by considering the situation of the person who posted the image. A statistical analysis showed that, in the conditions where the image quality and the evaluation by others did not match, the conformity rate showed negative correlation in a group of self-oriented subjects whereas it showed positive correlation in a group of others-oriented subjects ($p<0.1$). Also, the conformity rate and the Oxy-Hb signals of fNIRS are negatively correlated in the group of self-orientated subjects and positively correlated in the group of others-oriented subjects ($p<0.05$). In conclusion, our behavioral experiment and brain function measurement suggest that a self-oriented person tends not to be affected by the evaluation by others, while an others-oriented person can easily be affected by the evaluation by others.

Disclosures: **M. Asaka:** None. **Y. Hosokawa:** None. **A. Omura:** None. **H. Sato:** None. **H. Kawaguchi:** None.

Poster

152. Human Motivation and Emotion I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 152.13/Q8

Topic: G.03. Emotion

Support: NRF Korea Grant 2017M3C7A104182
NRF Korea Grant 2017R1D1A1A09000664

Title: Empathy and prosocial motivation for suffering others is improved by implicit empathy training

Authors: ***S. KIM**, S. KIM;
Korea Univ., Seoul, Korea, Republic of

Abstract: Given the importance of empathy in healthy relationship and sound social cognition, attempts have been made to develop strategies to enhance the ability to empathize with others. Most commonly used methods, however, rely on explicit instructions and self-regulatory resources, which can cause rather empathic over-arousal and ego-depletion that are associated with self-focused personal distress rather than other-focused empathic concern. Therefore, we devised an implicit training task to enhance empathy and examined its behavioral and neural effects on empathy for others. Forty-four healthy adults participated in this functional

neuroimaging study and were assigned to either the empathy or control training group. During the training task, participants read a series of written scenarios and complete a fragmented target word as soon as possible. The empathy version described a person in a suffering situation, and the control version described a person in an emotionally neutral situation. The target words were either empathy-related or neutral corresponding to each version. After completing the training task, participants viewed either empathy-evoking or neutral excerpts, in turn, taken from movies or tv dramas inside the scanner. Empathic concern and personal distress were rated after each clip. Outside the scanner, participants were presented with ten empathy-evoking excerpts they had watched in the scanner and rated their helping intentions. Behavioral results showed that the empathy group felt less personal distress to empathy-evoking situations, and reported greater helping intention for the sufferers compared to control group. Neuroimaging results revealed that relative to the control group, the empathy group showed increased activity in the anterior cingulate cortex, precuneus, dorsomedial prefrontal cortex, superior frontal gyrus, and temporo-parietal junction. Furthermore, these activations were negatively correlated with individual differences in personal distress levels reported during the task. These results indicate that the implicit empathy training we developed is effective in inducing greater empathic responses in subjective and neural domains and in increasing prosocial motivation.

Disclosures: S. Kim: None. **S. Kim:** None.

Poster

152. Human Motivation and Emotion I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 152.14/Q9

Topic: G.03. Emotion

Support: FNS Grant CR13I1_162720 / 1

Title: The voice of primates: Evolutionary perspective to the human affective brain

Authors: *C. DEBRACQUE[1], L. CERAVOLO[1], K. SLOCOMBE[2], Z. CLAY[3], T. GRUBER[1], D. GRANDJEAN[1];
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Abstract: “With many kinds of animals, man included, the vocal organs are efficient in the highest degree as a means of expression”. In 1872, Darwin already highlighted the importance of an evolutionary approach to improve our knowledge, especially of affects, about vocal expressions in humans. However, only few have relied on this approach to understand neural processes related to affective voice perception. To fill this gap, the present study aimed at

investigating how humans categorize affective primate vocalizations using functional Magnetic Resonance Imaging (fMRI). Twenty-five participants were exposed to affective vocalizations produced by humans, chimpanzees, bonobos and macaques related to threatening, distressful and affiliative situations. Participants were then asked, in two distinctive tasks, (1) to categorize the affective content of the stimuli and (2) to identify the species that expressed these vocalizations. Data analysis revealed enhanced brain activations in the bilateral inferior frontal gyrus, dorsolateral prefrontal cortex (DLPFC) and superior temporal gyrus (STG) and sulcus for correctly categorized distressful compared to affiliative vocalizations only for human relative to vocalizations of chimpanzee, bonobo and macaque ($p < .05$, voxel wise FDR corrected). Furthermore, our results showed that a subregion of the voice sensitive areas (right mid STG) was enhanced for correctly categorizing both human and chimpanzee as opposed to bonobo and macaque vocalizations ($p < .05$, voxel wise FDR corrected). Moreover, functional connectivity (FC; $p < .05$, seed-level FDR corrected, two-tailed) revealed coupled networks of brain regions including primary auditory cortex, putamen and pallidum known to be involved in vocal affective perception and recognition, and the right supramarginal gyrus and left superior parietal lobule, which may be related to intention processing. FC also showed an anti-coupled network involving the DLPFC and the inferior temporal gyrus involved in decision-making and categorization of auditory material. In conclusion, the categorization of positive or negative auditory vocalizations in human voices appears to rely on a large network of brain regions as previously proposed (Frühholz & Grandjean, 2013). Moreover, to our knowledge, this study is the first to demonstrate a common network in the voice sensitive areas between human and chimpanzee affective vocalizations, highlighting the importance of an evolutionary approach to understand affective processing in the human brain.

Disclosures: C. Debracque: None. **L. Ceravolo:** None. **K. Slocombe:** None. **Z. Clay:** None. **T. Gruber:** None. **D. Grandjean:** None.

Poster

152. Human Motivation and Emotion I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 152.15/Q10

Topic: G.03. Emotion

Support: National Science Foundation

Title: Increased amygdala-frontal resting state functional connectivity is associated with greater heart rate variability and reduced anxiety

Authors: K. FREDERIKS[1], S. KARK[1], J. PAYNE[2], *E. A. KENSINGER[1];
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Abstract: Recent work has shown that time-domain metrics of heart-rate variability (HRV), such as the Root Mean Square of the Successive Differences (RMSSD) between heart beats, are related to resting state functional connectivity (RSFC) amongst emotional regulation brain regions, such as the amygdala and frontal cortex (Sakaki et al., 2016). However, it is not known if frequency-domain metrics of HRV relate to these amygdala-frontal effects. Low and high-frequency HRV metrics are thought to reflect sympathetic and parasympathetic tone, respectively, implicating their utility to reveal differential effects of the autonomic nervous system on amygdala-frontal coupling. In the present study, 56 healthy young adults (24 males), underwent a resting-state functional connectivity scan, 5-minutes of resting electrocardiogram monitoring, and completed a trait anxiety survey. Whole-brain correlations demarcated regions that showed increased amygdala coupling as a function of individual differences in resting HRV metrics and subjective anxiety across individuals. Results revealed positive correlations between the strength of amygdala RSFC with the ventrolateral prefrontal cortex (VLPFC) with RMSSD (replicating prior work) but also high-frequency HRV, the latter relationship suggesting that amygdala-VLPFC coupling in HRV is likely related to enhanced parasympathetic tone. Conversely, a negative correlation was observed between amygdala-medial PFC coupling and a low-frequency HRV, suggesting those participants with greater amygdala-medial PFC coupling have reduced sympathetic activation. Finally, we observed a negative correlation between amygdala-orbitofrontal coupling and trait anxiety levels. Together, these results suggest that greater amygdala coupling with frontal areas tracks not only with enhanced objective well-being (i.e., HRV measures), but also subjective well-being (i.e., lower anxiety). These findings replicate prior work in a larger sample and add that these amygdala-frontal interactions are related to differential branches of the autonomic nervous system, as revealed by frequency-domain metrics of HRV, and subjective anxiety. Future work will be needed to address direction of influence to determine how HRV and subjective well-being influence and maintain one another.

Disclosures: **E.A. Kensinger:** None. **S. Kark:** None. **K. Frederiks:** None. **J. Payne:** None.

Poster

152. Human Motivation and Emotion I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 152.16/Q11

Topic: G.02. Motivation

Support: Qdai-jump Research Program (27818, 2015-2016)

Title: Presence of another person can affect neuronal responses and task performance

Authors: *Y.-H. TSENG[1], K. TAMURA[2], T. OKAMOTO[1,2];
[1]Grad. Sch. of Systems Life Sci., [2]Fac. of Arts and Sci., Kyushu Univ., Fukuoka, Japan

Abstract: Humans are social animals and cannot live alone. Understanding how social situations affect human behavior can often facilitate efforts to promote successful learning or working in various environments. The presence of another person is a fundamental but poorly understood factor in social situations. To elucidate the influence of another person's presence, we examined neuronal activity in 28 participants (14 women; age range, 18-26 years) as they performed common cognitive tasks under two conditions: a solitary condition under which participants performed cognitive tasks alone and a paired condition under which participants performed the tasks independently while another participant was present. The cognitive tasks consisted of three types of auditory oddball tasks: easy (1000 Hz, standard; 1500 Hz, target), difficult (1000 Hz, standard; 1050 Hz, target), and complex (1000 Hz, standard; 1500 Hz or 1050 Hz, target). The participants responded to oddball stimuli by pressing a numeric keypad that was concealed in a soundproof box. We recorded electroencephalograms and event-related potentials from the participants during the tasks. This study was approved by the Kyushu University ethics committee. The analyses of event-related potentials revealed that P300 amplitudes at the Pz electrode were higher under the solitary condition than under the paired condition at the difficult task. P300 amplitudes are associated with attention, so we can infer that the presence of another person diminished the participants' abilities to focus on the tasks. Furthermore, reaction times were longer under the paired condition than under the solitary condition. We also deduce that attention levels were lower when another person was present. A time-frequency analysis showed that alpha band amplitudes were higher under the solitary condition than under the paired condition at the difficult task. Greater alpha band activity is associated with relaxed states, so we infer that the participants were less relaxed when another person was present. Based on these results, we conclude that the presence of another person influences attention levels and the degree of relaxation during cognitive task performance.

Disclosures: Y. Tseng: None. **K. Tamura:** None. **T. Okamoto:** None.

Poster

152. Human Motivation and Emotion I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 152.17/Q12

Topic: G.03. Emotion

Support: NIH/NIA R01AG057204

Title: Empathic concern trajectories reflect underlying amyloid-β and hippocampal gray matter volume in cognitively normal older adults

Authors: *T. E. CHOW, C. R. VEZIRIS, R. LA JOIE, V. BOURAKOVA, K. P. RANKIN, J. H. KRAMER, B. L. MILLER, W. W. SEELEY, G. D. RABINOVICI, V. E. STURM;
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Abstract: Alzheimer's disease involves both cognitive and emotional symptoms, including changes in empathy. Deposition of amyloid-β, a pathological hallmark of Alzheimer's disease, is thought to begin a decade or more before clinical symptoms emerge. There has been limited research regarding the impact of amyloid-β on emotional processes, particularly in the preclinical stage of Alzheimer's disease. This study examined the relationship between amyloid-β and empathy trajectories in 88 older participants who were cognitively normal at their baseline research visit with the UCSF Hillblom Healthy Aging Network. A total of 24 amyloid-β positive participants (Aβ+; *M* = 69.4 years; *SD* = 5.2 years; 8 females) were identified based on ^{18}F-AV-45 (Florbetapir) or ^{11}C-Pittsburgh compound B (^{11}C-PiB) Positron Emission Tomography scans that were dichotomized (Aβ-/Aβ+) based on quantitative standardized uptake value ratio thresholds. The remaining 64 participants were amyloid-β negative (Aβ-; *M* = 70.0 years; *SD* = 7.2 years; 34 females). All participants were cognitively normal at a baseline visit based on multidisciplinary diagnostic assessments, including neuropsychological testing. Informants evaluated participants' current empathy levels at the baseline visit and on subsequent annual research visits using the Interpersonal Reactivity Index, a multidimensional empathy measure (Davis, 1983). We focused on a subscale that measures empathic concern, a form of affective empathy characterized by feelings of compassion and concern for others. Participants were followed for an average of 2.3 total research visits that took place across an average of 1.9 years. Longitudinal analyses revealed that amyloid-β positivity was associated with changes in empathic concern: Aβ+ participants demonstrated greater increases in empathic concern over time than Aβ- participants ($p = 0.02$) despite no initial group differences at baseline ($p = 0.68$). Voxel-based morphometry analyses revealed that smaller hippocampal gray matter volume was associated with greater increases in empathic concern over time in Aβ+ participants ($pFWE < 0.05$). These findings suggest there are longitudinal increases in affective empathy that accompany amyloid-β positivity and reflect atrophy in structures that are vulnerable in Alzheimer's disease.

Disclosures: T.E. Chow: None. **C.R. Veziris:** None. **R. La Joie:** None. **V. Bourakova:** None. **K.P. Rankin:** None. **J.H. Kramer:** None. **B.L. Miller:** None. **W.W. Seeley:** None. **G.D. Rabinovici:** None. **V.E. Sturm:** None.

Poster

152. Human Motivation and Emotion I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 152.18/Q13

Topic: G.03. Emotion

Title: Why TV commercials inside the advertising unit attract less attention

Authors: *S. TUKAIEV[1], I. SELEZNOV[2], A. POPOV[2];
[1]Dept. of Social Communication, Natl. Taras Shevchenko Univ. of Kyiv, Inst. of Journalism, Kyiv, Ukraine; [2]Dept. of Electronic Engin., Natl. Tech. Univ. of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute", Kyiv, Ukraine

Abstract: TV viewing affects the EEG parameters of the cortical activity. The main task of advertising is to cause emotions and be memorized by the viewer. The Detrended Fluctuation Analysis (DFA) has been widely used to quantify the presence and stability of the oscillatory activity over time in each region of the brain. The aim of the study was to reveal whether DFA allows analyzing the specific emotional features of cortical activity while watching the TV commercials depending on Ads placement. 91 healthy volunteers (62 women and 29 men) aged 18 to 26 years (Mage = 19.47, SD = 1.67 years) participated in this study. For the current experiment we used the set of negative TV news reports (4 plots each 1-1,5 minutes long) interrupted by a pause for three 30 seconds-long TV commercials (food and drink). We found that the character and degree of EEG changes during the perception of emotional stimuli significantly depend on the placement of a particular stimulus. The growth of the power spectral density of the theta-range indicates emotional activation. The changes in the alpha1 subband indicates the activation of the short-term memory. Depression of the alpha2-subrange indicates an increase in the level of attention. Depression of the alpha3-subband indicates activation of access to long-term memory, associative and semantic processes. The changes in the beta- and gamma-ranges are evidence of the activation of the processes of awareness, higher associative processes. Also the gamma band activity in the frontal areas of the right hemisphere, the pre and frontal regions of the left hemisphere can predict memorization of the TV commercial. DFA revealed an increase of oscillations stationarity in all bands above 1.00 while watching the Ads inside advertising unit. The last pointed on the nonstationarity of the process. It relates to the instability of EEG dynamical characteristic and may point on the global decrease of the informational cognitive component in the brain activity and explain why TV commercial inside TV attract less attention.

Disclosures: S. Tukaiev: None. **I. Seleznov:** None. **A. Popov:** None.

Poster

152. Human Motivation and Emotion I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 152.19/Q14

Topic: G.03. Emotion

Support: KEEN Program Transformation Grant
Saint Louis University

Title: The relationship between anxiety and emotionally ambiguous facial expressions

Authors: *H. M. WILKS, S. E. WILLIAMS, K. L. MEEKER, J. D. WARING;
Dept. of Psychology, St. Louis Univ., Saint Louis, MO

Abstract: Symptoms of anxiety range upon a continuum. Individuals may experience symptoms of anxiety at bothersome levels, but do not meet diagnostic criteria for an anxiety disorder. Studying individuals with elevated, but not clinically significant levels of anxiety may provide insights as to the variations in the interpretations of emotionally ambiguous faces (surprise and neutral). Socially anxious individuals interpret neutral faces negatively, indicating a negative interpretation bias. Furthermore, high trait anxiety is associated with a negative interpretation bias for surprise. The primary aim of the present study was to examine how response times and valence ratings of emotionally ambiguous faces (surprise and neutral) differ between individuals with normal to mild anxiety levels (low anxiety; LA) versus moderate to extremely severe anxiety levels (high anxiety; HA). The secondary aim was to examine the relationships of heart rate variability (HRV) and emotional regulation strategies to anxiety. Participants completed the Depression, Anxiety, and Stress Scale-21 Items (DASS-21) and Emotional Regulation Questionnaire (ERQ). Resting state HRV was collected. Participants were instructed to rate the emotional valence of happy, angry, surprised, and neutral human facial expressions. Preliminary analyses revealed our sample of college students had higher than normal levels of anxiety. Participants with HA (DASS-21>9) rated neutral faces more negatively and more slowly than participants with LA (DASS-21<10). Surprised faces took longer to rate versus neutral, and were rated more positively overall, but there were no differences between HA and LA participants. There was no relationship between anxiety level and HRV or emotional regulation strategy. Due to the prevalence of neutral faces encountered by individuals, it is important to investigate the role of anxiety in interpreting neutral faces. Preliminary results show HA participants rated neutral faces more negatively than did LA participants, implying either negative interpretation biases or a deficit in the ability to differentiate between neutral and angry facial expressions. Rather than rapidly interpreting neutral faces negatively, HA participants appear to spend more time processing neutral faces, contrary to the negative interpretation bias theory.

Disclosures: H.M. Wilks: None. **S.E. Williams:** None. **K.L. Meeker:** None. **J.D. Waring:** None.

Poster

152. Human Motivation and Emotion I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 152.20/Q15

Topic: G.03. Emotion

Support: NIH Grant P50 NS22343

NIH Grant T32DC7361

Title: The continued influence of neuroplasticity on attention and emotional expression in school-age children with perinatal stroke

Authors: *M. R. MEYER, S. EDWARDS, E. BEAVER, P. LAI;
Communication Disorders, Univ. of Nebraska at Kearney, Kearney, NE

Abstract: Children with PS provide a unique opportunity to study the neural basis of sociability and affect across communication systems. These children suffered a cerebrovascular event around the perinatal period (i.e. between the last trimester of gestation up to the first month after birth). The estimated prevalence rate is 1 in 4,000 live births and is more likely to occur in males than females, and in the left hemisphere than the right hemisphere. A perinatal stroke often results in a significant lesion that can be diagnosed *in utero*, at birth, or months after birth when symptoms are noted. How plastic is the brain with regards to the social and communicative domain? This particular group can provide clues towards a better understanding of the neural basis of affect and sociability and its neural flexibility. This study included 20 children in total between 7-14 years of age; 11 children with Right Hemisphere Injury (RHI) and 9 children with LHI Left Hemisphere Injury (LHI). The social and affective profile of these different groups was characterized by examining the production of facial expressions and eye gaze behaviors of the children during a social dialog with an experimenter. Eye gaze behaviors can signify how much an individual is willing to interact with the experimenter. The same type of question can be asked for facial expression, for example what caused the child to produce a smile when expressing joy. Results from the one-way analysis of variance (ANOVA) for both initiation of eye contact and facial expressions of the child was not statistically significant. For eye contact ($p=0.496$) and facial expression ($p=0.836$), both the LHI and RHI are producing similar communicative behaviors. These results mirror studies investigating language production in these two groups. In previous studies regarding the expression of emotions, children with RHI were flatter in their emotional expression compared to their LHI peers. As they develop and reach school-age, the RHI children are expressing emotions on their faces, a pattern not observed in younger children with RHI. The affective patterns observed in this study suggest the powerful role of neuroplasticity as it continues to shape behavior in school-age children as they overcome an early brain injury.

Disclosures: **M.R. Meyer:** None. **S. Edwards:** None. **E. Beaver:** None. **P. Lai:** None.

Poster

152. Human Motivation and Emotion I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 152.21/Q16

Topic: G.03. Emotion

Support: US-Israel binational science foundation

Title: Dissociating affective and semantic neural representations of valence

Authors: *X. LI, A. K. ANDERSON;
Dept. of Human Development, Cornell Univ., Ithaca, NY

Abstract: Valence, the negative and positive values associated with an event, is central to affect, judgement and decision-making studies. Here we examined whether there are multiple neural representations of valence, supporting affective (feeling) and semantic (knowing) features. Affective valence describes people's emotional response to an event, while semantic valence is more related to people's stored knowledge about an event. During self-reported emotional responses to external events, individuals may confuse these two ways to report and represent valence. However, the distinction between feeling and knowing has been previously studied using habituation. After repeated exposure to an image, affective valence as self-reported feeling and facial EMG decreased while semantic valence as reflected in knowledge-focused self-report did not decrease (Itkes, Kimchi, Haj-Ali, Shapiro, & Kron, 2017). In the present study, we used multi-echo BOLD fMRI and a within-subject design of affective and semantic valence report to study the dissociated and associated neural activations of feeling and knowing judgments during viewing of emotional scenes. Participants viewed a set of 108 pictures selected from IAPS database. Each picture was displayed twice, once in feeling and once in knowing conditions, where participants rated their feelings or knowledge about the picture on positive and negative valence scales. While there were similar magnitude of feeling and knowing ratings for each pictures, a contrast of feeling and knowing conditions showed greater recruitment of the premotor cortex, orbitofrontal cortex, inferior parietal cortex, insula and middle temporal cortex during feeling judgments, and higher recruitment in medial frontal cortex, inferior frontal cortex and inferior occipital cortex for knowing judgments . Valence judgments have both affective and semantic components that depends on distinct brain regions with feeling and knowing differentially associated with valence as an internal subjective experience and as an external semantic-perceptual representation.

Disclosures: X. Li: None. **A.K. Anderson:** None.

Poster

152. Human Motivation and Emotion I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 152.22/Q17

Topic: G.02. Motivation

Support: National Natural Science Foundation of China 31630034

Title: Compensation and its cousins: Parsing moral motives underlying guilt-induced behaviors

Authors: *B. SHEN[1], H. YU[2], X. ZHOU[2];
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Abstract: Guilt give rises to behaviors that span a wide range of moral spectrum. Three types of moral motives can be identified underlying these behaviors: 1) compensatory, aiming to alleviate victim's suffering; 2) reparative, aiming to restore the social tie with the victim; and 3) self-punitive, aiming to reduce aversive feelings caused by falling short of one's own moral standard. These motives oftentimes lead to the same external behavior and research on guilt has not systematically dissociated them, either behaviorally or neurally. Here we developed a novel interactive task where a receiver would receive mild electric shocks either due to their own fault or due to participants' fault. The participants, while undergoing fMRI scan, chose how many shocks they themselves would like to receive regardless of whom triggered receiver's shocks. Critically, this choice was made in 3 contexts: in the Changeable context participants can reduce the receiver's shocks by re-directing shocks to themselves; in the Public context, participants cannot modify the receiver's shock, but their choice can be seen by the receiver, making it possible to signal their intent to the receiver; in the Private context, neither can the participants change the receiver's shock, nor can their choice be seen by the receiver. After the task, participants rated how much they considered each candidate motive in choosing shocks for themselves, including "compensate my mistake" (compensatory), "seek forgiveness" (reparative), and "deserve punishment" (self-punitive). Behaviorally, compensatory motive was most pronounced in the Changeable context, whereas reparative motive was equally strong in the Changeable and Public contexts, but less so in the Private context where signaling was blocked. Self-punitive motive was present in all 3 contexts, but was not mixed with other motives only in the Private context. Neurally, we found dissociable brain networks whose activation resembled the patterns of different motives, with TPJ corresponding to the compensatory motive, while posterior cingulate and perigenual anterior cingulate cortex resembling the reparative motive. Moreover, activation in dorsomedial prefrontal cortex correlated with the strength of self-punitive motive in the Private context.

Disclosures: B. Shen: None. **H. Yu:** None. **X. Zhou:** None.

Poster

152. Human Motivation and Emotion I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 152.23/Q18

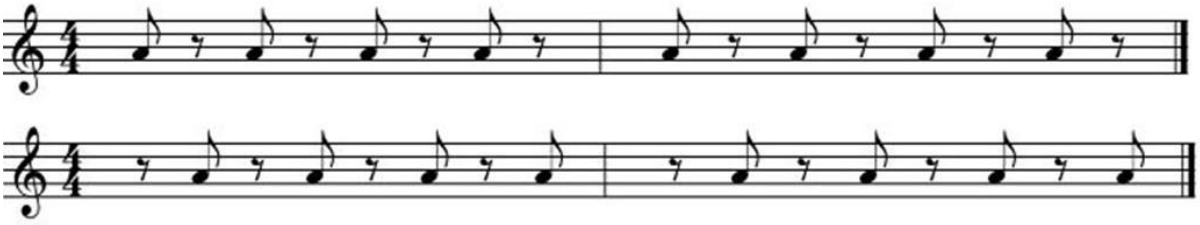
Topic: I.06. Computation/ Modeling/ and Simulation

Support: A-SAP, Photon Valley Center, Hamamatsu, Japan

Title: How direct or indirect social interaction affects the brain activities during the drum playing?

Authors: *H. EDA[1,2], T. SHIBUYA[3], M. UCHIYAMA[3], T. TANAKA[3], M. YAMAZAKI[4], N. OKAMOTO[5], Y. KURODA[6], M. SATOH[7];
[1]Med. Optics, Grad. Sch. For GPI, Hamamatsu, Japan; [2]Phonics Innovations Co., Ltd., Hamamatsu, Japan; [3]ATV Corp., Hamamatsu, Japan; [4]Grand Coeur Lab. LLC, Saitama, Japan; [5]Col. of Social Sciences, Ritsumeikan Univ., Kyoto, Japan; [6]Dept. of Mathematics, Kyoto Univ. of Educ., Kyoto, Japan; [7]Grad. Sch. of Med., Mie Univ., Tsu, Japan

Abstract: Introduction
How social interaction affects the brain activities during playing music is still under the research. Purpose of this study is to discuss how social interaction affects the brain activities during the music experiment performing by 2 players with Near InfraRed Spectroscopy (NIRS).
Method
We used Electronic drum (aFrame, http://www.aframe.jp/ , ATV corporation, Japan). A total of 6 subjects participated in this study. The consent form was obtained from all subjects. Experiment ethics and private information protection were fully considered. The subject was required to play in four Beats music on the Figure score, the first beat (FB) or the second beat (SB) of two eighth notes. Two subjects played the score four times. For example, player1 played FB, SB, FB, SB, and player2 FB, SB, SB, FB. For the third trial, player1 leaded and player2 followed the beat. For the fourth trial player1 followed and player2 leaded. Two conditions were considered. For indirect social interaction condition, 2 players played 2 drums for each, individually. They could hear another player's sound, but could not see the face. For direct social interaction condition, 2 players played the same 1 drum face to face. We put the NIRS sensors consisted of 16 Source-Detector pairs (16 channels) on the forehead and recorded the brain activation with NIRS system (Spectratech, Inc., Japan). After all tasks, they recall and reported their thinking or ideas by reviewing a video recorded their experiments.
Results
Playing performance was more affected by direct social interaction. The subjects who had music experience or high skill were not affected. Our study suggests that brain activations can be changed in direct / indirect social interaction during drum play and provide objective information about how social interaction affects each other.



Disclosures: H. Eda: None. **T. Shibuya:** None. **M. Uchiyama:** None. **T. Tanaka:** None. **M. Yamazaki:** None. **N. Okamoto:** None. **Y. Kuroda:** None. **M. Satoh:** None.

Poster

152. Human Motivation and Emotion I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 152.24/R1

Topic: G.02. Motivation

Title: An fMRI study on the encoding and retrieval of political fake news

Authors: *B. L. GONZALEZ, Y.-W. CHEN, T. CANLI;
Psychology, Stony Brook Univ., Stony Brook, NY

Abstract: The concept of "fake news" has gained attention in the past few years, but the neural processes underlying the encoding and retrieval of false news stories in the political context remain to be elucidated. We used functional magnetic resonance imaging (fMRI) to investigate the effect of "real" and "fake" negative political news statements on subsequent retrieval of information. In this pilot study, human participants (N=5; 2F, 3M; mean age: 28.4) were shown negative news statements in the form of a Twitter 'tweet' from two hypothetical political opponents in the scanner. Scanning included an encoding and a retrieval task. During the encoding task, participants were first asked to rate whether a given news item was "probably real" or "probably fake". They then received feedback as to whether the presented news item was indeed "real" or "fake". Participants were instructed to remember this information for the memory task that would follow. After the scan session, participants completed a set of questionnaires to assess their political orientation, attitudes, and beliefs, and were then debriefed. Data were analyzed according to whether candidate's party affiliation was congruent ("same-party") or incongruent ("other-party") with the participant's political orientation. We found that participants rated negative news associated with the same-party candidate as equally likely (50%) to be "real" or "fake". In contrast, participants rated negative news associated with the other-party candidate more often as "real" (72.5%) than as "fake" (27.5%). Imaging data suggest that encoding of negative political news was associated with activation in the superior and middle frontal gyrus, precuneus, cingulate cortex, superior and middle temporal gyrus, and the superior and inferior parietal lobe. These regions have previously been associated with motivated reasoning, decision-making, and conflict detection. Additional analyses specific to same-versus other-party news encoding and retrieval will be presented. Understanding how people process political fake news can lead to more insight on political cognition and decision-making. Bridging this gap between psychology, neuroscience, and political science can better help understand issues that are important to society but subject to partisanship.

Disclosures: B.L. Gonzalez: None. **Y. Chen:** None. **T. Canli:** None.

Poster

153. Human Motivation and Emotion II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 153.01/R2

Topic: G.03. Emotion

Title: Interoceptive imagination facilitates emotional face perception

Authors: *Y. CHAE;
Kyung Hee Univ., Seoul, Korea, Republic of

Abstract: Physiological feedback plays an important role in the perception of emotion, which is thought to be the subjective experience of a physiological reaction to emotional stimuli or a physiological reaction itself. We investigated whether enhanced interoceptive inference of corresponding bodily sensations facilitated emotional face recognition. Participants performed an emotional judgment task after a synchronization task inside a functional magnetic resonance imaging scanner. Participants were instructed to imagine feeling the bodily sensations of two specific somatotopic patterns: a fear-associated bodily sensation (FBS) and a disgust-associated bodily sensation (DBS). After completion of the task, participants were shown faces expressing various levels of fearfulness and disgust and instructed to classify the facial expression as fear or disgust. We found a stronger bias favoring the “fearful face” under the congruent FBS condition than under the incongruent DBS condition. The response to fearful versus intermediate faces increased in the fronto-insular-temporal network under the FBS, but not the DBS, condition. The fearful face elicited activity in the anterior cingulate cortex and extrastriatal body area under the FBS condition relative to the DBS condition. Furthermore, effective connectivity between the anterior cingulate cortex/extrastriate body area and the fronto-insular-temporal network was modulated according to the specific bodily sensation. Our findings suggest that somatotopic patterns of bodily sensation provide informative access to the collective visceral state in the fear processing via the fronto-insular-temporal network.

Disclosures: Y. Chae: None.

Poster

153. Human Motivation and Emotion II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 153.02/R3

Topic: G.03. Emotion

Support: Russian Science Foundation Grant no. 18-11-00336

Title: An EMG study of human affective state dynamics in a social videogame paradigm

Authors: D. V. TIKHOMIROVA, ***A. V. SAMSONOVICH**;
Cybernetics Dept., Natl. Res. Nuclear Univ. Mephi, Moscow, Russian Federation

Abstract: Future artificial social-emotional intelligence must replicate the laws of dynamics of human affects in social interactions. A general theoretical model describing these laws needs to be constructed and validated through empirical study of social interactions under controlled conditions, building on available knowledge. With this meta-goal in mind, here a virtual-reality-based paradigm of the game Teleport was implemented and used to study social interactions of a human participant with two other players, each of which was either another human participant or a Virtual Actor: a believable intelligent agent based on the eBICA cognitive architecture (Samsonovich, 2013, 2018). Achieving the game objective requires persistent cooperation with one player against the other. Exclusive partnership in this paradigm is only possible to establish using observable behavior: avatars look indistinguishable, players are anonymous. It was a priori hypothesized that (i) different mental states described by distinct moral schemas correspond to different social conditions of the player: e.g., looking for a partner vs. cooperating with the partner vs. betraying the partner; (ii) same significant events and same actions of others may elicit different affects in different mental states. These phenomena predicted by the proposed model based on eBICA were expected to be detectable via facial expressions. To test predictions, a study was conducted using 20 NRNU MEPhI Master students, age 22 to 24. During videogame sessions, EMG recordings were taken using Neuro-KM (Brainsys) from two facial muscles: the left M. Zygomaticus and the left M. Corrugator, in the frequency range of 7 to 70 Hz. The two signals were mapped to the Valence-Arousal affective space coordinates. In addition, facial expressions were extracted from the video of the face, recorded during each session, with FaceReader (Noldus) using Facial Action Coding System (FACS). This analysis also yielded Valence and Arousal values, in addition to eight specific affective measures. Combined data showed clear semantic correlates of significant events in the game. In particular, Valence bursts occurred in the first seconds of the next game round following a successful round. These bursts were absent after unsuccessful rounds, and gradually accumulated strength in a sequence of successes. These and other observed patterns of affective dynamics were accounted by the proposed model. In conclusion, the data obtained support the general eBICA model, allowing us to adjust its parameters and to further refine its structure. It is expected that the corrected model will possess a higher cross-paradigm, cross-domain generality.

Disclosures: D.V. Tikhomirova: None. **A.V. Samsonovich:** None.

Poster

153. Human Motivation and Emotion II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 153.03/R4

Topic: G.03. Emotion

Support: DFG Grant KR3691/8-1

Title: Interindividual differences in the neural response to social rejection predict aggressive behavior

Authors: *P. PETEREIT[1], U. M. KRÄMER[1,2];
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Abstract: Social rejection is experienced as aversive and threatening and can lead to different reactions, including dysfunctional responses as aggression or social avoidance. Here, we asked whether aggression and avoidance after rejection can be predicted from the neural reactivity to social rejection signals. We hypothesized that interindividual differences in the expectation of rejection relate to neural and behavioral reactions to rejection. To investigate this, participants (n=61, mean age (SD) = 22.3 (4.9), 51 females) played a get-acquainted game and received feedback from peers, while their EEG was recorded. One group received mainly negative/rejecting feedback ("rejection context"), whereas the other group mainly received positive/accepting feedback ("acceptance context"). Before each trial, participants indicated their expectation to be rejected or not. Afterwards, participants played a competitive reaction time task against another participant, where they had the opportunity to either aggress towards or avoid their opponent. Although participants in the rejection context adapted their expectations to some extent, they maintained an optimistic bias. The feedback-related negativity (FRN) and P300 proved to be sensitive to both context and feedback valence. Only in the rejection context, the FRN was enhanced to negative relative to positive feedback. P300 amplitudes were higher for positive feedback than for negative feedback in the rejection context, whereas the opposite was true in the acceptance context. Against our hypothesis, participants who had received more negative feedback were not more aggressive or avoidant than those who had received more positive feedback. However, the P300 amplitude difference between positive and negative feedback positively predicted aggressive behavior in the rejection context only (r = .37, p= .036). This suggests a weaker updating of social feedback expectations after negative compared to positive feedback in participants responding more aggressively after rejection.

Disclosures: P. Petereit: None. **U.M. Krämer:** None.

Poster

153. Human Motivation and Emotion II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 153.04/R5

Topic: G.03. Emotion

Support: ERC 819814 - RememberEx

Title: Cross frequency coupling between the human amygdala and hippocampus during successful encoding of negative emotional memory

Authors: M. COSTA[1], A. GIL.NAGEL[2], R. TOLEDANO[2], C. OEHRN[3], M. YEBRA[1], C. MÉNDEZ-BÉRTOLO[1], N. AXMACHER[4], S. MORATTI[5], *B. A. STRANGE[6];
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Abstract: Communication between the amygdala and the hippocampus has been proposed to support enhanced memory for emotional events. However, a mechanistic account of how these two structures interact during successful encoding of emotional – unpleasant – information is lacking. The aim of the present study is to investigate the oscillatory responses in amygdala and hippocampus associated with successful encoding of unpleasant scenes.
Using direct intracranial recordings from the amygdala and the hippocampus in 8 patients being evaluated for medication-resistant epilepsy, we investigated the oscillatory responses associated with subsequent memory of unpleasant and neutral scenes from the IAPS database. Retrieval was assessed by a recognition test 24 h after encoding. All patients included in the study have intracranial depth electrodes implanted into the amygdala and the hippocampus (3 right, 4 left, and 1 bilateral).
Encoding-related responses predicting subsequent recollection of unpleasant pictures, but not of neutral pictures, are observed in the amygdala from around 350ms post-stimulus onset in the fast gamma range (75-130 Hz). A lower-frequency gamma response (50-75 Hz) occurs in the hippocampus during successful encoding of both negative and neutral scenes at around 500ms. Gamma power in the two structures is significantly correlated during successful encoding of unpleasant pictures. Gamma is not only more pronounced for unpleasant remembered vs forgotten scenes, but its envelope is also more strongly modulated by alpha oscillations (11 Hz) in the amygdala and theta oscillations (8 Hz) in the hippocampus. We then calculated cross-frequency coupling between the amplitude of high-frequency activity and the phase of low-frequency oscillations within the two regions and between amygdala low frequencies phases and hippocampal gamma power. These analyses reveal that alpha phases in the amygdala (11 Hz)

modulate amygdala gamma power, while amygdala theta phases (6-8 Hz) modulate hippocampal gamma power during successful encoding of unpleasant pictures.
These results suggest that the amygdala modulates gamma oscillations in the hippocampus by phase amplitude coupling during the successful encoding of unpleasant pictures, thus playing a fundamental role in emotional memory formation. Our findings provide fundamental evidence for the understanding of the neural basis supporting negative memory formation.

Disclosures: **M. Costa:** None. **A. Gil.Nagel:** None. **R. Toledano:** None. **C. Oehrn:** None. **M. Yebra:** None. **C. Méndez-Bértolo:** None. **N. Axmacher:** None. **S. Moratti:** None. **B.A. Strange:** None.

Poster

153. Human Motivation and Emotion II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 153.05/R6

Topic: G.03. Emotion

Support: NRF-2017M3C7A1031333

Title: Neural substrates of negativity bias in the recognition of facial emotion

Authors: *G. KIM[1], S.-H. LEE[1,2];
[1]Dept. of Bio and Brain Engin., [2]Program of Brain and Cognitive Engin., Korea Advanced Inst. of Sci. and Technol. (KAIST), Daejeon, Korea, Republic of

Abstract: Recognizing emotion from the faces of others is critical for social interactions. However, it has been reported that patients with mood disorders such as depression or anxiety tend to interpret ambiguous facial expressions negatively. Recent studies also showed that this tendency, called negativity bias, can be observed in non-patient participants depending on their mental states. Moreover, cognitive models of mood disorders suggest that the negative feedback loop caused by the negativity bias is one of the major factors to maintain and worsen the disorder. However, the neural substrates involved in the negativity bias of facial emotion recognition remain elusive. To investigate the negativity bias-related neural substrates, we performed an event-related functional magnetic resonance imaging (fMRI) experiment. During the scan, the cortical activities were recorded while participants performed facial emotion recognition tasks with a series of graded stimuli morphed between prototypical neutral and angry or happy faces. Using the multi-voxel pattern analysis, we found that the response patterns of the right ventrolateral prefrontal cortex (VLPFC) could be used to decode prototypical angry and neutral faces, and that the participant with the higher negativity bias showed greater similarities between the neural response patterns for prototypical neutral and angry faces in the VLPFC. Additionally, the participants with higher negativity bias showed greater activation in the face-

selective regions in the superior temporal sulcus (STS). Moreover, activation in the STS was positively correlated with the neural pattern similarity between the neutral face and angry face conditions in the VLPFC. Collectively, these results suggest that the VLPFC and STS serve as neural substrates for the negativity bias in facial emotion perception. *This work was supported by the Brain Research Program (NRF*-2017M3C7A1031333*) through the National Research Foundation (NRF) of Korea.*

Disclosures: G. Kim: None. **S. Lee:** None.

Poster

153. Human Motivation and Emotion II

Location: Hall A

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Program #/Poster #: 153.06/R7

Topic: G.03. Emotion

Support: IBS-R015-D1
2019R1C1C1004512
18-BR-03

Title: Brain decoding of affective meaning through personal stories

Authors: *H.-J. KIM[1,2], C.-W. WOO[1,2];
[1]Ctr. For Neurosci. Imaging Res. (CNIR), Suwon, Korea, Republic of; [2]Sungkyunkwan Univ., Suwon, Korea, Republic of

Abstract: Sensory and affective responses can be shaped by multiple ingredients such as sensory and conceptual information, social and affective values, past memories, relevance to the 'self', etc. All these ingredients construct affective meaning, which could dramatically change the sensory and affective responses even to the same stimuli. Here we aim to understand how the ingredients of affective meaning are represented and processed in the brain by using participants' personal stories. Though most previous studies using naturalistic tasks have focused on external stimuli, such as movies and stories created by others, these external stimuli are less effective in evoking affective meaning compared to the self-generated ones, which could induce unique responses from each individual. To this end, we performed one-on-one interviews to create personal stories for each participant and used the stories to elicit personal thoughts and emotions that are closely related to participants' life. While collecting the functional Magnetic Resonance Imaging (fMRI) data, 52 participants (23 females, mean age = 22.5) underwent the free-thinking task and the story reading task; in the free-thinking task, participants were asked to think freely, and intermittently reported a few words that represented their thoughts at the moment. In the story reading task, participants were asked to read stories generated by themselves or other people. After the fMRI session, participants rated the words and stories from the tasks in terms of

valence and self-relevance. The high correlation between ratings of stories inside versus outside of the MRI scanner, $r = 0.724$, ensured that the post-scan ratings reflected the inside-scanner experience well.With the fMRI data, we developed a fMRI-based signature predictive of the valence and self-relevance ratings of each participant. We also tried to develop classifiers that discriminated self-generated vs. common stories to identify the brain activity patterns preferentially associated with self-relevant contexts. We further examined the brain activity and connectivity patterns of inter-subject synchronization with respect to valence and self-relevance across self-generated and common stories. This study identified the brain representations and dynamics of key ingredients of personal affective meaning, providing an important step towards developing brain models of internal thoughts and emotions. Ultimately, this study will help better understand patients with psychiatric and somatic disorders that are associated with dysfunctional affective meaning.

Disclosures: H. Kim: None. **C. Woo:** None.

Poster

153. Human Motivation and Emotion II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 153.07/R8

Topic: G.03. Emotion

Support: IBS-R015-D1
2019R1C1C1004512
18-BR-03

Title: Know pain, know gain: Brain representations of sensory pleasure and pain

Authors: *S.-A. LEE[1,2], J. HAN[1,2], M. CHOI[1,2], C.-W. WOO[1,2];
[1]Ctr. For Neurosci. Imaging Res. (CNIR), Suwon-Si, Korea, Republic of; [2]Sungkyunkwan Univ., Suwon-Si, Korea, Republic of

Abstract: Pleasure and pain are interconnected; as in the common saying, “no pain, no gain,” pain is often associated with pleasurable reward, while pleasant stimuli are also known to have strong analgesic effects. Based on these observations, pleasure and pain are hypothesized to be connected in the brain, but this has yet to be tested. Here, we aim to examine the commonalities and differences between the brain representations of pleasure and pain with a functional Magnetic Resonance Imaging (fMRI) experiment. We aim to develop and compare multivariate pattern-based brain signatures for sensory pleasure and pain based on whole-brain functional connectivity. Particularly, we used taste and tactile stimuli to effectively evoke sustained experience of sensory pleasure and pain.To this end, we first developed an 8-channel fluid delivery system that could reliably deliver tastant stimuli with different levels of viscosity (1-20

cps) to participants' oral cavity and could be controlled by a computer program. With this new device, we delivered hot chocolate and capsaicin to induce pleasant taste and orofacial pain, respectively. We also had the quinine (bitter taste) and water conditions as active and passive controls, respectively. Further, we included another pleasure condition with a different modality, soft-touch, in which we stimulated C-tactile afferents using a soft brush on participants' left forearm, allowing us to develop a pleasure marker generalizable across different sensory modalities. The time-course of each stimulus delivery was optimized to evoke the target sensation twice within the 14.5 minutes of scanning based on a pilot study with *N*= 13. Behavioral data from the main experiment with 50 participants (23 females, mean age = 25.0) showed that we successfully and reliably induced pleasant and unpleasant sensations during the MR imaging. With the fMRI data, we developed whole-brain functional connectivity-based brain signatures for different conditions, including pleasure and pain, and then cross-compared their predictive weights among different models. This study will help us better understand the neural mechanisms of pleasure and pain, ultimately providing specific brain targets for the treatment of patients with chronic pain and addiction.

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Poster

153. Human Motivation and Emotion II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 153.08/R9

Topic: G.03. Emotion

Support: IBS-R015-D1
2019R1C1C1004512
18-BR-03

Title: Understanding the brain representations of endogenous affective experiences

Authors: *B. E. KIM[1,2], C.-W. WOO[1,2];
[1]Ctr. For Neurosci. Imaging Res., Suwon-si, Korea, Republic of; [2]Dept. of Biomed. Engin., Sungkyunkwan Univ., Suwon-si, Korea, Republic of

Abstract: Brain representations of emotional valence have been studied in many different contexts, e.g., ranging from animal to human, from molecular to system-level research, etc. However, most of the previous studies focused on the emotional valence induced by external stimuli, though spontaneous and endogenous emotional experience might be more important for explaining individual differences in emotional traits and mental health. Here we examined whether the endogenous affective experiences are represented in the brain in a similar way to exogenous affective experiences with a functional Magnetic Resonance Imaging (fMRI)

experiment (N = 61). In the experiment, we conducted the Free Association Semantic Task, in which participants were asked to generate a 40 word-train of spontaneous thoughts for a given seed word. We collected total 160 words from each participant, and many of the words provided personally meaningful concepts and thought topics. After the generation of each word-train of spontaneous thoughts, we showed those words one by one and asked them to think about their personal meaning while fMRI data were collected. After we finished the scan, we asked participants to rate each word on multiple dimensions, such as valence and self-relevance. Behavioral results showed that the temporal dynamics of the affective dimension ratings were highly predictive of general negative affectivity (r = .49), suggesting the importance of dynamic characteristics of endogenous affective experience in explaining emotional traits. Next, we tried to develop unified fMRI pattern-based predictive models for emotional valence and self-relevance within and across individuals, but we failed to obtain well-performing and generalizable models, suggesting that the brain representations of endogenous affective experiences are heterogeneous across individuals and also across distinct internal states. To identify common, but local, brain representations of endogenous affective experiences in terms of valence and self-relevance, we used a Hidden Markov Model to model distinct, while locally consistent, brain states, which were then used as a basis set to model low dimensional structure of endogenous affective experiences in the brain. Further analyses with linear and non-linear dimensionality reduction methods, such as the principal component analysis and the uniform manifold approximation and projection, we are identifying individual's unique manifold representations of endogenous affective experiences. We expect these models to allow us to better understand how endogenous affective experiences are represented in the brain.

Disclosures: B.E. Kim: None. **C. Woo:** None.

Poster

153. Human Motivation and Emotion II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 153.09/R10

Topic: G.03. Emotion

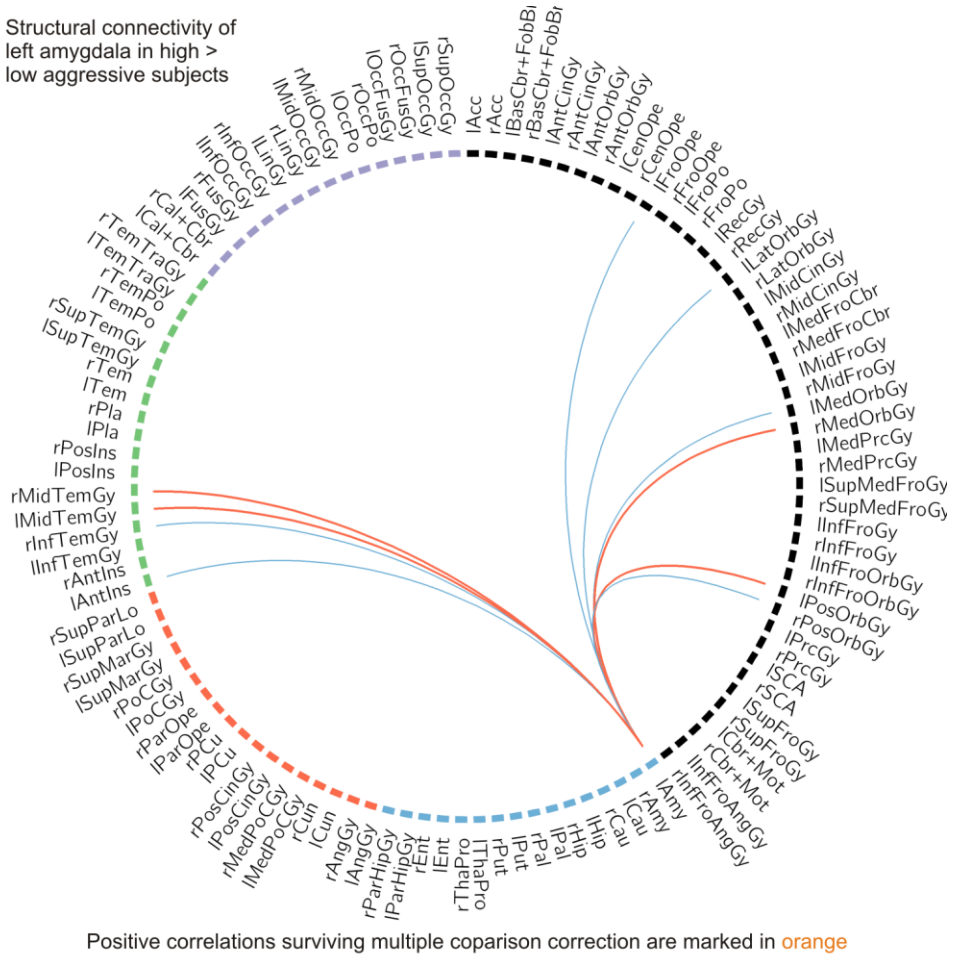
Support: DFG Grant KR3691/5-1

Title: Structural covariance of the amygdala is associated with trait aggression

Authors: M. GÖTTLICH[1], ***M. BUADES-ROTGER**[1], F. BEYER[2], J. WIECHERT[1], U. M. KRÄMER[1];
[1]Univ. of Luebeck, Luebeck, Germany; [2]Queen Mary Univ., London, United Kingdom

Abstract: Previous work has consistently linked aggressiveness with reduced gray matter volume in the amygdala. However, most conventional morphometric studies do not address

potential inter-regional correlations in morphology. Investigating the latter can offer additional insight into the neuro-structural architecture of aggressive behavior. Here, we tested whether whole-brain structural covariance of the amygdala is associated with self-reported trait aggression in a sample of 140 healthy young individuals (40% women). The bilateral amygdala showed a more widespread structural covariance network in high- compared to low-aggressive subjects. Specifically, amygdala volume was more strongly and positively correlated with both ventral and medial aspects of the orbitofrontal cortex as well as with the bilateral middle temporal gyrus in high- vs - low-aggressive individuals. These results survived stringent multiple comparison correction and were not accounted for by gender differences in trait aggressiveness. Importantly, our findings converge with other studies showing that functional connectivity of the amygdala with these same regions can predict aggressive behavior. This indicates a strong overlap between functional and structural patterns of aggression-related brain organization. Our results thus identify novel neuro-structural correlates of aggression and inform the study of structure-function relationships in this field.



Disclosures: **M. Göttlich:** None. **M. Buades-Rotger:** None. **U.M. Krämer:** None. **F. Beyer:** None. **J. Wiechert:** None.

Poster

153. Human Motivation and Emotion II

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Program #/Poster #: 153.10/R11

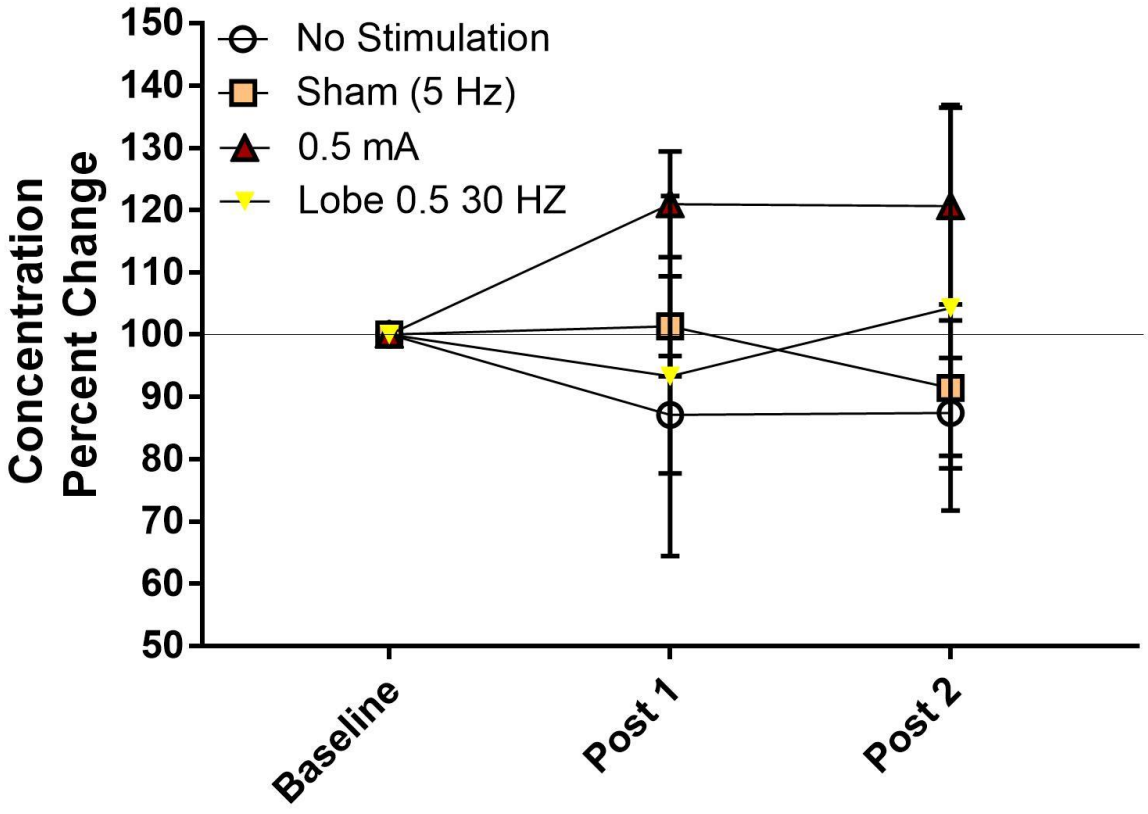
Topic: G.03. Emotion

Support: National Institute of General Medical Sciences, (NIGMS), P20 GM103429 from the National Institutes of Health

Title: Increased savivary alpha amylase following intermittent transdermal VNS

Authors: D. SHELTON[1], B. E. HARRIS[2], G. COLLIER[2], M. MENNEMEIER[3], ***R. W. ROOSEVELT**[1];
[1]Psychology, [2]Arkansas Tech. Univ., Russellville, AR; [3]Univ. of Arkansas for Med. Sci., Little Rock, AR

Abstract: Transdermal electrical stimulation of the auricle branch of the vagus (TVNS) has been proposed as a potential alternative to invasive vagus nerve stimulation (VNS). To be viable, TVNS must functionally activate the LC and that activity should be observable at distal structures. Previously, we demonstrated intermittent TVNS's capacity to increase heart rate variability, here we demonstrate increased concentrations of salivary alpha amylase. Participants received active TVNS consisting of 30 HZ, 5 mA stimulation in 6 second trains followed by 24 seconds of no stimulation for 10 minutes or one of three control conditions. Control 1- no stimulation, Control 2- 5 HZ stimulation at the auricle, Control 3- 30 Hz Stimulation at the ear lobe. Salivary samples were collected prior to stimulation, immediately following, and at 10 minutes post-stimulation and frozen until assay. Following assay, concentrations were converted to percent change from baseline and analyzed using ANOVA. The active TVNS group had significantly elevated salivary alpha amylase following stimulation compared to the control conditions ($F(3, 98=3.78, p=0.01)$. These observations support the hypothesis that TVNS functionally activates the LC and may provide an additional approach to modulating CNS function in a non-invasive and non-pharmacological manner.



Disclosures: **D. Shelton:** None. **B.E. Harris:** None. **G. Collier:** None. **R.W. Roosevelt:** None.

Poster

153. Human Motivation and Emotion II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 153.11/R12

Topic: G.03. Emotion

Support: NIH Grant MH098348

Title: Violence exposure contributes to sex differences in the neural response to stress

Authors: *E. DAVIS[1], A. M. GOODMAN[2], M. N. ELLIOTT[3], M. A. SCHUSTER[4], S. TORTOLERO EMERY[5], S. MRUG[2], D. C. KNIGHT[2];
[1]Univ. of Alabama At Birmingham, Birmingham, AL; [2]Univ. of Alabama at Birmingham, Birmingham, AL; [3]RAND, Santa Monica, CA; [4]Kaiser Permanente, Pasadena, CA; [5]Univ. of Texas, Houston, TX

Abstract: The neural response to stress differs between men and women within multiple brain regions (e.g. prefrontal cortex, amygdala, and hippocampus) that play an important role in emotion expression and regulation (Seo et al., 2013; Seo et al., 2017). However, prior research has not focused on whether violence exposure contributes to sex differences in stress-elicited neural activity. Therefore, the present study investigated the relationship between sex, prospectively assessed violence exposure (ages 11, 13, 16, and 19), and the neural response to psychosocial stress as young adults (mean age = 20.03 ± 1.51). In the present study, 301 participants (149 Men, 152 Women) completed the Montreal Imaging Stress Task (MIST), a psychosocial stress task designed for the neuroimaging environment. Results demonstrated significant sex differences in violence exposure, such that men had greater violence exposure than women [$t(299)=3.35$, $p<0.01$]. Further, a linear mixed effects model revealed significant sex differences in the neural response to stress within the dorsolateral prefrontal cortex (dlPFC) and dorsomedial prefrontal cortex (dmPFC). Specifically, men had greater neural reactivity within the dlPFC and dmPFC than women. In addition, we assessed the interaction between violence exposure and sex on the neural response to stress. Results demonstrated a significant interaction between sex and violence exposure within the right parahippocampal gyrus. The present study demonstrates the relationship between violence exposure and the neural response to stress.

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Poster

153. Human Motivation and Emotion II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 153.12/R13

Topic: G.03. Emotion

Title: Effect of mortality salience on the neurocognitive processing of guilt and shame

Authors: *Z. XU[1,2,3], R. ZHU[1,2,3], C. LIU[1,2,3];
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Abstract: It is the unique human capacity to know that one day we will not exist. Thinking about death impacts many kinds of human social behaviors, generating various adaptive phenomena. Previous studies showed that mortality salience modulated individual experience of emotion; however, the underlying neural substrates remain obscure. This study focused on two important self-conscious emotions involving self and others: guilt and shame. Using fMRI technique, we investigated how mortality salience affected the neural processing of these two emotions. Sixty-five healthy volunteers were randomly assigned to one of the two conditions of the mortality salience manipulation. After mortality salience priming or negative affect priming, participants

recalled the shame events, guilt events and neutral events they wrote down in an online questionnaire before coming to the laboratory and relived emotions in these events. Our behavioral results showed that mortality priming made people feel more guilty and ashamed for guilt events. In addition, we found that no matter comparing guilt condition vs. neutral condition or comparing shame condition vs. neutral condition, mortality salience group showed greater activation within ventromedial prefrontal cortex (vmPFC) region than control group. Further psychophysiological interaction (PPI) analysis revealed that mortality salience increased vmPFC connectivity with precuneus and middle temporal gyrus (MTG) in guilt condition comparing with neutral condition. But when comparing shame condition vs. neutral condition, mortality salience decreased vmPFC connectivity with precuneus and posterior cingulate cortex (PCC). Our findings indicated that, while guilt and shame have many similar characteristics, mortality salience triggered different regulation mechanisms for the two emotions, suggesting the different significance of guilt and shame in the face of death.

Disclosures: **Z. Xu:** None. **R. Zhu:** None. **C. Liu:** None.

Poster

153. Human Motivation and Emotion II

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Program #/Poster #: 153.13/R14

Topic: G.03. Emotion

Support: the National Science Foundation of China 31730038, 31861143040
the 111 Project B07008

Title: Neuroticism associates brain functional network efficiency

Authors: *M. PENG;
Beijing Normal Univ., Beijing, China

Abstract: Neuroticism, a stable personality trait, associated with various anxious and depressive psychiatric disorders. Prior studies mainly focused on the dysfunction of limbic and frontal cortex to explain high neurotic individuals' tendency to worry and to respond poorly to stress. Moreover, some structural evidence showed that the alteration not only occurred in the aforementioned regions, but also extended to other regions. However, studies concerning how functional changes of wider brain areas affect neuroticism are rare, especially with multivariate pattern analysis methods. Using resting state fMRI and the Big Five Aspects Scale questionnaire, we investigated the relationship between the changes in the whole-brain resting state functional network structure, measured by local efficiency, and neuroticism in 391 subjects. The results showed that the local efficiency in the left insula and left cerebellum was associated with neuroticism. Further, we built support vector regression models for neuroticism using local

efficiency features, and showed that the models could significantly predict the neuroticism score. The identified local efficiency regions contributing to the neuroticism prediction consisted of a wide range of regions, including not only the limbic system and frontal areas (e.g., left anterior cingulum cortex, left insula, left inferior frontal orbital cortex, right superior frontal cortex) as many studies found with univariate methods, but the motor system (e.g., supplement motor area, right precentral gyrus) and the visual system (e.g., right inferior occipital cortex, left rolandic operculum). This finding allowed a more comprehensive understanding of the neural basis of neuroticism.

Disclosures: M. Peng: None.

Poster

153. Human Motivation and Emotion II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 153.14/R15

Topic: G.03. Emotion

Support: Stanford Center on Compassion and Altruism Research and Education

Title: Neural evidence for positive "compassion carry-over"

Authors: T. SRIRANGARAJAN[1], A. GENEVSKY[2], B. MERRITT[3], T. JINPA[4], *B. D. KNUTSON[1];
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Abstract: Can simply extending compassion to someone “carry over” to affectively color future encounters? To assess whether individuals could extend compassion to strangers and whether their efforts might leave a lasting impression, we paired a simple but novel compassion induction task with a subsequent affect misattribution task -- first during behavioral experiments (n=22; n=13), and later during the acquisition of functional magnetic resonance imaging (FMRI) data (n=21).
In Experiment 1, extending compassion (versus neutrality) towards targets’ faces increased positive responses to neutral stimuli (i.e., modern art) presented immediately after the faces (100 msec). In Experiment 2, extending compassion (versus neutrality) towards targets’ faces again increased positive responses to neutral stimuli presented immediately after the faces -- even though the faces were presented subliminally (33 msec). These subliminal results suggested that positive affective “carry over” from previously extending compassion did not require conscious awareness. In Experiment 3, extending compassion (versus neutrality) increased activity in circuits implicated in reward processing (including the nucleus accumbens (NAcc), and medial

prefrontal cortex (MPFC)), as well as social inference (i.e., the left temporal parietal junction (TPJ)). Subliminal exposure to compassion targets again increased positive affective responses to subsequently-presented neutral stimuli, while increasing activity in subcortical regions implicated in reward processing (i.e., the NAcc).
Together, these findings suggest that people can extend compassion to strangers in a laboratory setting and that positive responses to compassion targets can "carry over" into future encounters -- even in the absence of awareness. The findings thus demonstrate potentially hidden but lasting benefits of cultivating compassion.

Disclosures: T. Srirangarajan: None. **A. Genevsky:** None. **B. Merritt:** None. **T. Jinpa:** None. **B.D. Knutson:** None.

Poster

153. Human Motivation and Emotion II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 153.15/R16

Topic: G.03. Emotion

Title: Cerebellar tDCS in Parkinson's disease: Modulation of negative emotions recognition

Authors: F. RUGGIERO[1], ***S. MARCEGLIA**[2], F. MAMELI[1], M. VERGARI[1], S. MRAKIC-SPOSTA[3], M. NIGRO[1], S. BARBIERI[1], A. PRIORI[4], R. FERRUCCI[5];
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Abstract: Introduction: Emotional-processing impairments, resulting in a difficulty to decode emotions from faces especially for negative emotions, are characteristic non-motor features of Parkinson's disease (PD) that could stem from a disturbance of facial mimicry or from incongruent feedback conflicting with the internal simulation of the observed facial expression. There is limited evidence about the specific contribution of the cerebellum to the recognition of emotional contents in facial expressions even though patients with cerebellar dysfunction often lose this ability. Also, cerebellar deficits are common in mental illnesses associated with defective emotional processing. In this work we aimed to evaluate the role of the cerebellum in recognizing facial expressions in PD.
Methods: Nine PD patients (aged 42-77; 5 males; H&Y range 1-3) were enrolled and received anodal and sham cerebellar tDCS (2mA, 20 min), once a day for 5 consecutive days, in two separate cycles at intervals of at least 1 month. The Facial Emotion Recognition Task was administered at baseline (T0) and after cerebellar tDCS on day five (T1). We used 64 facial expressions from the NimStim Face Stimulus Set, consisting of 16 Caucasian adults (8 men) expressing anger, happiness, sadness and neutral expression. We generated two alternative sets

of pictures consisting of 32 trials (8 faces, 4 men). Pictures were presented in random order and each facial expression was shown three times (total 96 trials, 24 for each emotion category). Reaction times (RTs) were used to investigate tDCS effects (three-way ANOVA, factors “time”, “stimulation”, “facial expression”, and Tukey’s honest post-hoc test, $p<0.05$).
Results: Anodal cerebellar tDCS significantly enhanced sensory processing in response to negative facial expressions (sadness) by about 16% [(mean±SE) anodal vs sham: -16%±6 vs -3%±5.5; $p=0.03$] but left positive emotion (happiness) and neutral facial expressions unchanged ($p>0.05$).
Conclusions: Our results show that cerebellar tDCS modulates the way PD patients recognize specific facial expressions thus suggesting that the cerebellum plays a crucial role in recognition of negative emotions, and corroborating previous knowledge on the link between social cognition and the cerebellum. Understanding the disruption of facial emotion recognition in PD is crucial for improving quality of life for both patients and caregivers, as this impairment is associated with heightened interpersonal difficulties and the possibility of modulating emotional recognition by cerebellar tDCS in PD patients might be relevant for developing novel therapeutic approaches.

Disclosures: **F. Ruggiero:** None. **S. Marceglia:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Newronika srl, spin-off company. **F. Mameli:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Newronika srl, spin-off company. **M. Vergari:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Newronika srl, spin-off company. **S. Mrakic-Sposta:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Newronika srl, spin-off company. **M. Nigro:** None. **S. Barbieri:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Newronika srl, spin-off company. **A. Priori:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Newronika srl, spin-off company. **R. Ferrucci:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Newronika srl, spin-off company.

Poster

153. Human Motivation and Emotion II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 153.16/R17

Topic: G.03. Emotion

Support: University of Illinois Veterans Center Grant

Title: Cultivating affective resilience: Translational benefits of a novel cognitive-emotional intervention in veterans

Authors: *S. D. DOLCOS, Y. HU, H. BERENBAUM, F. DOLCOS;
Univ. of Illinois at Urbana-Champaign, Champaign, IL

Abstract: Experiential factors influence well-being and evidence shows that specific forms of training can produce strong and enduring beneficial effects. Training can also shape the structure and function of our brains. Abundant evidence highlights the important role of cognitive and emotion regulation (ER) in the experience of subjective well-being, but translation of the beneficial effects of ER from laboratory studies to real-life benefits in affective resilience is still in early phases. Here, we report supportive evidence from a novel cognitive-emotional intervention targeting the development of self-regulation skills aimed at increasing resilience against emotional distress in a group of returning veterans. This intervention was informed by our own research on the effectiveness of two ER strategies - focused attention and cognitive reappraisal - and involved training in applying them to scenarios presenting emotional conflicts, constructed with both external (visual stimuli) and internal (autobiographical memories) cues. The intervention was administered in a total of nineteen veterans (all males), in two sessions per week, for 5-8 weeks, and was preceded and followed by cognitive/executive and clinical assessments, to examine basic and transfer effects; a subsample of ten participants also underwent resting state fMRI scans. Preliminary results showed overall enhanced psychological well-being and executive function, following training. The resting state fMRI results showed that our cognitive emotional intervention facilitated enhanced functional connectivity between regions associated with ER (prefrontal cortex) and basic emotion processing (amygdala), and decreased connectivity between control and visual regions. Overall, our findings suggest that resilience and well-being can indeed be learned through training, and that intervention-related improvements, manifested in both behavioral change and neuroplasticity in the resting state functional connectivity, can translate in real-life benefits in dealing with emotional challenges. Future research is needed to tap further into the potential benefits of our training and to determine whether it can produce changes with long-lasting consequences.

Disclosures: S.D. Dolcos: None. **Y. Hu:** None. **H. Berenbaum:** None. **F. Dolcos:** None.

Poster

153. Human Motivation and Emotion II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 153.17/R18

Topic: G.03. Emotion

Support: University of Illinois

Title: Immediate and long-term effects of focused attention: A functional MRI investigation

Authors: *F. DOLCOS, S. JUN, Y. KATSUMI, S. CHEN, P. BOGDAN, M. O'BRIEN, S. BUETTI, A. LLERAS, K. FREEMAN BOST, S. DOLCOS;
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Abstract: Emotion regulation (ER) can modulate both immediate (emotional experience) and long-term (episodic memory) effects of emotion. Previous studies have mainly focused on reappraisal and suppression, strategies known to engage extensive cognitive resources. However, to help individuals with limited cognitive resources available, it is essential to also examine ER strategies that can be effectively engaged with relatively less cognitive effort. Here, we investigated the effects of a strategy considered more efficient in controlling emotional responses: focused attention (FA). Forty-eight healthy adults rated the emotional content of negative and neutral pictures (immediate effect) under different attentional manipulation conditions, cued immediately before image onset. A subsample (N=24) also had fMRI and eye-tracking data recorded simultaneously. One week later, participants' memory for the pictures was also tested in a recognition memory task (long-term effect). Behaviorally, FA was successful in decreasing both the emotional experience and the memory for negative images. Moreover, individual differences in eye-gaze predicted emotional ratings, thus suggesting the utility of this measure in predicting the behavioral impact of FA. Preliminary analyses of brain imaging data show that focusing away from the emotional aspects of negative images increased activity in executive/attentional control regions, including the dorsolateral prefrontal cortex (dlPFC) and lateral/medial parietal cortex, and decreased activity in the amygdala. Overall, these findings demonstrate that FA is effective in decreasing both the immediate experience and the long-term memory for negative stimuli, and that these effects are linked to modulation activity in regions associated with top-down (dlPFC) and bottom-up (amygdala) neural processing.

Disclosures: F. Dolcos: None. **S. Jun:** None. **Y. Katsumi:** None. **S. Chen:** None. **P. Bogdan:** None. **M. O'Brien:** None. **S. Buetti:** None. **A. Lleras:** None. **K. Freeman Bost:** None. **S. Dolcos:** None.

Poster

153. Human Motivation and Emotion II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 153.18/R19

Topic: G.03. Emotion

Title: Neurophysiological synchrony as generalizable biomarker of group experience and performance

Authors: *S. M. RENNIE[1], L. GAUDREAU[2], R. LEONE[2], D. BHATT[2], M. L. PLATT[3]; [1]Univ. of Pennsylvania, Philadelphia, PA; [2]Univ. of Pennsylvania, Philidelphia, PA; [3]CCN, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Synchrony the coupling of dynamical systems, is a fundamental property of interacting biological systems at all scales, whether it be populations of cells or groups of people. Humans are exquisitely social. Our brains and physiology are uniquely shaped to support collective action and seek shared experiences. In humans behavioural and physiological synchrony emerges from individuals acting or experiencing together. Our ability to examine the biological mechanisms of dynamic interactions amongst humans has been limited by theoretical and technological barriers both in the lab and the real world. To overcome these limitations we developed and validated a wearable platform that allowed us to simultaneously record multi-channel, multi-timescale, physiological signals including electrodermal activity (EDA), respiration, electromyography (EMG) electrocardiography (ECG), electroencephalography (EEG) and movement (ACC) simultaneously in freely behaving human groups. Using this platform we tested the hypothesis that biological synchrony constitutes a set of generalizable biomarkers of performance and experience in two separate types of human group interactions. First when individuals act together as a team, in this case during rowing training, where we could precisely measure behavioural and physiological synchrony and relate this directly to experience and performance. Second, when groups of humans share collective experiences during a range of different dance and music performances where we could relate measured synchrony to a range of individual differences such as tendency to cooperate.

Disclosures: S.M. Rennie: None. **L. Gaudreau:** None. **R. Leone:** None. **D. Bhatt:** None. **M.L. Platt:** None.

Poster

153. Human Motivation and Emotion II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 153.19/R20

Topic: G.03. Emotion

Support: CNPq (National Council for Scientific and Technological Development), Project n°426861/2016-7

Title: Postural and autonomic responses during observation of emotional body expressions

Authors: *A. A. NOGUEIRA-CAMPOS[1], M. R. A. CORREIA[2], L. A. IMBIRIBA[2]; [1]Dept. of Physiol., Federal Univ. of Juiz De Fora, Juiz de Fora, Brazil; [2]Sch. of Physical Educ. and Sports, Federal Univ. of Rio de Janeiro, Rio de Janeiro, Brazil

Abstract: Emotional body expressions pictures have been largely used as stimuli to unveil the effect of emotion over the physiology. Their observation has a high recognition level and recruits motor regions, besides emotional ones. Herein, we aimed to investigate postural and cardiorespiratory responses during the observation of positive, negative and neutral body expressions pictures. Thirty-nine healthy adult volunteers (21 men, 23.05±3.4 of age) stood upright on a force platform (AccuSway Plus, AMTI, EUA) with arms along their body and feet together, while pictures were presented by means of a projection-controlled screen positioned in front of them. During stabilometric and cardiorespiratory recordings, 20 positive (happiness), 20 negative (anger) and 20 neutral body expressions pictures were presented. Pictures presentation was set in a blocked-protocol by each category, interposed by a gray screen displayed at the beginning and at the end of each block. Finally, a psychometric report was performed in which participants evaluated each picture according to the level of emotional expression recognition by a three alternative-forced-choice task (happiness, anger or neutral), and in two dimensions (valence and arousal) by the Self-assessment Manikin scale. The data were collected by a custom software (Matlab, Mathworks) and the block comparisons were assessed by analysis of variance with Newman-Keuls post hoc test at 95 % confidence level ($p<0.05$). The pictures had a level of recognition superior to 70% for all blocks. Positive pictures had higher level of valence (6.01±0.93) followed by neutral (5.03±0.57), and negative (4.29±0.92) one. However, the neutral pictures had the lowest level of arousal (2.32±1.43), followed by positive (3.15±1.5) and negative (3.83±1.92). There was a statistically significant reduction in the body sway area between the negative (184.72±69.73mm²) compared to the positive (216.42±128.77mm²) and neutral (219.75±98.1mm²) blocks. The mean power frequency in the medial-lateral axis showed that the negative (0.26±0.08Hz) block also had a significant increase compared to the neutral (0.23±0.07Hz) one. In addition, the negative (9.76±4.17ms) block showed a significant increase for the RMSSD parameter of heart rate variability when compared to the positive (8.93±4.1ms) one. Thus, the observation of negative pictures of body expressions prompted postural and cardiac alterations compatible with the activation of defensive adjustments. Taken together, these changes may reflect the motor and emotional contagion of the observer to the emotional stimuli based on body expressions.

Disclosures: **A.A. Nogueira-Campos:** None. **M.R.A. Correia:** None. **L.A. Imbiriba:** None.

Poster

153. Human Motivation and Emotion II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 153.20/S1

Topic: G.03. Emotion

Title: EEG source localization of independent components associated with multimodal emotion perception

Authors: *K. M. BECKER[1], D. C. ROJAS[2];
[2]Psychology, [1]Colorado State Univ., Fort Collins, CO

Abstract: Facial movements and vocal expressions instantaneously convey emotion, with emotional percepts occurring via the simultaneous integration of affective vocal and facial information. These separate but often complementary channels activate a distributed constellation of brain areas, which are known to be sensitive to emotional facial expressions and are distinct from those devoted to prosody recognition. While much research has focused on the independent contributions of these channels to emotion perception, fewer studies have focused on disentangling the core components underlying multimodal affect perception and their localization in the brain. The current study sought to quantify and localize the electrical sources underlying this dynamic process by extracting components maximally correlated with the behavioral task from 39 electrodes using independent component analysis (ICA). The components were then projected to source space using the sLORETA algorithm. Subjects' (n=26) were presented with either affective faces (F), voices (V), or simultaneous faces and voices (F+V) while their brain activity was measured with EEG. Vocal prosodic stimuli consisted of emotional vocalizations of the vowel /a/ produced in neutral, angry, and happy voice tones. These stimuli were used to create seven conditions, three bimodal (face and voice), three voice only (one for each prosody) and one face only condition. Component activity was significantly correlated with all stimuli types between approximately 200-800 ms. One component was significantly correlated with the F+V stimulus condition and this activity was localized to occipitoparietal areas bilaterally. Additionally, this component was also correlated with the unimodal face and voice only stimulus conditions, but the time courses of these correlations appeared to be anti-correlated with the time course of the F+V stimulus condition. Collapsed across stimulus type, one component was correlated with both anger and happiness, but in opposing directions across the post-stimulus time period. Component activity was localized activity to frontotemporal and parietal regions in the right hemisphere and temporal areas associated with language processing in the left hemisphere. These results indicate that brain activity can be decomposed and correlated with voices, faces, and simultaneously presented faces and voices. Moreover, the brain appears to exhibit a unique and distributed pattern of neural activity when processing multimodal emotional stimuli, which is distinct from that witnessed during the processing of unimodal stimuli.

Disclosures: K.M. Becker: None. **D.C. Rojas:** None.

Poster

154. Drugs of Abuse: Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 154.01/S2

Topic: G.08. Drugs of Abuse and Addiction

Support: Ministerio de Economía y Competitividad (Plan Nacional I+D, PSI2015-68600-P)
Ministerio de Sanidad, Servicios Sociales e Igualdad (Plan Nacional de Drogas, PND-132400)
Universitat Jaume I (Projectes d'investigació científica i desenvolupament tecnològic 2017, UJI:17I389.01/1)

Title: Cerebellar perineuronal nets digestion with chondroitinase ABC in cocaine-induced memory: Creating conditions for change

Authors: *J. GUARQUE-CHABRERA, I. MELCHOR-EIXEA, A. SANCHEZ-HERNANDEZ, M. MIQUEL;
Área de Psicobiología, Univ. Jaume I, Castellon de la Plana, Spain

Abstract: Perineuronal nets (PNNs) are cartilage-like structures of extracellular matrix molecules that enwrap in a net-like manner the cell-body and proximal dendrites of special subsets of neurons. PNNs stabilize their incoming connections and restrict plasticity. Consequently, they have been proposed as a candidate mechanism for drug-induced learning and memory. Indeed, PNNs are considered to contribute to the maintenance of drug-induced conditioned memories after prolonged drug abuse. In the cerebellum, PNNs surround both inhibitory and excitatory neurons in the deep cerebellar nuclei (DCN) but only inhibitory Golgi cells in the cerebellar cortex. Previous studies from the lab showed an increase in PNN expression in both the apical region of the cerebellar vermis and the lateral DCN (LDCN), only in those animals exhibiting preference towards cocaine-associated cues. The present research aimed to investigate the role of the cerebellar PNNs in drug-induced conditioned memories. For this purpose, we use the enzyme chondroitinase ABC (ChABC) in order to digest cerebellar PNNs at different time points of the learning process to ascertain whether their degradation can affect drug-induced memories.
Our results show that PNN degradation using ChABC in lobule VIII of the vermis prior to conditioning promoted the acquisition of cocaine-induced preference conditioning. However, digesting PNNs in dorsal vermal area once drug memory was already acquired did not produce any effect over preference memories. Thus, to assess the hypothesis of a memory transfer from the cerebellar cortex to the DCN once memory is acquired, we digested bilaterally PNNs in the LDCN in order to impair memory expression. Preliminary results showed a blockade in the expression of cocaine-induced conditioned preference, supporting the memory transfer hypothesis. Moreover, the enzymatic degradation prior to extinction increased the number of rats reaching the extinction criterion after the first extinction test.
These findings indicate that PNN digestion in the dorsal cerebellar cortex might facilitate both the acquisition of drug-induced conditioned preference or its extinction. However, PNN degradation within the LDCN would block the expression of cocaine-induced memories. Therefore, the absence of PNNs in the cerebellum induces a plasticity state that could be manipulated to optimize the disruption of drug-induced memories.

Disclosures: J. Guarque-Chabrera: None. **I. Melchor-Eixea:** None. **A. Sanchez-Hernandez:** None. **M. Miquel:** None.

Poster

154. Drugs of Abuse: Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 154.02/S3

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA Grant DA042029

Title: Effects of chemogenetic manipulation of the VTA dopamine projection to lateral amygdala on cocaine-cue conditional learning

Authors: *D. M. SMITH, M. M. TORREGROSSA;
Psychiatry Dept., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Drug craving and relapse can be triggered by environmental cues previously associated with drug-use. Thus, determining the circuitry underlying drug-cue associations is critical for developing effective treatments for addiction. Many studies investigating associative learning processes have shown that an auditory conditioned stimulus (CS) is encoded by both auditory thalamus and auditory cortex inputs to the lateral amygdala (LA). However, the LA inputs that encode the reinforcing interoceptive effects of a drug that act as the US during associative conditioning are not well characterized. Previous research indicates that dopamine (DA) release in the LA likely contributes to encoding the reinforcing interoceptive effects of cocaine since DA in the LA maintains cocaine-cue associations. To investigate this hypothesis, we expressed hM4Di-coupled designer receptors (DREADDs) in the VTA and silenced terminals in the LA by local microinjection of the DREADD agonist clozapine-N-oxide (CNO) to investigate the role of this projection in cocaine-cue conditional learning. Rats underwent 5 days of 1h self-administration sessions where an active press resulted in a cocaine infusion (1 mg/kg) paired with a 10s tone/light cue. Prior to each self-administration session, rats received intra-LA microinjections of CNO or vehicle (ACSF). Their seeking behavior was extinguished before the rats underwent both cue-induced reinstatement and cocaine-primed reinstatement. Inhibiting the VTA DA projection to the LA led to slower acquisition of cocaine self-administration. Moreover, while cocaine-primed reinstatement did not differ between animals that received CNO versus vehicle during self-administration, prior CNO treatment did reduce cue-induced reinstatement.
These results suggest that the VTA DA projection to the LA is necessary for facilitating CS-US associative learning, but is not sufficient to encode the positive reinforcing effects of cocaine. These findings contribute to our understanding of the role of the VTA DA to LA projection in pairing the CS with the reinforcing interoceptive effects serving as the US in cocaine-cue conditioning.

Disclosures: D.M. Smith: None. **M.M. Torregrossa:** None.

Poster

154. Drugs of Abuse: Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 154.03/S4

Topic: G.08. Drugs of Abuse and Addiction

Title: Hippocampal MeCP2 and histone acetylation mediate cocaine-induced shifts in behavioral learning and control

Authors: E. HARVEY, M. M. COBB, ***P. J. KENNEDY**;
Psychology, Univ. of California Los Angeles, Los Angeles, CA

Abstract: The transition from recreational drug use to addiction involves pathological learning processes that support a persistent shift from flexible, goal-directed to habit behavioral control. Here we examined the molecular mechanisms supporting altered function in hippocampal (HPC) and dorsolateral striatal (DLS) memory systems following abstinence from repeated cocaine. After 3 weeks of cocaine abstinence we tested new behavioral learning in male rats using a dual-solution maze task, which provides an unbiased approach to assess HPC- vs. DLS-dependent learning strategies. Dorsal hippocampus (dHPC) and DLS brain tissues were collected after memory testing to identify transcriptional adaptations associated with cocaine-induced shifts in behavioral learning. Our results demonstrate that following prolonged cocaine abstinence rats show a bias towards the use of an inflexible, habit memory system (DLS) in lieu of a more flexible, easily updated memory system involving the HPC. This memory system bias was associated with upregulation of methyl CpG binding protein 2 (MeCP2) in the HPC and DLS as well as decreased and enhanced permissive histone acetylation at brain-derived neurotrophic factor (BDNF) and MeCP2 promoter regions in the HPC, respectively. Using viral-mediated gene transfer, we knocked down MeCP2 in the dHPC during cocaine abstinence and new maze learning. This manipulation restored HPC-dependent behavioral control. These findings provide a systems-level understanding of altered plasticity and behavioral learning following cocaine abstinence and inform mechanisms mediating the organization of learning and memory more broadly.

Disclosures: **E. Harvey:** None. **M.M. Cobb:** None. **P.J. Kennedy:** None.

Poster

154. Drugs of Abuse: Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 154.04/S5

Topic: G.08. Drugs of Abuse and Addiction

Title: Cognitive training and cocaine addiction in rats: Timing is everything!

Authors: *M. SOLINAS, M. WAHAB, L. VIRGINIE;
INSERM U1084/University of Poitiers, Poitiers, France

Abstract: Drug addiction is often associated with cognitive deficits and cognitive training has been proposed to help individuals to resist their desire to use drugs. On the other hand, an important body of literature suggests that, under certain conditions, exerting a cognitive effort could exhaust self-control resources and lead to bad decision-making and excessive drug intake. Therefore, in this study, we investigated the effects of cognitive training immediately or 2h before cocaine self-administration of cocaine.
In a first experiment, we investigated the effects of cognitive exercise immediately before cocaine self-administration on drug taking behavior. For this, adult male rats were allowed to self-administer either cocaine or saline in sessions that were immediately preceded by sessions in which rats had to perform a cognitive task involving behavioral flexibility. These sessions started and ended with 45min attentional set-shifting phases and, in between, rats could self-administer cocaine or saline for 150min. In a second experiment, we investigated the effect of cognitive training separated from cocaine self-administration on drug taking behavior. For this, rats underwent cognitive training in the same 45min attentional set-shifting task 2h before the start of the 150min cocaine self-administration session, giving them the time to recover from cognitive effort. For both experiments, control groups performed a non-cognitive task were they could obtain food without exerting any cognitive effort.
We found that rats that underwent a cognitive training immediately before access to the drug took significantly more cocaine than control rats and continued to seek for cocaine even when the drug was not available. Notably, cocaine seeking was increased before self-administration and was correlated with the intensity of subsequent self-administration suggesting that rats in this group were anticipating cocaine availability. In contrast, we found that rats that were allowed to rest for a period of 120 minutes after the cognitive training took significantly less cocaine than control animals that performed a non-cognitive training.
Therefore, similarly to what is described in humans, exerting a cognitive effort before having access to the drug has negative effects on cocaine self-administration. In contrast, when cognitive exercise is followed by a recuperation period, it has positive on cocaine taking. These results suggest that cognitive training may have beneficial effects on drug addiction but, if

cognitive exercise leads to excessive cognitive load at time when the individuals have access to the drug, it could also have negative effects.

Disclosures: M. Solinas: None. **M. Wahab:** None. **L. Virginie:** None.

Poster

154. Drugs of Abuse: Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 154.05/S6

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA/NIH

Title: Identifying genetic targets within activity-dependent neuronal ensembles in the prefrontal cortex following cocaine place conditioning

Authors: *L. R. WHITAKER, C. N. MILLER, C. CANN, R. MADANGOPAL, R. A. M. MARINO, M. VENNIRO, F. J. RUBIO, C. T. RICHIE, B. T. HOPE;
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Abstract: Learned associations between rewards, such as food or drugs of abuse, and reward-predictive stimuli, are critical to motivated behavior. Associative learning is thought to be encoded by functional alterations within activity-dependent neuronal ensembles. The specific mechanism by which learned associations are encoded within activity-dependent ensembles remains unknown. The immediate early gene, Fos, can be used to identify activity-dependent ensemble neurons. We used a cocaine conditioned place preference (CPP) procedure to study the learned association between an environmental context and cocaine reward in mice. To identify brain regions active during expression of cocaine CPP we used Fos immunohistochemistry and identified the prelimbic cortex as a particularly active region. We then used fluorescence activated cell sorting (FACS) to identify genetic targets that distinguish Fos-positive ensemble neurons from Fos-negative neurons in the prefrontal cortex following exposure to the paired or unpaired context. Next, we examined the stability of Fos-expressing PFC ensembles using Fos-tTa transgenic mice in conjunction with the AAV-TRE3G-histone H2BmCherry virus. Our results suggest that PFC ensembles are dynamically encoded. In future, we will use the Fos-tTa system to identify, characterize, and manipulate functional targets that we have identified using FACS within neuronal ensembles that encode associative learning.

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Poster

154. Drugs of Abuse: Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 154.06/S7

Topic: G.08. Drugs of Abuse and Addiction

Support: DA014339
DA045335
DA007244

Title: Effects of prolonged cocaine abstinence on neural activity in the prelimbic cortex and nucleus accumbens core

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Abstract: In individuals with substance use disorders (SUDs), the length of time away from drug use plays a critical role in the propensity to relapse. In animal models, cocaine-associated stimuli elicit drug-seeking which increases as a function of abstinence duration, and has been attributed to alterations in neural activity in brain reward circuitry. We have previously reported that 1 month of experimenter-imposed abstinence from cocaine self-administration is associated with robust increases in the proportion of cells in the nucleus accumbens (NAc) core and prelimbic cortex (PrL) that are phasic to cocaine-associated cues, and an increase in drug seeking (Hollander and Carelli, J Neurosci, 2007; West et al., Eur J Neurosci, 2014). However, animal models also show reduced resting state functional connectivity between the medial prefrontal cortex, which includes the PrL, and NAc following 1-month abstinence from cocaine (Lu et al., Brain Connect, 2014), paralleling hypofrontality in individuals with SUDs. However, whether these seemingly divergent neuroadaptations interact and the respective role of each in persistent cocaine seeking has not yet been assessed. In preliminary studies, adult male Sprague Dawley rats were trained to self-administer cocaine (n=5, ~1 mg/kg/inf, 2 h per session) or saline/water (n=3, volume matched), paired with an audiovisual cue (20 s) during 14 daily sessions before undergoing 30 days of experimenter-imposed abstinence. Next, rats underwent a test session consisting of non-contingent presentations of the audiovisual cue (15 min), extinction (lever press resulted in cue but no drug; 2 h), and resumption of self-administration (2 h). Using *in vivo* electrophysiological methods, we simultaneously recorded local field potentials (LFPs) in the PrL and NAc core to examine synchronized oscillatory dynamics to assess functional connectivity between these regions. Preliminary data show that 1-month cocaine abstinence led to reduced spontaneous synchronous PrL and NAc core activity (reflected in PrL—NAc core

coherence) in the beta (12-20 Hz) and high gamma (>70 Hz) ranges compared with saline/water controls. These reductions were not seen on the first day of abstinence. Ongoing investigations are examining whether incubation of cocaine craving (i.e. heightened cocaine seeking following 1-month versus 1-day abstinence) is associated with alterations in PrL—NAc core activity and functional connectivity at resting state (i.e. spontaneous neural activity) or to cocaine-associated cues.

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Poster

154. Drugs of Abuse: Learning and Memory I

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Program #/Poster #: 154.07/S8

Topic: G.08. Drugs of Abuse and Addiction

Support: K99 DA042934
R21 DA045335

Title: Targeted optogenetic stimulation of the prelimbic cortex to nucleus accumbens core pathway restores behavioral flexibility following a history of cocaine exposure

Authors: *E. A. WEST, M. NIEDRINGHAUS, T. J. SLOAND, R. M. CARELLI;
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Abstract: The ability to shift behavior in response to changing outcomes (behavioral flexibility) is necessary for survival. Substance use disorders (SUDs) are characterized by the inability to stop reward-seeking despite maladaptive consequences. As we have previously shown, neural encoding in nucleus accumbens (NAc) core, which receives dense glutamatergic input from prelimbic cortex (PrL), during learning predicted subsequent behavioral flexibility (West and Carelli 2016). In addition, PrL neural encoding during learning predicted subsequent behavioral flexibility, and suppressing transmission in PrL-NAc core neurons during learning (using retrograde Cre virus in the NAc core and Cre-on halorhodopsin virus in the PrL) is sufficient to induce deficits in behavioral flexibility. Finally, prior exposure to cocaine (14 days; 2-hr self-administration; 0.33 mg/inf, ~25 mg/kg) followed by a month of experimentally imposed abstinence suppressed coherent activity in the PrL-NAc core connection and induced deficits in behavioral flexibility compared to controls. We used a reinforcer devaluation task, a canonical measure of behavioral flexibility, following a history of cocaine to determine if optogenetic stimulation of the PrL-NAc core pathway is sufficient to reverse behavioral flexibility deficits

following prior drug exposure. We utilized a viral paradigm to express channelrhodopsin (ChR2) in PrL neurons that directly project to NAc core (retrograde Cre virus in NAc core and Cre-on ChR2 virus in the PrL). Rats (Long-Evan males, n=8 ChR2 and n=6 mCherry control) self-administered cocaine as described above, followed by 18 days of abstinence. High frequency (83Hz) blue light was delivered into the PrL for 3 days (10s on/10s off, 20 cycles/day; days 18-20 of abstinence) to excite the PrL-NAc core pathway. Next, rats underwent Pavlovian conditioning. One conditioned stimulus (CS+1) predicted food, while a different conditioned stimulus (CS+2) predicted sugar, while two other stimuli did not predict anything (CS-1 and CS-2). Sugar pellets were paired with Lithium Chloride (0.3 M) to induce a conditioned taste aversion. Finally, we measured the rats' ability to avoid the cue paired with sugar pellets (i.e., CS+2) under extinction. We found that high frequency stimulation of PrL neurons projecting to the NAc core prior to behavioral training and testing restored cocaine-induced deficits in behavioral flexibility. These findings suggest the PrL and its connection with the NAc core is causally linked to behavioral flexibility and this pathway may serve as a putative target for potential therapeutics to restore behavioral flexibility in SUD patient populations.

Disclosures: E.A. West: None. **M. Niedringhaus:** None. **T.J. Sloand:** None. **R.M. Carelli:** None.

Poster

154. Drugs of Abuse: Learning and Memory I

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Topic: G.08. Drugs of Abuse and Addiction

Support: NSFC Grant 81501108
973 program 2015CB553504

Title: A small interference peptide disrupts drug induced long term place preference in rats

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Abstract: Objective: Maladaptive learning and memory process that associate neutral environmental stimuli over time with the drugs rewarding effects are known to have an important role in inducing craving and relapse to drug seeking behaviors. A small interference peptide named Tat-GluR2$_{3Y}$ with very low risk of side effects could prevent relapse by block AMPA receptor endocytosis hence disrupt drug-reward associative memory. However, the practical therapeutic strategies of Tat-GluR2$_{3Y}$ for long-term relapse prevention in clinic management need to be further explored. Conditioned Place Preference (CPP) assesses the drug reward-

mediated associative learning in animals and is used to identify relapse prevention medications. **Methods and results:** Here we used methamphetamine or morphine induced-CPP rats models and applied Tat-GluR2$_{3Y}$ at the different stages with different administration strategies, to further demonstrate the effect of Tat-GluR2$_{3Y}$ in the drug reward associative learning and develop the practical therapeutic strategies of Tat-GluR2$_{3Y}$ for long-term relapse prevention for methamphetamine or morphine use disorders. We found that Tat-GluR2$_{3Y}$ paired methamphetamine could disrupt the drug-associated memory and prevent reinstatement when peptide was given before extinction training. Moreover, the preventive effect could last for 1-2 months. With similar strategies, Tat-GluR2$_{3Y}$ could disrupt the morphine-induced place preference. **Conclusions:** Tat-GluR23$_{Y}$ could disrupt the methamphetamine or morphine-induced place preference and prevent reinstatement with similar strategies and at specific time window.

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Poster

154. Drugs of Abuse: Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 154.09/S10

Topic: G.08. Drugs of Abuse and Addiction

Support: UH2NS096833

Title: Brain region-specific differences in the transcriptome of cocaine- and methamphetamine-associated memories

Authors: *M. HAFENBREIDEL[1], M. E. JONES[1], S. B. BRIGGS[1], S. E. SILLIVAN[1], S. KHAN[2], M. D. CAMERON[2], G. RUMBAUGH[3], C. A. MILLER[1];
[1]Neuroscience; Mol. Med., [2]Mol. Med., [3]Neurosci., Scripps Res., Jupiter, FL

Abstract: Drug-associated memories are persistent and perpetuate substance use disorders (SUD), as they induce motivation to seek drug. We previously reported that methamphetamine (METH)-associated memories can be disrupted by direct actin depolymerization systemically or in the basolateral amygdala (BLA) by Blebbistatin (Blebb), which inhibits the actin motor ATPase nonmuscle myosin II (NMII). The effect is specific, as it does not have the same effect on other aversive or appetitive memories, including cocaine (COC). Further, it appears to be region-specific, as it does not have the same effect in dorsal hippocampus (dHPC) or nucleus accumbens (NAc). Therefore, we aimed to determine the differences between METH and COC to identify mechanisms contributing to the selective effect of NMII inhibition on BLA-specific METH-associated memories. We first assessed the impact of half-life, as that is one difference

between METH and COC. Clearance rates of METH (2mg/kg) and COC (15mg/kg) were first determined following CPP conditioning, as METH exposure has previously been shown to slow subsequent clearance rates. Using mass spectrometry, METH's brain concentration was confirmed to be higher than COC's 15 minutes after injection on the final conditioning day, and its clearance rate was slower (below detection at ~8hr for METH and ~2hr for COC). Mini-pumps were then programmed to infuse COC at a rate that mimicked METH's clearance rate during CPP conditioning, followed by systemic Blebb administration prior to the first retention test. However, mimicking METH's half-life did not render the COC-associated memory susceptible to NMII inhibition, but a CPP was absent at the second retention test, confirming our previous report that NMII inhibition disrupts reconsolidation. We next used RNA-seq to identify potential unique transcriptional changes with tissue samples collected from BLA, NAc, and dHPC after the final CPP training session for METH, COC, or saline. A relatively large difference emerged between METH and COC-conditioned samples in BLA, as 282 differentially expressed genes (DEGs) had an adjusted p-value of <0.05, whereas less than 10 DEGs emerged when comparing METH or COC to saline, suggesting that METH and COC oppositely drive transcriptional changes. Moreover, there were no statistically significant DEGs in NAc or dHPC between METH and COC conditions. Sequencing validation and bioinformatic analyses are currently being performed. Identification of the mechanism(s) responsible for METH-associated memory's selective vulnerability to NMII inhibition may yield avenues to target other pathogenic memories, including other SUDs and traumatic memories.

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Poster

154. Drugs of Abuse: Learning and Memory I

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Topic: G.08. Drugs of Abuse and Addiction

Support: TRF Research Career Development Grant (RSA5980041)

Title: Melatonin attenuates learning and memory impairment and accompanying changes in mouse hippocampus after methamphetamine administration

Authors: *S. MUKDA[1], N. VESCHSANIT[1], T. LWIN[1], P. CHANCHAROEN[1,3], S. NGAMPRAMUAN[1], P. GOVITRAPONG[1,2,4];
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Abstract: Methamphetamine (METH) has become a major public health problem for its high potential for abuse and neurotoxic effects. It has been shown that methamphetamine is directly neurotoxic and can cause changes of structure and function in the brain. The cognitive skills including attention, decision making, emotional memory, and working memory impairment in correlate to METH use have been reported. The purpose of this work aims to approach the vulnerabilities of addiction emphasizing on the cognitive impairment. Evidence has shown that melatonin can improve the cognitive function in the mild cognitive impairment patients. However, little information about the study of melatonin effects on METH-induced cognitive impairment has been documented. Thus, in this study, the effect of MEL on METH-induced cognitive impairment and accompanying changes in mouse hippocampus after methamphetamine administration were investigated. Adult male ICR mice were received METH (1 mg/kg body weight) or saline subcutaneously (s.c.) once daily for 7 consecutive days, followed by saline or MEL (s.c.) administration once daily for another 14 days. The Morris water maze (MWM) test was used to evaluate the effect of MEL on METH-induced learning and spatial memory impairment. The results showed that mice treated with METH spent longer time to reach the platform when compared with the control and other groups. Mice treated with MEL after induced by METH spent less time of the escape latency when compare with METH-treated animals, whereas MEL alone showed no effect on the time spend to reach the platform. Moreover, the effects of MEL on METH-induced change in proteins involved in memory function, including BDNF, TrkB, NMDA receptors, and phosphorylated CaMKII in the mouse hippocampus were investigated. The results found that METH-induced change in these proteins level in the mouse hippocampus, but could be restored by MEL. Taken together, these results suggested that exposure to METH causes learning and memory impairment and accompanying changes in the proteins involved in memory function in the mouse hippocampus, which can be attenuated by treated with MEL. On the basis of these results, MEL could be an effective and promising cognitive enhancer in the treatment of METH use disorders in human.

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Poster

154. Drugs of Abuse: Learning and Memory I

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Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 154.11/S12

Topic: G.08. Drugs of Abuse and Addiction

Support: R01 DA047870

Title: Experience with methamphetamine induces changes in credit assignment during multi-cue reinforcement learning

Authors: S. KOLLI, A. STOLYAROVA, A. B. THOMPSON, *A. IZQUIERDO;
Psychology, UCLA, Los Angeles, CA

Abstract: Repeated experience with powerful reinforcers in the form of drugs of abuse can result in impaired performance and decreased reward procurement in laboratory tasks, very commonly reversal learning paradigms (Calu et al. 2007; Ersche et al. 2011; Izquierdo et al. 2010; Jentsch et al. 2002), where behavior needs to be flexibly and rapidly adapted to new task demands. Yet there are several instances in which drug exposure can potentiate some forms of reinforcement learning (RL; Stolyarova et al. 2014), depending on the behavioral paradigm and treatment regimen used.One aspect of RL has remained largely overlooked: learning from the appropriate representation of reward prediction errors (RPEs) and high learning rates themselves are insufficient for successful behavioral change if the organism does not know what to learn about. To guide adaptive behavior the subject needs to correctly apply RPEs to choices that were causal to reward receipt. Under naturalistic conditions, where rewards are delayed in time or multiple cues are encountered, discovering which choices are responsible for the rewards obtained can present a challenge, known as the *credit assignment problem*. In the present work, we trained methamphetamine (meth)-pretreated and control rats on a 3-alternative visual discrimination task that offered us an opportunity to investigate changes in RL and credit assignment mechanisms that occur long after drug exposure. Consistent with a previous report, we found attenuated learning from negative feedback in protracted meth withdrawal. However, learning from positive feedback and exploitation (reliance on learned outcome values) were increased in this treatment group. Our results also revealed that exposure to meth potentiated contingent learning, as evidenced by the increased assignment of credit for outcomes to immediately preceding choices. These alterations combined can account for both increased reward sensitivity and reversal learning impairments reported previously. In addition to shedding new light on flexible RL after drug exposure, our results suggest that the influence of previous experience goes beyond affecting reward sensitivity and behavioral vigor and can similarly alter the assignment of credit for outcomes to their causal choices.

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Poster

154. Drugs of Abuse: Learning and Memory I

Location: Hall A

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Topic: G.08. Drugs of Abuse and Addiction

Support: Natural Science Foundation of China # 81871046
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Natural Science Foundation of China #81821092

Title: The neuronal ensembles in the infralimbic prefrontal cortex is involved in the effect of memory retrieval-extinction procedure on methamphetamine seeking

Authors: *Y. XUE[1], Y.-Y. CHEN[2], J. SHI[3], L. LU[4];
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Abstract: Drug addiction is a chronic relapsing disorder and the persistent existence of drug memory is a major cause of relapse among addicts. Increasing evidence showed that destroying drug memory can significantly reduce the recurrence of drug seeking induced by drug-related cues, which is an effective treatment strategy for preventing relapse. Recently, it has been demonstrated that the memory conditioned stimulus (CS) retrieval-extinction paradigm, which performed daily extinction training within the consolidation time window after CS retrieval of drug memory successfully resulted in erasure of drug memory. This procedure significantly attenuated the reinstatement of cocaine, heroin and alcohol seeking in rats as well as the cue-induced craving in heroin and nicotine addicts. However, the neural mechanisms underlying the inhibitory effects of memory CS retrieval-extinction procedure on relapse remains largely elusive and this procedure cannot be always effective under all conditions, which limited its further application in clinical practice. In recently years, methamphetamine abuse has become increasingly severe, placing a heavy burden to both society and individuals. Nonetheless, there is still a lack of effective means of treating methamphetamine addiction in clinic. Therefore, we used the rat self-administration model to explore the effect of memory CS retrieval-extinction on methamphetamine addiction memory and the relapse of methamphetamine seeking. We further explored its neural mechanisms using chemogenetic technique and labeling method of neuronal ensembles. Our findings will provide behavioral paradigms and theoretical basis for the treatment of methamphetamine addiction.

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Poster

154. Drugs of Abuse: Learning and Memory I

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Topic: G.08. Drugs of Abuse and Addiction

Support: This work is supported Department of Health and Human Services/ National Institutes of Health/ National Institute on Drug Abuse/ Intramural Research Program.

Title: Escalated compulsive methamphetamine taking in the presence of punishment: Expression of stress-related genes in the rat hippocampus and prefrontal cortex

Authors: *S. JAYANTHI[1], J. A. HERNANDEZ[3], M. T. MCCOY[2], B. LADENHEIM[1], J. L. CADET[1];
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Abstract: Stress-induced relapses to methamphetamine (METH) taking behaviors after varied lengths of forced or voluntary abstinence is a major complication of METH addiction. Susceptibility to stress is likely related to longstanding neuroadaptations that are secondary to dysfunctional epigenetic and transcriptional alterations in brain regions like the prefrontal cortex (PFC) and hippocampus that mediate cognitive aspects of decision making and memory formation that are impaired in METH addicted humans. To mimic DSM criteria of human METH addiction, we have used a rat model of drug self-administration (SA) accompanied with response-contingent punishment in rats that had escalated their METH intake. Male Sprague-Dawley rats were trained to self-administer METH (0.1mg/kg/injection) or saline intravenously for 9 h/day for 21 days. METH-trained rats escalated their intake of the drug during this process. Thereafter, lever presses for METH were punished by mild foot-shocks for 8 days (0.18-0.36 mA). Contingent footshocks helped to segregate METH-trained animals into two phenotypes: one METH SA group continued to compulsively press the lever for METH [shock- resistant (SR), addicted], whereas the other group progressively decreased or stopped their METH intake [shock-sensitive (SS), not addicted]. To mimic the adverse consequences of punishment, groups of saline SA rats were also yoked to the METH groups (YSR and YSS) and received footshocks each time that METH SA rats were punished. Rats were euthanized 2 hours after the last METH plus shock session. Quantitative PCR was used to measure mRNA in the rat PFC and hippocampus. In the hippocampus, we found that, in comparison to control and resistant rats, SS rats exhibited increased mRNA expression of *Crh* (corticotrophin-releasing hormone), *Avpr1b* (arginine vasopressin receptor 1b) and glucocorticoid receptors (*Nr3c1* and *Nr3c2)*. Sensitive rats also showed increased PFC expression of *Avpr1a* (arginine vasopressin receptor 1a) in comparison to their respective yoked-shock control and resistant rats. Interestingly, the SR and YSR rats showed significant decreases in hippocampal and PFC *Crhr1*, *Crhr2*, *Avp*, *Avpr1b*, *Nr3c1* and *Nr3c2* mRNA levels in comparison to control rats. Differential alterations in the expression of stress-related mRNAs in these two brain regions support their potential involvement in cognitive dysfunctions observed in METH addicted individuals.
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Poster

154. Drugs of Abuse: Learning and Memory I

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Program #/Poster #: 154.14/S15

Topic: G.08. Drugs of Abuse and Addiction

Support: Illinois State University School of Biological Sciences
Phi Sigma Society, Beta Lambda Chapter

Title: Modafinil and methylphenidate activate dopamine transients in the rat striatum

Authors: S. AVULA, ***B. M. SMITH**, A. A. SALZMAN, N. WORBY, P. A. GARRIS;
Sch. of Biol. Sci., Illinois State Univ., Normal, IL

Abstract: Psychostimulants with high abuse potential activate dopamine (DA) transients, sub-second to second extracellular signals that are generated by burst firing of DA neurons and mediate reward learning. Drugs of abuse are hypothesized to elicit a greater activation of DA transients than natural rewards, thereby hijacking reward circuits and causing the pathological overlearning of drug predictive cues. The effects of the psychostimulants modafinil and methylphenidate on DA transients are not established. Modafinil, a wakefulness-promoting psychostimulant used to treat sleep-related disorders such as narcolepsy and shift-work disorder, is thought to exhibit low abuse potential. In contrast, methylphenidate, a psychostimulant used to treat attention deficit hyperactivity disorder, exhibits high abuse potential. Here we investigate the effects of modafinil and methylphenidate on DA transients and the presynaptic mechanisms of DA release and uptake. Previous work has demonstrated that psychostimulants with high abuse potential, such as amphetamine and cocaine, increase DA release and decrease DA uptake in the rat striatum. These presynaptic effects, in turn, increase the amplitude and duration of DA transients. Fast-scan cyclic voltammetry at a carbon-fiber microelectrode was used to record DA transients and electrically evoked DA transient-like signals in the rat striatum, a brain region involved in reward learning. These evoked signals were kinetically analyzed to determine parameters for DA release and uptake. In awake rats, modafinil (150mg/kg) and methylphenidate (5mg/kg) administered intraperitoneally augmented electrically evoked phasic-like DA signals and ambulation, and increased the frequency of DA transients. At the same dose and route of administration, modafinil and methylphenidate augmented electrically evoked transient-like DA signals in anesthetized rats. Kinetic analysis indicated that both modafinil and methylphenidate increase DA release and decrease DA uptake. Taken together, our results demonstrate that both modafinil, a psychostimulant with low abuse potential, and methylphenidate, a psychostimulant with high abuse potential, activate DA transients, and increase and decrease the presynaptic mechanisms of DA release and uptake, respectively. Further study is required to determine the relative potencies of these psychostimulant effects.

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Poster

154. Drugs of Abuse: Learning and Memory I

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Program #/Poster #: 154.15/S16

Topic: G.08. Drugs of Abuse and Addiction

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

Title: The effect of contextual cocaine and nicotine conditioned stimuli on memory consolidation: Role of the noradrenergic system

Authors: M. WOLTER, T. SPEIGEL, B. WINTER, *F. LERI;
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Abstract: Contextual stimuli (CS) paired with the effects of cocaine or nicotine enhance memory consolidation. The neurochemical action of these drugs also enhance monoamine transmission in several memory systems. Our parallel findings with cocaine, nicotine and their CSs suggest that the effects of these drugs and their CSs may facilitate memory storage by activating overlapping neurobiological systems during memory consolidation. One system likely to be involved in the acute effects of such drugs, as well as exposure to their CSs, is noradrenaline (NA) because of its roles in drug reinforcement and memory enhancement produced by emotional stimuli. The current study tested this hypothesis in male Sprague-Dawley rats performing an object recognition memory task. The CSs were generated using a within-subjects conditioning protocol to produce a drug-conditioned context (CS+) and a vehicle-conditioned context (CS-). Post-sample injections of cocaine (20 mg/kg) or nicotine (0.4 mg/kg) enhanced object recognition memory, and these effects were impaired by co-administration of the β-noradrenergic receptor antagonist propranolol (5 and 10 mg/kg). More interestingly, the previously reported memory enhancing effects of post-sample exposure to the cocaine or nicotine CS+ was also blocked by propranolol (10 mg/kg). Overall, these data indicate that cocaine, nicotine and their CSs promote object memory consolidation by enhancing noradrenergic transmission, indicating a neurochemical function of adrenergic tone that may impact the persistence and maintenance of addictive behaviours.

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Poster

154. Drugs of Abuse: Learning and Memory I

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Topic: G.08. Drugs of Abuse and Addiction

Support: Natural Sciences and Engineering Research Council of Canada

Title: Memories that linger: Effects of acute and conditioned opiate withdrawal on memory consolidation

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Abstract: Opiate withdrawal can be associated to a context through classical conditioning to produce conditioned withdrawal. To explore the role of conditioned withdrawal in memory processes, this research investigated whether conditioned withdrawal could impact memory consolidation. Two experiments in males Sprague-Dawley rats compared the effects of naltrexone-precipitated withdrawal and conditioned morphine withdrawal on consolidation of object recognition memory. In Experiment 1, 1 and 3 mg/kg naltrexone was administered immediately, or 6 hours, post-sample to morphine-naïve and morphine-dependent animals (osmotic mini-pumps; 10 mg/kg/day). The post-training effects of naltrexone were re-tested 7 days following removal of the pumps. In Experiment 2, morphine-naïve and morphine dependent rats were confined for 2 hours in a distinctive chamber (CS+) following naltrexone injections (1 or 3 mg/kg) and in another chamber (CS-) following vehicle injections. This was repeated for 10 days: 5 naltrexone/CS+ pairings and 5 vehicle/CS- parings. The effects of immediate or delayed (6 hrs) post-sample exposure to the CS+ and CS- were tested during dependence, and 7 days following removal of pumps.Experiment 1 found that 3 mg/kg naltrexone enhanced object recognition memory when administered immediately, but not 6 hours, post-training in morphine dependent and post-dependent, but not morphine-naïve, rats. During conditioning in the CS+, Experiment 2 found that naltrexone supressed locomotor activity, caused rapid body weight loss, and increased frequency of wet dog shakes in morphine-dependent rats only. When confined in the CS+ without naltrexone injections, rats displayed suppressed locomotion, weight loss and wet-dog shakes. More importantly, exposure to CS+ immediately, but not 6 hours, post-training enhanced object recognition memory during dependence and post-dependence. These experiments indicate that both acute precipitated and conditioned withdrawal have significant and persistent faciliatory effects on memory consolidation. This suggests that conditioned effects on memory processes can play a significant role in addictive behaviours.

Disclosures: **N. Baidoo:** None. **M. Wolter:** None. **B. Winters:** None. **M. Holahan:** None. **F. Leri:** None.

Poster

154. Drugs of Abuse: Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 154.17/S18

Topic: G.08. Drugs of Abuse and Addiction

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Title: VTA-DH projections mediate morphine-induced memory retrieval

Authors: Y. HAN[1,4], Y. ZHANG[2,5], H. KIM[3], M. V. CENTENO[6], A. L. GUEDEA[1], V. S. GRAYSON[1], C. GAO[4], M. MARTINA[3], J. A. ROGERS[2], A. V. APKARIAN[3], J. M. RADULOVIC[1];
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Abstract: The long-term prescription of opioids to patients suffering from chronic pain has proved to be of limited success in alleviating pain, while creating an epidemic of addiction and associated deaths. Memories of contexts associated with use of opioid often retain their rewarding features even after prolonged abstinence, and are thus viewed as important causes of long-term opioid craving. The dorsal hippocampus (DH) plays a central role in the processing of context memory, and may therefore become a region related to addiction-related behaviors. Accumulating evidence about mesolimbic ventral tegmental area (VTA) plays an important role in mediating addiction, which send dopaminergic projections to DH, carrying information about reward. Furthermore, VTA neurons also release other signaling molecules, such as γ-aminobutyric acid (GABA) and glutamate, which may play an important role in addiction. We therefore investigated the role of VTA-DH circuits in morphine-induced conditioned place preference. We demonstrated that chemogenetic inhibition of VTA-DH projections impaired the reinstatement of morphine-induced place preference in a neurotransmitter- and sex-specific manner. These studies begin to elucidate novel circuit mechanisms of opioid addiction.

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Poster

154. Drugs of Abuse: Learning and Memory I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 154.18/DP10/T1

ControlExtraData.DynamicPosterDisplay:
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Topic: G.08. Drugs of Abuse and Addiction

Support: CEBRA-DA042581
T32-DA007261

Title: Hippocampal activity dynamics during contextual reward association in virtual reality place conditioning

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Abstract: Exposure to environmental contexts associated with drug use can induce cravings that promote continued use and/or relapse. Opioid abuse is marked by high relapse rates, suggesting that contextual memories formed during opioid use may be particularly strong. While it is known that reward-seeking behavior is controlled by the mesolimbic reward circuit, little is understood about how contextual memories are altered by drug use. The dorsal hippocampus (dHPC) is necessary for multiple types of contextual learning and the place-specific activity of CA1 place cells map out space in a given environment. Here we developed a virtual reality (VR) morphine CPP (Mor-CPP) paradigm and used *in vivo* two-photon calcium imaging to record the activity of CA1 pyramidal neurons. This novel paradigm allows us to examine the neuronal representation of context as animals develop morphine-paired environmental associations and investigate changes in the hippocampal encoding before, during, and after drug-pairing. We found increased neuronal activity, including more place cells, in water-rewarded contexts following real-time operant conditioning, but not after Mor-CPP training, suggesting different neuronal representation of contexts associated with natural reinforcers or morphine.

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Poster

154. Drugs of Abuse: Learning and Memory I

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Topic: G.08. Drugs of Abuse and Addiction

Support: NSERC Grant
Queen Elizabeth II Graduate Scholarship in Science and Technology

Title: Enhancement of object memory consolidation in rats by exposure to heroin or a heroin-paired context is unaffected by naltrexone

Authors: *A. E. HUFF, M. WOLTER, N. BAIDOO, B. D. WINTERS, F. LERI;
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Abstract: The memory enhancing hypothesis of drugs of abuse proposes that drugs promote addictive behaviours through facilitation of memory consolidation processes. Because addictive behaviours are also profoundly affected by environmental stimuli paired with the effects of drugs, it is possible that drug-paired stimuli can also impact memory consolidation processes. The current study tested this hypothesis by comparing the effects of immediate and delayed post-training exposure to heroin, to immediate and delayed exposure to a heroin-paired context (CS+), on spontaneous object recognition (SOR) memory. Four within-subject experiments were performed in Sprague-Dawley male rats demonstrating that: 1) immediate, but not delayed, post-training administration of heroin (0.3 and 1 mg/kg) enhanced SOR; 2) rats displayed a hyperlocomotion response when tested drug free in a context that was repeatedly paired with the effects of 1 mg/kg heroin (CS+); 3) immediate, but not delayed, post-training confinement to the heroin CS+ enhanced SOR; and 4) the effect of the CS+ on SOR memory was more resistant to extinction than the effect of the CS+ on locomotion. To investigate the role of endogenous opioid receptors in unconditioned and conditioned memory consolidation, four additional experiments were conducted to explore the effect of naltrexone on heroin, and heroin CS+, induced SOR enhancement. To our surprise, co-administration of naltrexone (3 mg/kg) with heroin (1 mg/kg) did not prevent heroin-induced enhancement of SOR and administration of naltrexone (3 mg/kg) prior to immediate post-training confinement in the heroin CS+ did not block the heroin CS+-induced enhancement of SOR. Because naltrexone (3 mg/kg) blocked the analgesic effect of heroin (1 mg/kg) in the hot plate test, these findings in rats indicate that a heroin-paired context has multiple effects on behavior, and that similarly to heroin itself, it can impact memory consolidation processes through neurochemical systems that do not appear to be regulated by endogenous opioid receptors.

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Poster

154. Drugs of Abuse: Learning and Memory I

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Program #/Poster #: 154.20/T3

Topic: G.08. Drugs of Abuse and Addiction

Support: FAPERJ
CNPq
CAPES

Title: Post trail inhibition of dopamine reverses morphine conditioning and prevents sensitization

Authors: *M. P. CARRERA[1], R. J. CAREY[2], J. B. LEITE, Jr.[1], J. M. MELLO BASTOS[1], R. SAMUELS[1];
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Abstract: Morphine (MOR) has substantial pro-dopamine effects and, in rodents, this activation is expressed as hyper-locomotion. With repeated treatments, this effect undergoes conditioning and sensitization. The aim of this study was to assess whether an inhibitory dopaminergic post-trial treatment induced by a dopamine auto-receptor agonist dose of apomorphine (0.05 mg/kg) (APO) given post-trial during re-consolidation/consolidation could reduce MOR conditioning/sensitization. In experiment 1 rats initially received MOR (10 mg/kg) or vehicle (VEH) in a paired/unpaired protocol immediately before 30 min. arena tests on five successive days (induction phase). Subsequently, the groups were tested for conditioning for 5 min. on four successive days. Separate sets of the MOR paired, MOR unpaired and VEH groups received either immediate or delayed APO/VEH post-trial treatments. Selectively, in the immediate APO post-trial treatment MOR paired group, the MOR conditioned response was eliminated after one APO post-trial treatment. In all other MOR paired groups the conditioned response remained robust and unchanged over the four conditioning tests. The immediate post-trial APO treatment had no effect on the baseline response of the MOR unpaired or VEH groups. Experiment 2 was designed to assess the effects of post-trial APO on sensitization induced by 5-10 daily MOR (10 mg/kg) injections. Following each 30 min. arena test rats received post-trial APO or VEH treatments either immediately or 15 min. delayed. With repeated MOR treatments the immediate post-trial VEH and delay post-trial VEH and APO groups developed a progressive hyper-locomotion indicative of potent sensitization effects. The MOR immediate post-trial APO group initially showed an increase in locomotion and then substantially declined indicative a reversal of the sensitization. In a MOR challenge test, sensitization in the immediate post-trial APO group was markedly attenuated. These results showed the importance of dopamine in consolidation/re-

consolidation processes, specifically in the mediation and maintenance of MOR conditioning and sensitization.

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Poster

155. Mechanisms Underlying Alcohol Consumption II

Location: Hall A

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Program #/Poster #: 155.01/T4

Topic: G.08. Drugs of Abuse and Addiction

Support: PAPIIT IN301717 (UNAM, Mexico)

Title: Effects of systemic and intra-accumbal administration of 5-HT_{1B} receptor agonist CP94253 on oral self-administration of ethanol in rats

Authors: ***J. C. JIMÉNEZ**[1], F. CORTÉS-SALAZAR[2], L. N. CEDILLO ZAVALETA[3], R. RUÍZ GARCÍA[4], A. BARRIENTOS-NORIEGA[5], F. MIRANDA-HERRERA[6];
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Abstract: INTRODUCTION. The mesocorticolimbic dopamine (DA) system plays a key role in mediating addictive effects of EtOH. This system is comprised of the DAergic neurons in the ventral tegmental area (VTA) that project their axons to nucleus accumbens (nAcc), prefrontal cortex, amygdala and other limbic structures. Although mesocorticolimbic DA system plays the main neurochemical substrate for regulating the addictive effects of EtOH, other neurotransmitters systems interact with DA such as serotonin (5-HT). A few years ago, it was reported that activity of 5-HT_{1B} receptors may influence DA neurotransmission in VTA and nAcc. We have previously shown that the systemic and intra-VTA administration of 5-HT_{1B} agonist CP94253 reduced oral self-administration of EtOH in rats. The nAcc is innervated by serotonergic fibers and contain several types of serotonin receptors including the 5-HT_{1B} subtype. It has been suggested that the addictive properties of EtOH are modulated by 5-HT_{1B} receptors in the nAcc. The present study was designed to assess the effects of systemic and intra-accumbal administration of the 5-HT_{1B} receptor agonist CP94253 on the oral self-administration of EtOH in rats. METHOD. Male Wistar rats (250-300 g) were used. Rats were water deprived for 24 h, and then trained a lever-press for water reinforcement on a FR1 schedule by 3 days. Then, rats were trained to *lever-press for EtOH (0.01 ml of EtOH in water at 12%)* on a FR1 schedule by 3 days. After this training, the reinforcement contingency was changed to FR3 for

EtOH access until response rate remained stable at 80%. After this training, a group of rats received a systemic injection of 5-HT_{1B} receptor agonist CP94253 (2.0, 4.0 and 8.0 mg/kg, each dose per session test) before rats were under FR3 schedule of reinforcement for EtOH access. Another group of rats received intra-accumbal injection of CP94253 (0.625, 1.25 and 2.5 μg, each dose per session test), cannulae were implanted at nAcc shell (AP +2.0 mm of Bregma, ML ± 0.8 mm, DV -4.5 mm). RESULTS. The data showed that both systemic and intra-accumbal injections reduces oral self-administration of EtOH. These findings suggest that the 5-HT_{1B} receptors activation may modulate the reduction of oral self-administration of EtOH in rats.

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Poster

155. Mechanisms Underlying Alcohol Consumption II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 155.02/T5

Topic: G.08. Drugs of Abuse and Addiction

Title: GABA-B receptor in the central amygdala as a target for treating compulsive-alcohol drinking in rats

Authors: *E. DOMI, L. XU, J. WISKERKE, S. TOVAINEN, E. BARBIER, E. AUGIER, M. HEILIG;
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Abstract: Compulsive alcohol intake, defined as use of alcohol despite harmful social, health and economic consequences, is a major component in the transition to alcohol addiction. The few pharmacological treatments currently in use in alcohol use disorders (AUDs) are not designed for treating the compulsion for alcohol seeking and their efficacy is limited. There is growing evidence that GABA-B receptor agonists may be effective in the treatment of AUDs. The purpose of this study was to investigate the role of GABA-B activation in compulsive alcohol drinking. We first established an operant model of alcohol-compulsivity in rats that mimics compulsive alcohol drinking in humans. Alcohol reward was associated to a footshock punishment and compulsive like behavior was measuerd by quantifying the persistance to lever press for alcohol despite the negative consequence. Overtime, only a subpopulation of rats did not decrease their alcohol drinking despite the footshock aversion (footshock-resistant) and were defined as alcohol-compulsive rats. This sub-population of rats showed a hyper neuronal activity in the central amygdala (CeA), a brain region that represents an integrative hub for alcohol use disorders. Moreover, increased activation of cFos in this region was positively correlated to the number of alcohol responses in alcohol compulsive rats. CeA´s activity is modulated by GABA-transmission, therefore we tested the GABA-B agonist baclofen in compulsive alcohol self-

administration. Systemic baclofen, dose dependently (1, 3 mg/kg) reduced significantly compulsive alcohol intake. Furthermore, we examined the impact of GABA-B receptors in the central amygdala on compulsive drinking by infusing baclofen (70 ng/0.3 ul) in this sub region prior to the alcohol session. Acute Baclofen microinjections reduced aversion resistance of alcohol drinking and prevented c-Fos expression in the CeA. Control experiments showed that baclofen did not affect saccharin intake or locomotor activity confirming its specific role on alcohol compulsive intake. Altogether, our results suggest that the CeA represents an important region driving compulsive drinking and GABA-B receptor represents a promising target for treating one of the most important features of AUDs.

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Poster

155. Mechanisms Underlying Alcohol Consumption II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 155.03/T6

Topic: G.08. Drugs of Abuse and Addiction

Support: RIN 100% region

Title: Risky alcohol consumption and its effects on the brain an *in vivo* longitudinal study

Authors: *A. LANQUETIN[1], A. DRIEU[1], C. FREYSSAINGE[1], D. VIVIEN[1], A.-L. PITEL[2], M. RUBIO[1];
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Abstract: Introduction: Neuroimaging and neuropsychological studies revealed structural and functional brain alterations associated with Alcohol Use Disorder (AUD). In 50 to 80 % of AUD patients, these brain alterations result in cognitive and/or motor impairments. However, the consequences of risky alcohol consumption (not reaching AUD but superior to the recommendations) have been less studied in both humans and rodents. In this longitudinal study, we aimed at studying the effects of risky alcohol consumption in mice at different time points. **Mat & meth**: Mice were divided into 3 groups: control (drinking water), risky alcohol consumption (10% ethanol solution *ad libitum*) and risky alcohol consumption with repeated periods of abstinence (ethanol replaced by water before and during the test week). From 6 weeks to 12 months alcohol exposure, every 3 months, and we conducted i) a battery of behavioral tests to measure motor abilities (balance beam), anxiety (open field) and memory (Y-maze, fear conditioning); ii) MRI examinations to study regional brain volumes. Beverage intake and body weight were measured all along the experiment and did not show any difference between groups (~6 ml/mouse/day).

Results: Behavioral alterations were significant after 6 months of risky alcohol consumption, as revealed by persistent memory impairments. After 9 and 12 months of alcohol exposure, balance abilities were gradually altered in the two groups with risky alcohol consumption. Anxiety levels did not differ between groups at any time. Brain volumes in various regions classically affected by alcohol consumption did not show any between-group difference after 6, 9 or 12 months of alcohol exposure.
Conclusion: Our results show that risky alcohol consumption, even when not reaching AUD, drives a series of behavioral alterations which are less severe but compatible with the deficits described in AUD patients. Interestingly, these deficits do not seem to be related to macrostructural brain alterations. Microscopic analyses to study neuronal density, microgliosis and astrogliosis that could explain the behavioral deficits observed are ongoing.

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Poster

155. Mechanisms Underlying Alcohol Consumption II

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Program #/Poster #: 155.04/T7

Topic: G.08. Drugs of Abuse and Addiction

Support: R01AA024527
R01DA047870

Title: Chronic intermittent voluntary alcohol consumption affects sensitivity to negative feedback but not overall probabilistic discrimination learning during prolonged withdrawal

Authors: *C. G. AGUIRRE[1], K. DAS[1], M. CERVANTES[1], I. SPIGELMAN[2], A. IZQUIERDO[1];
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Abstract: Alcohol Use Disorder (AUD) is a chronic relapsing brain disease characterized by persistent use despite negative consequences. Preclinical models can elucidate some of the underlying mechanisms involved in cognitive deficits seen in individuals with AUD through the use of alcohol (ethanol, EtOH) consumption models with high predictive validity. Previous studies report that alcohol exposure impairs cognitive flexibility, but have not considered probabilistic reversal learning (PRL). Here, male (n=16) and female (n=16) Long-Evans rats underwent either 10 weeks of voluntary intermittent 10% EtOH consumption, with access to both water and 10% EtOH for a 24-hour period (3 days/week) and only water on remaining days, or water-drinking only (H2O) controls using a 2-bottle choice procedure. Rats were then tested for PRL via touchscreen response, where they selected between two visual stimuli, assigned as

the Better (B) or Worse (W) options, rewarded with probability pR(B)=0.70 or pR(W)=0.30, respectively. There was a significant within-subject effect of day on EtOH consumption, indicating escalation. Although the EtOH-exposed cohort required more pretraining sessions to reach criterion than the H20 cohort, there were no group differences on sessions to reach criterion for discrimination learning (reversal learning is still ongoing). Both groups learned, collected more rewards, while decreasing initiation omissions. Trial-by-trial analyses were conducted to assess win-stay/lose-shift strategies during the first 400 trials of the PRL task. Rats can either select the same stimulus after a reward (i.e. Win-Stay) or switch to a different stimulus following a loss (i.e. Lose-shift). We found a significant effect of drinking group on lose-shift strategy, with the EtOH group displaying reduced negative feedback sensitivity relative to the H2O group and reduced ability to follow the rule (i.e. rewarded after choosing Better option). The EtOH group was more likely to exhibit perseverative behavior, selecting the same stimulus regardless of the outcome. Ongoing work is aimed at probing subregional frontocortical plasticity underlying long-term flexible learning, as this is still poorly understood following alcohol exposure.

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Poster

155. Mechanisms Underlying Alcohol Consumption II

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Program #/Poster #: 155.05/T8

Topic: G.08. Drugs of Abuse and Addiction

Title: Behavioral characterization of mice with selective dopamine D3 receptor knockout on striatal D1 projection neurons

Authors: *C. E. TERESI[1,2], M. E. BOCARSLY[1], L. K. DOBBS[1,2], Z. Z. FREYBERG[3], V. A. ALVAREZ[1,2];
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Abstract: Dopamine transmission in the striatum plays an important role in reward learning and reinforcement. Dopamine transmission is mediated by a family of G protein-coupled receptors with five identified genes expressing D1 through D5. The subfamily of D2-like receptors (D2, D3, D4) have been implicated in alcohol and drug addiction. The focus thus far has largely been placed on D2 receptors (Drd2 gene), but increasing evidence points to a contribution of the D3 receptor (Drd3 gene) in alcohol drinking and reward. Consistent with this idea, the distribution of Drd3 expression is enriched in mesolimbic areas known to influence motivation and reward-

motivated learning, such as the nucleus accumbens, amygdala, and olfactory tubercle. The current study aims to examine the selective role of striatal D3 receptors in modulating behavior and alcohol drinking. To this end, genetically engineered mice with a conditional Drd3 gene were crossed with mice expressing Cre recombinase in D1 receptor-expressing neurons in order to target the deletion of Drd3 to direct pathway medium spiny neurons (dMSNs). We first confirmed a reduction of Drd3 expression in this selective knockout of Drd3 gene (D1-Drd3 KO) using qPCR and RNAscope methodologies. Following this confirmation, we behaviorally characterized this mouse line compared to littermate controls using open field, elevated zero maze, and light/dark box tests. Our results suggest that most behavioral responses remain intact, while there is a trend towards increased risk seeking. To assess ethanol consumption, a two-bottle choice and intermittent drinking test was performed. Preliminary results indicate no differences in consumption between the D1-Drd3 KO and littermate control mice, though heterozygotes and homozygotes D1-Drd3 KO mice did consume significantly more on the first day of access to ethanol when compared with controls. We are currently examining the drinking patterns of selective D3R KO mice using a “drinking in the dark” drinking paradigm and combined pharmacology with locomotion testing. Thus far, it appears that D1-Drd3 KO mice show no differences on basal locomotor activity but show reduced locomotor activation in response to a D1-like receptor agonist across different doses. We will continue to explore this difference as well as the locomotor responses to a D2-like receptor agonist and selective D3 receptor agonists.

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Poster

155. Mechanisms Underlying Alcohol Consumption II

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Program #/Poster #: 155.06/T9

Topic: G.08. Drugs of Abuse and Addiction

Support: AA024208

Title: Operant ethanol self-administration paradigm measuring both appetitive and consummatory behavior in rats

Authors: H. YANG, R. PATWELL, ***E. M. STARR**, E. J. GLOVER;
Dept. of Psychiatry, Univ. of Illinois at Chicago, Chicago, IL

Abstract: Traditional operant ethanol self-administration procedures obtain measures of appetitive behavior but lack robust measures of consummatory behavior. Instead these procedures rely on indirect measures of consumption that are frequently inaccurate. Recent work

in mice suggests that operant responding for access to a lickometer-equipped spout containing ethanol produces accurate measures of both appetitive and consummatory behaviors. To expand upon this work, we developed a similar operant self-administration paradigm in rats. Adult male Long-Evans rats were first trained to operantly self-administer 10% sucrose on a fixed ratio (FR) 1 schedule of reinforcement where one press on the active lever resulted in 30 seconds (30S) of access to the spout. After responding was maintained, rats were transitioned to a FR1 15S schedule and then to a FR3 15S schedule. Once FR3 responding was maintained, sucrose was slowly faded out of the reinforcer and replaced with ethanol until a final concentration of 10% ethanol was reached. Robust lever pressing and licking was observed in response to 10% sucrose reinforcer. Responding decreased but was maintained for 10% ethanol with an average of 25 ± 4 active lever presses resulting in the delivery of the ethanol spout 8 ± 1 times and an average of 213 ± 31 licks per 30 min session. In contrast, inactive lever presses occurred on average 1 ± 0.3 times per session. Rats that underwent the same training paradigm but for 20% ethanol with no sucrose fade successfully learned the operant procedure. Interestingly, while the number of reinforcer deliveries was only slightly reduced to an average of 6 ± 1, licks were substantially lower at 44 ± 6 per session. Together these data demonstrate the utility of an operant ethanol self-administration procedure that measures both appetitive and consummatory behavior to uncover individual and group differences in the motivation to drink ethanol that cannot be adequately revealed by lever pressing and reinforcer delivery alone.

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Poster

155. Mechanisms Underlying Alcohol Consumption II

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Program #/Poster #: 155.07/T10

Topic: G.08. Drugs of Abuse and Addiction

Support: SIP: 20180296
CONACYT 267454
BEIFI-IPN 20171334

Title: Salvia divinorum extract increase alcohol consumption and tonic immobility and decrease food intake in Wistar rat

Authors: *A. MIRANDA-PÁEZ[1], P. VÁZQUEZ-LEÓN[2], U. ARENAS-MARTÍNEZ[1]; [1]Fisiología (ENCB), Inst. Politécnico Nacional, Mexico City, Mexico; [2]Fisiología y Farmacología, Ctr. de Ciencias Basicas, Univ. Autónoma de Aguascalientes, Aguascalientes, Mexico

Abstract: Kappa-opioid (KOP) system has been found key as inhibitor to seek and consume certain drugs of abuse like cocaine, alcohol and opioids. Unfortunately, the semi-synthetic kappa-opioids have adverse effects as sedation, aversion and depression when used for the treatment of addiction. Salvinorin A (Sal-A) is the first exogenous found in nature kappa-opioid receptor (KOPr) agonist. Noteworthy, even being a non-nitrogenous molecule is the main active principle of the sage *Salvia divinorum* (*S. divinorum*, Lamiaceae; formerly Labiatae). Based on studies which state a key role of the KOP system on consume of drugs of abuse, we hypothesized that the *Salvia divinorum* extract (SDE) containing mainly Sal-A modify the alcohol consumption pattern in adult male Wistar rats. Additionally, we assessed food intake as rewarded naturally behavior and tonic immobility (TI) as indicative of anxiety-like behavior.
Materials and Methods. 32 adult male Wistar rats divided in four groups (8 subjects each): Control = alcohol naïve + vehicle (CONT), alcohol naïve + *Salvia divinorum* extract (ANSDE), alcohol consumption + vehicle (ACVEH) and alcohol consumption + *Salvia divinorum* extract (ACSDE) were evaluated for alcohol and food consumption and on tonic immobility test after vehicle or *Salvia divinorum* extract injected (1 mg/kg, intraperitoneally).
Results. *Salvia divinorum* extract produced a significant increase on voluntary alcohol intake mainly in rats with a history of ethanol consumption. Additionally, prolongs the tonic immobility duration and decrease food intake.
Conclusion. *Salvia divinorum* extract produced increase on alcohol consumption especially in rats with ethanol consumption history, increased tonic immobility as indicative of anxious-like behavior, as well as elicited a transient anorexigenic effect in male Wistar rats.
Keywords. *Salvia divinorum*; alcohol; food; tonic immobility.

Disclosures: A. Miranda-páez: None. **P. Vázquez-León:** None. **U. Arenas-Martínez:** None.

Poster

155. Mechanisms Underlying Alcohol Consumption II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 155.08/T11

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH/NIAAA grant AA07611.

Title: Systemic and intra-dorsal striatal administration of the AMPA receptor antagonist NBQX disrupts binge-like alcohol consumption in C57BL/6J mice

Authors: *M. R. WATSON, D. P. GARCY, S. L. BOEHM, II;
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Abstract: In the United States alone, over 15 million people suffer from alcohol use disorder (AUD; National Survey on Drug Use and Health, 2015). AUD is a chronic disease that is

clinically characterized by continued alcohol seeking despite negative consequences, or compulsive alcohol seeking (American Psychiatric Association, 2013). Synaptic restructuring occurs following long-term use of alcohol leading to hyperactivation of ionotropic glutamate receptors. Glutamatergic afferents bind post-synaptic AMPA-gated GABAergic medium spiny neurons in the dorsolateral striatum (DLS), a brain region involved in the development of behavioral compulsivity. The AMPA receptor (AMPAR) antagonist, NBQX, has been shown to reduce alcohol consumption when administered systemically or into the DLS of rats (Corbit, Nie, & Janak, 2014; Ruda-Kucerova et al., 2018). However, much remains unknown about the role of AMPARs in the control of binge-like alcohol drinking or compulsive alcohol drinking in mice. Here we investigated the effects of systemic and intra-DLS administration of NBQX on binge-like alcohol drinking and/or compulsive-like quinine adulterated drinking using a mouse model of binge drinking, Drinking-in-the-Dark (DID). For the systemic NBQX study, mice (N=40) were allowed free access to 20% alcohol for two hours each day for four days. On day five mice were injected (I.P.) with saline or NBQX (3, 10, or 30 mg/kg; n=10) before DID. At 30 mg/kg mice drank significantly less alcohol compared to controls, (one-way ANOVA, $p < 0.01$). Animals in the 30 mg/kg condition also drank significantly less alcohol in the first two hours than in the second two hours (paired t-test, $p < 0.01$). To test the hypothesis that AMPARs in the DLS are involved in compulsive binge-drinking, adult male C57BL/6J mice (N=20) received bilateral intra-DLS infusion of NBQX (2.0 µg/µl) on days 7 and 9 of a 9-day DID procedure in which the alcohol was adulterated with quinine (0.5 mM) on one of the days (7 or 9) in counterbalanced fashion. Intra-DLS NBQX significantly reduced alcohol consumption, quinine-adulterated alcohol consumption, and overall locomotor activity (two-way RM ANOVA, $p < 0.05$). These results suggest that DLS AMPARs are important in the control of both binge-like alcohol consumption, and compulsive-like aversion resistant alcohol consumption, although the exact mechanism requires further investigation.

Disclosures: **M.R. Watson:** None. **D.P. Garcy:** None. **S.L. Boehm:** None.

Poster

155. Mechanisms Underlying Alcohol Consumption II

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Program #/Poster #: 155.09/T12

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant AA021445

Title: Evidence for separable anterior insula pathways that control compulsion-like and regular, alcohol-only drinking

Authors: *T. DE OLIVEIRA SERGIO, K. LEI, C. KWOK, L. NAKAYAMA, L. LI, S. GHOTRA, J. YU, S. A. WEGNER, D. DAREVSKY, F. W. HOPF;
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Abstract: Compulsion-like alcohol drinking (CLAD), where intake persists despite bad consequences, is a major contributor to the enormous harm and costs of alcohol use order. We have shown that insula-related cortical inputs to NAc, a conflict processing circuit, is critical for the expression of CLAD but not RAD, and we hypothesized that the Locus Coeruleus related adaptive stress response circuit would also be critical, since CLAD could be considered reward-directed responding in the presence of stressors. Indeed, we find that insula-to-LCA inputs are critical for CLAD but not regular, alcohol-only drinking (RAD) or saccharin-quinine intake. Aversion-resistant intake was also significantly reduced by inhibiting insula-to-brainstem inputs or alpha1 receptors (alpha1Rs) systemically or within the mPFC with prazosin, manipulations that also did not impact RAD. However, while insula-to-brainstem and systemic or mPFC alpha1R manipulation reduced CLAD but not RAD, we unexpectedly found that inhibition of alpha1Rs in insula decreased both CLAD and RAD, which was also observed when insula was inhibited more globally with muscimol/baclofen. Similarly, unilateral ablation of LC cells also decreased both CLAD and RAD. Our findings together suggest that the insula has two throughputs that are perhaps mediated by somewhat dissociated sets of insula throughputs, one dedicated primarily to CLAD, the other mediating RAD and whose specific contribution to CLAD is at present undefined. Ongoing studies are working to better delineate the identity and other functional consequences of these pathways for different forms of drinking Supported by AA021445 (FWH).

Disclosures: T. De Oliveira Sergio: None. **K. Lei:** None. **C. Kwok:** None. **L. Nakayama:** None. **L. Li:** None. **S. Ghotra:** None. **J. Yu:** None. **S.A. Wegner:** None. **D. Darevsky:** None. **F.W. Hopf:** None.

Poster

155. Mechanisms Underlying Alcohol Consumption II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 155.10/T13

Topic: G.08. Drugs of Abuse and Addiction

Support: P20 GM113109-01A1

Title: The role of mu-opioid receptors in individual differences in voluntary alcohol consumption and conditioned fear learning

Authors: *A. PAJSER, H. FISHER, C. L. PICKENS;
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Abstract: PTSD and alcohol abuse frequently co-occur, but the cause of this co-morbidity is unclear. Alcohol exposure could alter problem drinkers' brains to promote the development of anxiety disorders, problem drinking could occur because of pre-existing anxiety (e.g.: self-medication), or a pre-existing factor could affect both anxiety and alcohol consumption. Previous research in our lab has found an association between the amount of alcohol a rat will voluntarily consume and their fear expression when tested in extinction, such that rats that consume higher levels of alcohol show lower levels of fear (both in limited and extended training fear conditioning). Because the opioid system can affect both alcohol consumption and fear learning, we chose to examine whether the individual differences in voluntary alcohol consumption and fear responding we have previously observed are due to individual differences in the opioid system. As an initial study, we investigated the role of the mu-opioid receptor system by administering naltrexone before fear conditioning sessions in high and low drinkers. Male Long-Evans rats received chronic intermittent alcohol access (CIA) or water-only access for 6 weeks (PND 26-66). We divided the rats (based on their drinking in early adulthood- the last 2 weeks of access) into high alcohol drinkers (consumed >2 g/kg/24-h alcohol), low alcohol drinkers (consumed<1.5 g/kg/24-h alcohol), or water drinkers (no alcohol access) Rats were then food-restricted and began behavioral training 9 days after the final alcohol access period, with fear conditioning taking place 15 days after the final alcohol access period. The rats were trained to lever-press and then received single day of fear conditioning. Half of the rats in each of the 3 drinking groups received a 1 mg/kg subcutaneous injection of naltrexone 10 minutes prior to a fear conditioning and the remaining rats received a saline injection at the same time. The rats were tested for conditioned fear (measured with conditioned suppression of lever-pressing) two days after the end of fear conditioning, with no pre-test injections. Generally, naltrexone administration prior to fear conditioning increased fear expression in the extinction test. Individual differences data will be discussed. Future studies will investigate the role of other components of the opioid system, such as the kappa- and delta- opioid receptors, using similar methods.

Disclosures: **A. Pajser:** None. **H. Fisher:** None. **C.L. Pickens:** None.

Poster

155. Mechanisms Underlying Alcohol Consumption II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 155.11/T14

Topic: G.08. Drugs of Abuse and Addiction

Support: NIMH Grant MN099085
NIDA Grant DA043461

Title: Nucleus accumbens D1- vs D2 receptor-containing medium spiny neurons differentially control binge-like alcohol drinking in male and female rats

Authors: *C. E. STRONG, D. P. HAGARTY, K. J. SCHOEPFER, A. BREA GUERRERO, S. M. CAJUSTE, K. L. MANN, J. L. BREEDLOVE, M. KABBAJ;
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Abstract: Alcohol is one of the most commonly abused drugs worldwide, yet effective treatment options are lacking. Furthermore, gender differences exist in alcohol consumption, withdrawal, and relapse. In order to improve treatment options for people with alcohol use disorder (AUD), the neuroadaptations that mediate alcohol's addictive properties need to be fully understood in both sexes. The nucleus accumbens (NAc), considered the hub of reward circuitry, is comprised of two main subsets of medium spiny neurons (MSNs) based on receptor subtype; dopamine 1 receptors (D1Rs) are stimulatory G protein-coupled receptors (GSα) while dopamine 2 receptors (D2Rs) are inhibitory G protein-coupled receptors (Giα). Previous reports indicate that in male rats, alcohol intake leads to enhanced excitatory transmission of GSα D1R-containing MSNs and enhanced inhibitory transmission on Giα D2R-containing MSNs, in part, through activation of mTOR signaling on D1 but not D2 MSNs. While it remains unclear how sex influences alcohol's actions on D1 vs D2 NAc MSNs, recent reports suggest that mTOR activation in the NAc occurs in male but not female rats. As such, the aim of this study is to determine the mechanism mediating sex differences in alcohol consumption by examining D1 vs D2 NAc MSNs. Male and female rats intermittently drank alcohol under a 2-bottle choice paradigm with 20% alcohol and water for 7-weeks. After 4-weeks, cre-dependent DREADDs viruses were infused into the NAc of transgenic male and female rats expressing cre-recombinase on the promoter regions of either the drd1a (D1R) or drd2 (D2R) genes. On the last session, we used clozapine-N-oxide (CNO) to selectively activate or inhibit D1 or D2 NAc MSNs and examine the effect on alcohol intake. Preliminary data suggests that binge-like alcohol drinking is controlled in a cell-type specific manner as changes in alcohol consumption were observed after DREADDs activation in both sexes. Future directions include examining mTOR pathway activation within D1 vs D2 MSNs in the NAc to better understand neural mechanisms mediating alcohol addiction in both sexes.

Disclosures: C.E. Strong: None. **D.P. Hagarty:** None. **K.J. Schoepfer:** None. **A. Brea Guerrero:** None. **S.M. Cajuste:** None. **K.L. Mann:** None. **J.L. Breedlove:** None. **M. Kabbaj:** None.

Poster

155. Mechanisms Underlying Alcohol Consumption II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 155.12/T15

Topic: G.08. Drugs of Abuse and Addiction

Support: U01 AA020912
P50 AA022538

Title: Regulation of binge alcohol drinking by anaplastic lymphoma kinase and signal transducer and activator of transcription 3

Authors: *K. HAMADA[1], A. W. LASEK[2];
[2]Psychiatry, [1]Univ. of Illinois At Chicago, Chicago, IL

Abstract: Of the estimated $249 billion financial burden caused by alcohol use disorder (AUD), over $190 billion are related to binge drinking, highlighting the importance of research aimed at understanding and mitigating binge drinking. The contribution of the neuroimmune system to AUD has become a major focus of interest in recent years and robust immune activation has been found in individuals with AUD. Signal transducer and activator of transcription 3 (STAT3) plays a critical role in the regulation of the immune response and is activated by anaplastic lymphoma kinase (ALK) in response to alcohol exposure in vitro. The goals of this study were to determine if: 1) binge ethanol drinking activated ALK and STAT3 signaling in the brain, and 2) to determine if inhibition of their signaling could reduce binge drinking. The drinking in the dark test was used as a mouse model of binge ethanol consumption. Male and female C57BL/6J mice were given access to 20% ethanol in a single bottle on their home cages for 2 hours on days 1-3 and 4 hours on day 4. Brains were collected at 0 and 24 hours after the final drinking session on day 4 and specific brain regions, including the hippocampus, were dissected for analysis of gene expression by qPCR and protein levels and phosphorylation by western blotting. ALK and STAT3 activation, as measured by tyrosine phosphorylation, were dynamically altered in the hippocampus after binge alcohol drinking. In addition, the expression of STAT3 target genes, such as Gfap and Socs3, were also increased following binge drinking. To determine if inhibition of ALK and STAT3 can alter binge ethanol drinking, mice were treated with the ALK inhibitor, alectinib, or the STAT3 inhibitors, stattic or niclosamide, prior to drinking sessions in the drinking in the dark test. Systemic treatment with either ALK or STAT3 inhibitors decreased binge-like drinking. These results indicate that ethanol exposure activates ALK and STAT3 signaling and that these pathways play an important role in binge drinking. Pharmacological inhibitors targeting ALK and STAT3 may represent viable new therapeutic approaches to reducing excessive alcohol consumption.

Disclosures: K. Hamada: None. **A.W. Lasek:** None.

Poster

155. Mechanisms Underlying Alcohol Consumption II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 155.13/T16

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH R01AA026306
NIH R01AA027213

Title: Encoding and enhancement of the motivation to consume alcohol by the central nucleus of the amygdala

Authors: *P. H. JANAK[1], T. KIM[2], D. J. OTTENHEIMER[1], **K. M. FRASER**[2];
[1]Solomon H. Snyder Dept. of Neurosci., [2]Psychological & Brain Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: Alcohol use disorder is a chronically relapsing disorder that poses significant threats to personal and societal well-being. The central nucleus of the amygdala (CeA) is a key site of neural plasticity that has been implicated in promoting the transition from recreational alcohol intake to compulsive drinking. However, the ways by which neurons in this region contribute to alcohol intake *in vivo* are still unclear. Here we used *in vivo* electrophysiology and optogenetics in a rat model of voluntary alcohol consumption to investigate CeA neural responses to alcohol intake.
To acclimate rats to the taste and pharmacological properties of alcohol, rats were allowed to drink unsweetened alcohol (15% v/v) freely in their homecage for 4-6 weeks on an intermittent access schedule (final average 24 hour intake 6.5 g/kg alcohol consumed). For the *in vivo* electrophysiology experiments, male Long-Evans rats were then implanted with drivable microelectrode arrays targeting the CeA and allowed to freely enter a port for delivery of 15% ethanol. Of 201 neurons in the CeA that we recorded, 17% of the neurons were inhibited upon port entries while 7% of the neurons were excited. In addition, 23% of the neurons were inhibited upon the first lick after the port entry response while 6.5% of the neurons were excited. We also found neurons with activity tightly correlated with the licking cycle, suggesting that CeA neurons track alcohol drinking. To delineate contributions of CeA to motivation to consume alcohol, we infused AAV5-hsyn-ChR2 into the CeA and stimulated the CeA (1s, 20 Hz, 8-12 mW) during consumption of alcohol using a closed loop design. We found that CeA stimulation time-locked to consumption, but not in an unpaired manner, increased alcohol consumption. Activation of the CeA did not increase motivation to consume water, suggesting stimulation of the CeA may be specific to motivationally salient rewards. Together, these data suggest the CeA is engaged to promote consumption of alcohol prior to the development of physical dependence.

Disclosures: P.H. Janak: None. **T. Kim:** None. **K.M. Fraser:** None.

Poster

155. Mechanisms Underlying Alcohol Consumption II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 155.14/T17

Topic: G.08. Drugs of Abuse and Addiction

Support: R01-DA009411-17
2T32MH014654-40

Title: Adolescent stress increases adult ethanol self-administration and alters ventral tegmental area GABA signaling

Authors: *D. A. CONNOR[1], R. WITTENBERG[1], J. DROGEN[2], A. MAK[2], C. M. GOTZ[2], M. M. THOMPSON[2], J. A. DANI[1];
[1]Dept. of Neurosci., [2]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Stress is a major risk factor for ethanol use disorders, and early-life stress positively predicts ethanol alcohol use disorders in adulthood. Several pieces of evidence indicate that stress exposure during adolescence can lead to long-term neuronal adaptations: (1) the mesolimbic system undergoes maturation during adolescence, (2) adolescent individuals show greater physiological response to stressors compared with adults, and (3) stress is known to act directly on mesolimbic substrates of motivation and reward. However, the cellular mechanisms linking early-life stress to long-term changes in addiction-related behavior remains unclear. Herein, we investigated if adolescent stress results in long-term changes in adult ethanol self-administration and changes VTA GABA signaling. Using Long-Evans rats we modeled exposure to stress using the chronic variable stress (CVS) paradigm, a non-habituating stress protocol consisting of pseudorandom daily exposure to alternating stressors: restraint, elevated platform, oscillating rocker platform, predator odor (fox urine), and overnight wet bedding. Rats were exposed to CVS for 14 days— adolescent: post-natal day (PND) 28 until PND 42, adult: PND 83 until 96. We found that rats exposed to CVS during adolescence showed increased self-administration of sweetened 4% and unsweetened 10% ethanol in adulthood. In contrast, adult rats exposed to CVS showed no long-term changes in ethanol self-administration. Furthermore, we found that adolescent CVS exposure resulted in a long-term increase in ethanol-induced inhibition of VTA DA neurons. Adolescent exposure was associated with long-term deficits in chloride homeostasis in VTA GABA neurons, as measured by decreased chloride extrusion. In sum, we show that adolescents are vulnerable to long-term effects of stress on ethanol self-administration concomitant with changes in VTA GABA signaling within midbrain reward-related circuitry.

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Poster

155. Mechanisms Underlying Alcohol Consumption II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 155.15/T18

Topic: G.08. Drugs of Abuse and Addiction

Support: CIHR

Title: The 5-HT_{2C} receptor agonist lorcaserin, alone and in combination with naltrexone, reduces binge-like alcohol drinking in C57BL/6J mice

Authors: *R. I. TABBARA[1,4], P. J. FLETCHER[4,1,2], A. D. LÊ[5,3,2];
[1]Psychology, [2]Psychiatry, [3]Pharmacol. and Toxicology, Univ. of Toronto, Toronto, ON, Canada; [4]Biopsychology, [5]Neurobio. of Alcohol, Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada

Abstract: Alcohol (ethanol; EtOH) use disorder is one of the top preventable causes of death worldwide. Studies in humans and animal models have implicated the serotonin (5-HT) system in the pathophysiology of this disorder. The success of lorcaserin (Lorqess, Belviq), a 5-HT_{2C} receptor agonist, in the treatment of obesity has sparked interest in its ability to attenuate the reinforcing properties of drugs-of-abuse, including those of alcohol. We investigated the effects of lorcaserin on EtOH intake using the Drinking-in-the-Dark (DID) procedure, an animal model of binge-like drinking that leads to pharmacologically relevant blood EtOH concentrations (> 1.0 mg/ml) in a short period of time. We compared the effects of lorcaserin to those of the FDA-approved drug naltrexone, and examined the effects of combining lorcaserin and naltrexone on EtOH drinking. Adult male C57BL/6J mice received EtOH access (20% v/v) for 2 hrs in the home-cage during the first 3 days of the DID procedure, beginning 3 hrs into the dark cycle. On day 4, mice were either injected with lorcaserin (vehicle, 0.375, 0.75, or 1.5 mg/kg; SC), naltrexone (vehicle, 1, 3, or 10 mg/kg; IP), or a combination of lorcaserin (vehicle, 0.375, or 0.75 mg/kg; SC) and naltrexone (vehicle, 0.33, or 1 mg/kg; IP), prior to a 4-hr EtOH access. Intake was measured at 1, 2, and 4 hr to monitor temporal changes in consumption. Looking at the 4 hr interval, lorcaserin dose-dependently reduced EtOH drinking, whereas naltrexone reduced drinking at the highest dose (10 mg/kg). Combining lorcaserin (0.375 mg/kg) and naltrexone (1 mg/kg) reduced drinking to a greater extent than either drug alone. These results suggest that lorcaserin and naltrexone can have additive effects on binge-like EtOH drinking, and support continued research into the potential of lorcaserin as a pharmacological treatment for alcohol use disorder.

Disclosures: R.I. Tabbara: None. **P.J. Fletcher:** None. **A.D. Lê:** None.

Poster

155. Mechanisms Underlying Alcohol Consumption II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 155.16/T19

Topic: G.08. Drugs of Abuse and Addiction

Title: Escalation of ethanol consumption: Effects on the expression of DAT (SLC6A3) and ABCG2

Authors: *D. MUSKIEWICZ[1], N. FROMMANN[1], B. PATEL[1], A. SIMON[1], H. AMAWI[1], A. TIWARI[1], F. S. HALL[2];
[1]The Univ. of Toledo, Toledo, OH; [2]Pharmacol., Univ. of Toledo Col. of Pharm. and Pharmaceut. Sci., Toledo, OH

Abstract: Background: Escalation of ethanol (EtOH) intake is a key criterion for diagnosis of alcohol dependence (AD). To model alcohol escalation associated with AD, we established conditions producing maximum EtOH absolute escalation (AE) in C57BL/6J mice (Expts. 1-3). Additionally, since AUD is highly heritable, Experiment 4 examined escalation in three strains of mice that differ in ethanol consumption: a low preferring strain (DBA/2), a moderately preferring strain (FVB/NJ), and a high-preferring strain (C57BL/6J). Escalation conditions for the final experiment were chosen based on the results of Experiments 1-3. Additionally, dorsal and ventral striatal samples were taken from these mice for Western analysis of protein expression, which examined the dopamine transporter (DAT; SLC6A3) and the ATP-binding cassette transporter G2 (ABCG2).
Methods: Male and female experimentally naïve C57BL/6J mice were used in Experiments 1-3 (N=10 per strain/sex group). Experiment 4 compared male and female C57BL/6J, DBA/2 and FVB/NJ mice (N=5 per strain/sex group). Exp. 1 examined the effect of four distinct EtOH concentrations (4%-32% v/v) using two-bottle, 24-hr access, 2 d/wk. Exp. 2 examined different intervals of availability of 16% EtOH (1, 2, or 3 days of 24-hr access, or continuous access). Exp. 3 examined the effects of a 4-bottle preference (4%, 8% and 32% EtOH v/v; and water) with 3 d of 24-hr access/wk. Exp. 4 examined AE in the three strains using ideal conditions determined by experiments 1-3. Western analysis was performed under standard conditions for DAT and ABCG2 and quantification analysis performed using Image J Software.
Results: Exp. 1-3 revealed optimal conditions to be 16% EtOH, three days per week for six weeks. In Experiment 3, the availability of more concentrations greatly increased absolute consumption, but there was no escalation (AC). In Experiment 4, FVB/NJ strain mice showed greater AE compared to C57BL/6J mice. Escalation was associated with increased ABCG2 expression in dorsal and ventral striatum while DAT was downregulated.
Discussion: Although C57BL/6J mice show high levels of consumption, these findings suggest that other strains, such as FVB/NJ, may better demonstrate escalation of EtOH intake because they begin at lower levels of ethanol consumption, but reach similar levels to C57BL/6J mice. Critical factors involved in escalation may include DAT and ABCG2, but further experiments are needed to determine whether these changes are determinants of escalation or compensatory mechanisms. Moreover, changes in ABCG2 indicate that escalation of ethanol consumption may affect responses to other drugs through regulation of ABCG2.

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Poster

155. Mechanisms Underlying Alcohol Consumption II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 155.17/T20

Topic: G.08. Drugs of Abuse and Addiction

Support: R01AA024112
P50DA037844

Title: Binge-like alcohol consumption during a within-session intermittent access paradigm

Authors: *C. P. KING, P. J. MEYER;
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Abstract: Patterns of drug-taking behavior are important in determining drug-directed motivation. Previously, intermittent-access schedules of cocaine reinforcement result in a pattern of spiking brain drug concentrations, and lead to enhanced motivation for the drug, compared to continuous access schedules in which drug is freely available (Zimmer et al. 2012). However, it is not known whether intermittent access to alcohol similarly increases its motivational effects. Here, we tested within-session intermittent access to alcohol altered patterns of drug taking, and whether it subsequently altered the motivation to work for alcohol using a series of progressive ratio procedures. First, we allowed male and female Long-Evans rats (n=32) to drink alcohol in their home cages on alternating days (adapted from Simms et al. 2010) to establish consumption. We then trained rats to lever press for 30-second presentations of retractable bottle containing alcohol (20% v/v) over 14 sessions for either 90 minutes (short-access), 315 minutes (long-access), intermittently in six 15-minute bins over 315 minutes (intermittent access), or water for 90 minutes. During training, we found that intermittent access led to fewer responses but more alcohol consumption per reinforcer than the other groups. However, during subsequent progressive-ratio tests, in which responses for each subsequent bottle presentation were slowly increased, we found no changes in breakpoint or responding. These results indicate that intermittent access schedules can potentiate “binge-like” behavior when drug is available during self-administration, thus enhancing the level of intoxication in a given 15-minutes period. Surprisingly, these behavioral effects did not result in increased responding during progressive-ratio. These findings support the notion that intermittent alcohol both within-session and between-session (Carnicella et al. 2014; Simms et al. 2010; Wise 1973) are important factors for regulating intake as a critical component of alcohol use.

Disclosures: C.P. King: None. **P.J. Meyer:** None.

Poster

155. Mechanisms Underlying Alcohol Consumption II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 155.18/U1

Topic: G.02. Motivation

Support: AA021445

Title: A cortical hypothesis for succeeding and failing at compulsion-like alcohol drinking

Authors: D. DAREVSKY, ***F. W. HOPF**;
UCSF, San Francisco, CA

Abstract: Intake despite negative consequences (compulsivity) is central to the great harm of alcohol use disorder and other addictions. Previous work converges on the central role of the anterior insula (AI), which encodes emotions and importance, and the medial prefrontal cortex (MPF), which organizes efficient action, for driving compulsive alcohol responding across rodent and human. We have shown that rat alcohol drinking under moderate but palpable challenge shows less variable, more stereotyped responding in nearly every drinking measure, suggesting adoption of a more automatic, session-long response strategy to overcome challenge. Interestingly, higher challenge dropped alcohol intake more than 50%, yet retained aspects of lower variability and greater drive seen with moderate challenge, including more consistent lick-intake relation, tongue control, and earlier bout initiation. These session-level measures suggest a greater overall commitment to drinking that was similar under moderate and higher challenge. In contrast, higher-challenge consumption had disrupted bout generation and greater lick-time variability, akin to studies showing timing disruption by MPF inhibition. Nonetheless, higher-challenge bouts that started faster persisted longer, despite a “danger zone” across the first seconds of licking with slowed licking and higher probability of stopping. Indeed, persistent higher-challenge bouts continued with similar duration and timing as longer moderate-challenge and alcohol-only bouts, and contributed a majority of intake. Together, these and complementary findings support a novel model where AI and MPF are both critical for compulsion-like intake, with AI providing session-long commitment regardless of challenge level, and MPF contributing more to intra-bout measures, especially increased drive at bout initiation, which can fail under higher challenge.

Disclosures: D. Darevsky: None. **F.W. Hopf:** None.

Poster

156. Neural and Behavioral Mechanisms of Addiction: Amphetamine

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 156.01/U2

Topic: G.08. Drugs of Abuse and Addiction

Title: Sex differences in methamphetamine intake and changes in molecular neuroadaptations associated with relapse to drug seeking behaviors

Authors: *A. DAIWILE, S. JAYANTHI, B. LADENHEIM, M. MCCOY, J. CADET;
Mol. Neuropsychiatry Res. Br., Natl. Inst. On Drug Abuse, Baltimore, MD

Abstract: Methamphetamine (METH) is an extremely addictive drug that continues to wreak havoc in the lives of addicted individuals. There is evidence of sex differences in patterns of abuse, amount of METH taken, as well as in relapse rates among METH users who meet DSM criteria for addiction. The molecular bases of these sex differences have remained elusive. In the present study, we have used male and female Long Evans rats that were trained to self-administer METH (0.1 mg/kg/infusion, IV) on an FR-1 schedule for 20 days using a pattern of two 3-h sessions/day. After completion of SA training, we measured drug seeking behaviors assessed for 3h on withdrawal day 3 (WD3) and 30 (WD30). Both female and male rats escalated the number of METH injections over the time of training. There were significant effects of sex, training day, and their interaction for METH intake, indicating that male rats took more METH than female rats. We found that male rats also showed steeper escalation of METH intake during the first nine days of training than females. Inspection of the intake of individual animals revealed two METH SA phenotypes consisting of *low* and *high* METH takers. Interestingly, 47% (8/17) of females while 73% (11/15) of males were *high* METH takers. We did not find any sex differences in cue-induced METH seeking after extended abstinence. Nucleus accumbens (NAc) tissues were dissected 24 hrs after the second METH seeking tests at WD30 to measure mRNA expression by quantitative PCR. There were higher mRNA levels of dynorphin and hypocretin/orexin receptors (*Hcrtr1/2*) in females than males whereas there was higher basal expression of vasopressin mRNA in males. In addition, there were significant correlations between *Hcrtr1, Hcrtr2, Crhr2, and Avpr1b* mRNA levels and cue-induced METH seeking behaviors of females but not of males. The study thus provides partial documentation of the complex nature of sex differences in molecular responses to METH self-administration.
Acknowledgement: This work is supported Department of Health and Human Services/ National Institutes of Health/ National Institute on Drug Abuse/ Intramural Research Program.

Disclosures: A. Daiwile: None. **S. Jayanthi:** None. **B. Ladenheim:** None. **M. McCoy:** None. **J. Cadet:** None.

Poster

156. Neural and Behavioral Mechanisms of Addiction: Amphetamine

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 156.02/U3

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH R15 DA 038295

Title: Dopamine D_1 and D_2 receptors mediate the discriminative stimulus effects of 4-methylmethcathinone (4-MMC) in male and female Sprague-Dawley rats

Authors: R. L. BURROUGHS, J. D. ZUARTH GONZALEZ, K. J. CARGILE, *L. E. BAKER; Dept. of Psychology, Western Michigan Univ., Kalamazoo, MI

Abstract: Recreational use of illicit synthetic cathinones ("bath salts") poses a significant public health risk. Considering the likelihood of concomitant use of these substances with other drugs, the abuse related health consequences are especially concerning. Drug discrimination is a well-established preclinical behavioral method predictive of interoceptive stimulus (subjective) drug effects. Preclinical drug discrimination studies of the synthetic cathinone, 4-methylmethcathinone (4-MMC) have demonstrated its effects are comparable to those of cocaine, methamphetamine, and MDMA. However, few studies have directly examined the specific neurotransmitter receptor actions underlying the discriminative stimulus effects of 4-MMC, and none of these studies have utilized female rodents. The present study investigated the contribution of D_1 and D_2 dopamine receptors to the discriminative stimulus effects of 4-MMC in both male and female rats. Twelve female and eight male adult Sprague-Dawley rats were trained to discriminate 3 mg/kg 4-MMC from saline using a fixed ratio 20 schedule of food reinforcement. After dose-response curves were determined with 4-MMC (0.375-3 mg/kg, 15 min I.P.) in each group, a series of stimulus antagonism tests were conducted with the D_1 dopamine antagonist, Sch 23390 (0.1 mg/kg, 30 min I.P.) or the D_2 DA antagonist, haloperidol (0.5 mg/kg, 60 min I.P.) administered as a pretreatment with each 4-MMC dose. Both Sch 23390 and haloperidol produced a downward shift in the 4-MMC dose response curve, but did not attenuate discrimination of the training dose. These results indicate both D_1 and D_2 dopamine receptors contribute to the discriminative stimulus effects of 4-MMC, but antagonism is surmountable. Additional tests with serotonin receptor antagonists (MDL 100, 907, WAY 100,635, pirenperone) with varying receptor selectivity are in progress to determine the contribution of serotonergic receptors to the interoceptive stimulus effects of 4-MMC. These preclinical findings regarding the psychopharmacology of 4-MMC may serve to inform clinical treatment of illicit synthetic cathinone abuse.

Disclosures: R.L. Burroughs: None. **J.D. Zuarth Gonzalez:** None. **K.J. Cargile:** None. **L.E. Baker:** A. Employment/Salary (full or part-time):; Western Michigan University. B. Contracted

Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; National Institute on Drug Abuse. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NIDA Drug Control Supply.

Poster

156. Neural and Behavioral Mechanisms of Addiction: Amphetamine

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 156.03/U4

Topic: G.08. Drugs of Abuse and Addiction

Support: MOST 107-2410-H-431-004
MOST 106-2410-H-431-006

Title: Rewarding and aversive effects of methamphetamine to test the reward comparison hypothesis and the paradoxical effect hypothesis of abused drugs

Authors: *A. C.-W. HUANG[1], C.-L. HUANG[1], A. KOZŁOWSKA[2], J.-C. CHEN[3], C.-W. WU[4]; [1]Dept. of Psychology, Fo Guang Univ., Yilan County, Taiwan; [2]Dept. of Human Physiology, Sch. Medicine, Collegium Medicum, Univ. of Warmia and Mazury in Olsztyn, Warszawska Av, 30, 10-082 Olsztyn, Poland; [3]Dept. of Substance Abuse and Psychiatry, Tri-Service Gen. Hosp. Beitou Br., Taipei, Taiwan; [4]Dept. of Pharmacy, Keelung Hospital, Ministry of Hlth. and Welfare, Keelung City, Taiwan

Abstract: A small amount of research has examined the opposite effect of aversion to abused drugs to balance the reward effect for drug taking. An aversive behavioral model of abused drugs in terms of conditioned taste aversion (CTA) was challenged by the reward comparison hypothesis (Grigson, 1997). To test the reward comparison hypothesis, the present study tested the rewarding or aversive neural substrates involved in methamphetamine-induced conditioned taste suppression. The behavioral data showed that methamphetamine induced conditioned suppression on conditioning and reinstatement but extinguished it on extinction. A higher level of stressful aversive corticosterone occurred on conditioning and reinstatement but not extinction. The c-Fos or p-ERK immunohistochemical activity showed that the cingulate cortex area 1 (Cg1), infralimbic cortex (IL), prelimbic cortex (PrL), basolateral amygdala (BLA), nucleus accumbens (NAc), and dentate gyrus (DG) of the hippocampus were overexpressed in aversive CTA induced by methamphetamine. These data may indicate that the Cg1, IL, PrL, BLA, NAc, and DG probably mediated the paradoxical effect—reward and aversion. Altogether, our data conflicted with the reward comparison hypothesis, and methamphetamine may simultaneously induce the paradoxical effect of reward and aversion in the brain to support the

paradoxical effect hypothesis of abused drugs. The present data implicate some insights for drug addiction in clinical aspects.

Disclosures: A.C. Huang: None. **C. Huang:** None. **A. Kozłowska:** None. **J. Chen:** None. **C. Wu:** None.

Poster

156. Neural and Behavioral Mechanisms of Addiction: Amphetamine

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 156.04/U5

Topic: G.08. Drugs of Abuse and Addiction

Support: DA038058

Title: The role of reverse transport of dopamine in complex behavioral phenotypes in *Drosophila melanogaster*

Authors: *S. J. MABRY[1], A. SHEKAR[2], J. AGUILAR[4], A. GALLI[3], H. MATTHIES[5]; [1]Univ. of Alabama Birmingham, Birmingham, AL; [2]Dept. of Pharmacol., [3]Dept of Molec Physio & Biophys, Vanderbilt Univ., Nashville, TN; [4]Pharmacol., Vandebilt Univ., Nashville, TN; [5]Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: The dopamine (DA) transporter (DAT) is an *SLC6* family presynaptic membrane protein that maintains DA homeostasis in the brain via active reuptake of DA. Although the conventional role of this transporter, DA reuptake, is well-characterized, little is known about its less prevalent and unconventional role, reverse transport of DA (DA efflux). In this study, we begin to define the context and mechanisms underlying DA efflux via the DAT. We uncover that N-terminal phosphorylation of DAT is required to promote constitutive DA efflux (CDE) which might be physiologically-relevant; however, phosphorylation alone is not sufficient. We have shown that the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) protein Syntaxin 1 (Stx1) interacts with the DAT N-terminus and this interaction supports CDE. Blockade of phosphorylation of Stx1 via pharmacological inhibition of casein kinase 2 (CK2) or via genetic manipulation prevents CDE. We translated these findings to *Drosophila melanogaster* and found that *in vivo*, efflux and its behavioral consequences (increased locomotion and courtship behaviors) can be inhibited by pharmacological inhibition of CK2. In conclusion, we have uncovered new mechanisms underlying constitutive efflux, and identified key proteins in the reverse transport pathway as therapeutic targets in both patients that harbor hDAT disease variants exhibiting constitutive efflux, and those that abuse psychostimulant drugs like AMPH.

Disclosures: **S.J. Mabry:** None. **A. Shekar:** None. **J. Aguilar:** None. **A. Galli:** None. **H. Matthies:** None.

Poster

156. Neural and Behavioral Mechanisms of Addiction: Amphetamine

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 156.05/U6

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant EB003320
NIH Grant DA034184
NIH Grant DA09397

Title: Repeated intermittent exposure to conditions of uncertainty increases glutamate signaling in the nucleus accumbens

Authors: P. MASCIA[1], Q. WANG[1], J. BROWN[1], K. M. NESBITT[2], R. T. KENNEDY[3], *P. VEZINA[1];
[1]The Univ. of Chicago, Chicago, IL; [2]Chem., Towson Univ., Towson, MD; [3]Chem., Univ. of Michigan, Ann Arbor, MI

Abstract: Recently we reported that nucleus accumbens dopamine tracks uncertainty during operant responding for non-caloric saccharin and that intermittent exposure to such conditions of uncertainty leads to subsequent sensitization of the locomotor and nucleus accumbens dopamine effects of amphetamine and promotes the subsequent self-administration of the drug. Here we show that nucleus accumbens glutamate signaling is similarly affected by uncertainty. Extracellular levels of glutamate tracked uncertainty in a task in which nose poking for saccharin on an escalating variable ratio schedule of reinforcement was associated with progressively increasing variance between performance of the operant and payout. Furthermore, exposure to uncertainty subsequently increased phosphorylation by PKC and CaMKII in the nucleus accumbens. Notably, we have shown that phosphorylation by these enzymes of AMPA receptor residues in this site is necessary for expression of enhanced drug seeking. As these effects, as well as those observed with DA, are similar to those produced by exposure to abused drugs, the present results point to the recruitment of both DA and glutamate signaling pathways in the nucleus accumbens in drug and behavioral addictions.

Disclosures: **P. Mascia:** None. **Q. Wang:** None. **J. Brown:** None. **K.M. Nesbitt:** None. **R.T. Kennedy:** None. **P. Vezina:** None.

Poster

156. Neural and Behavioral Mechanisms of Addiction: Amphetamine

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 156.06/U7

Topic: G.08. Drugs of Abuse and Addiction

Support: SAF2016- 75347-R
PND 2016I004
LDC is a recipient of a fellowship from Ministerio de Educación, Cultura y Deporte.

Title: Cross reinstatement by 3 4 methylenedioxypyrovalerone MDPV and cocaine of mice conditioned to both drugs

Authors: L. DUART-CASTELLS[1], C. BLANCO-GANDÍA[2], ***M. H. BUENROSTRO-JAUREGUI**[3], D. PUBILL[1], M. RODRÍGUEZ-ARIAS[2], E. ESCUBEDO[1];
[1]Dept. of Pharmacology, Toxicology and Therapeut. Chemistry, Pharmacol., Univ. of Barcelona, Barcelona, Spain; [2]Dept. of Psychobiology, Univ. of Valencia, Valencia, Spain; [3]Dept. of Psychology, Univ. Iberoamericana, Mexico City, Mexico

Abstract: 3,4-Methylenedioxypyrovalerone (MDPV) is a cathinone that acts as cocaine, blocking the dopamine transporter. We previously reported that MDPV increases vulnerability to cocaine abuse and the existence of cross-sensitization between both drugs. Now, the aim of the present study was to compare both drugs in the extinction and reinstatement phase after being subjected to a conditioning place preference paradigm (CPP). In parallel, we sought to determine the expression of some neuroplasticity markers 24h after the test and 2h after a single injection was given one week after the CPP test, simulating an early reinstatement before drug-seeking extinction. Male adult OF-1 mice showed a similar CPP score to cocaine (10 mg/kg i.p.) or to MDPV (2 mg/kg, s.c.). In extinction, MDPV-conditioned mice persisted responsive longer (16 weeks) than those conditioned with cocaine (6 weeks). Our results showed that both, MDPV and cocaine, induced the reinstatement with priming-doses up to 50% of the initial dose used in the conditioning phase. A cross-reinstatement was evidenced for both drugs, although the relapse was always higher within the same drug. 24h after the CPP test, the main difference between both drugs was the long-lasting expression of ΔFosB protein in animals conditioned to MDPV, but not to cocaine. Probably this factor reflects the longest extinction time of the cathinone. When analyzing the response of the conditioned mice 2h after a challenge dose of cocaine or MDPV, some differences must be highlighted. Firstly, G9a mRNA expression decreased after a cocaine injection in all animals but increased after MDPV challenge in cocaine-conditioned animals. Differences in Arc mRNA expression were only observed in cocaine-conditioned animals so that its expression decreased when cocaine was reinstated. Finally, a decrease in c-

Fos RNAm expression was observed, again, only in cocaine-conditioned animals after being re-exposed to this drug. Overall, it seems that MDPV-conditioned mice develop tolerance in such a way that they did not respond to a new drug exposition, meanwhile, the cocaine-conditioned mice were more reactive to a new exposition. To sum up, although both psychostimulants are similar enough to produce a cross-reinstatement, the neuroplasticity mechanisms that they activate differ notably.

Disclosures: L. Duart-Castells: None. **C. Blanco-Gandía:** None. **M.H. Buenrostro-jauregui:** None. **D. Pubill:** None. **M. Rodríguez-Arias:** None. **E. Escubedo:** None.

Poster

156. Neural and Behavioral Mechanisms of Addiction: Amphetamine

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 156.07/U8

Topic: G.08. Drugs of Abuse and Addiction

Title: The effects of methamphetamine exposure on anxiety-like behavior, corticosterone levels, and tyrosine hydroxylase levels in adolescent and adult mice

Authors: M. L. NEWBY, H. A. ORTMAN, *J. A. SIEGEL;
The Univ. of St. Thomas, Saint Paul, MN

Abstract: Methamphetamine (MA) has neurotoxic effects that can lead to deficits in behavior and changes in the stress response system. The effects of MA are modulated by age, and relatively little research has examined the effects of MA in adolescents compared to adults. As the brain is developing during adolescence, the effects of MA may differ from the effects in adults. Previous research shows that acute MA exposure in adolescence increases anxiety-like behavior. However, the research on the effects of acute MA exposure in adults is conflicting, with some studies showing decreased anxiety-like behavior and others showing increased anxiety-like behavior. Acute MA exposure in adolescence does not alter corticosterone levels while acute MA exposure in adults has been shown to increase corticosterone or have no effect on corticosterone levels. Furthermore, age modulates the effects of MA on the brain dopamine system, with adolescent rodents showing relative resistance to the neurotoxic effects of MA on the dopamine system compared to adults. Previous research from our lab examined the effects of acute MA exposure (4 mg/kg) in adolescent and adult mice on anxiety-like behavior, corticosterone levels, and hippocampal tyrosine hydroxylase levels. The current research expands on our previous work and examines the acute effects of MA exposure (2 mg/kg and 4 mg/kg) during adolescence and adulthood on anxiety-like behavior, plasma corticosterone levels, and striatal tyrosine hydroxylase levels in male C57BL/6J mice. Current experiments are ongoing to study the effects of adolescent and adult MA exposure on behavior in the open field test to evaluate locomotor activity and anxiety-like behavior. Plasma corticosterone and striatal

tyrosine hydroxylase levels will be measured following behavioral testing. Based on previous data, we expect both the low and high dose of MA to increase anxiety-like behavior, and to have no effect on plasma corticosterone levels, in adolescent and adult mice. Furthermore, we predict that MA will have no effect on tyrosine hydroxylase levels in adolescent mice, but will decrease tyrosine hydroxylase levels in adult mice. These findings will contribute to a greater understanding of how various doses of MA alter behavior, the stress response system, and the dopamine system and how these effects of MA may differ between adolescent and adult mice.

Disclosures: M.L. Newby: None. **H.A. Ortman:** None. **J.A. Siegel:** None.

Poster

156. Neural and Behavioral Mechanisms of Addiction: Amphetamine

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 156.08/U9

Topic: G.08. Drugs of Abuse and Addiction

Support: University of Vermont Department of Psychological Science

Title: Methamphetamine and habit formation in gonadally-intact female rats

Authors: *H. SCHOENBERG[1], L. BAUSCH[1], I. MINEARO[1], A. KIRSHENBAUM[2], D. J. TOUFEXIS[1];
[1]Dept. of Psychological Sci., Univ. of Vermont, Burlington, VT; [2]St. Michael's Col., Colchester, VT

Abstract: It has been demonstrated that multiple drugs of abuse can accelerate the transition from goal-directed to habitual behavior, and that this is not limited to drug-taking itself, but is true also for natural rewards like sucrose. Pre-treatment with low doses of amphetamine or methamphetamine (METH) prior to training in male rats has been reported to facilitate the early expression of habitual responding for food reinforcers. Previously, we showed that low-dose METH pre-exposure in ovariectomized (OVX) and OVX cyclic-estrogen replaced rats does not advance habitual responding at training levels subthreshold to habit formation for females. In order to determine if factors other than, or in addition to, estrogen in the estrous cycle may interact with METH and advance habit, we tested intact female rats at the same subthreshold training level as our OVX females. Results found that METH did not accelerate habit formation in these females. Thus, unlike published studies in male rats, habit formation in female rats does not appear to be enhanced by METH exposure. However, in very preliminary results we found that METH pre-exposed intact females, trained to levels that usually result in habitual responding, showed a strong statistical trend towards remaining goal-directed. Thus, we are undertaking follow-up studies to determine if METH exposure delays habit formation in female rats.

Disclosures: **H. Schoenberg:** None. **D.J. Toufexis:** None. **L. Bausch:** None. **I. Minearo:** None. **A. Kirshenbaum:** None.

Poster

156. Neural and Behavioral Mechanisms of Addiction: Amphetamine

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Program #/Poster #: 156.09/U10

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA035435-02
NIH Grant P20GM113109

Title: The effects of n-acetylcysteine and differential rearing on amphetamine seeking

Authors: ***T. D. FORT**, T. J. MOSER, J. T. GOMENDOZA, J. P. RACK, M. ALLISON; Psychological Sci., Kansas State Univ., Manhattan, KS

Abstract: Previous research has demonstrated that enriched rearing conditions (EC) can reduce amphetamine (AMP) self-administration (SA) and cue-induced reinstatement when compared to isolated rearing conditions (IC). However, there has been little work done using longer AMP access SA procedures often used to model the escalation of drug intake characteristic of substance abuse disorders. The current study used 6-hr SA procedures to elucidate the effect that differential rearing may have on overall AMP self-administration, relapse following an incubation period, and the extent to which relapse can be mitigated by treatment with *N-acetylcysteine* (NAC). Given that differential rearing alters glutamate homeostasis, the impact of differential rearing and NAC treatment on astrocyte density will be evaluated via immunohistochemical (IHC) staining of GFAP+ cells in the mPFC and NAcc. We hypothesize that enrichment will protect against escalation and will result in the greatest GFAP expression. Sprague-Dawley rats were raised in their respective environmental conditions for 30 days. EC rats were raised communally, given novel arrangements of toys, and handled daily. IC rats were single-housed in hanging cages, lacked novel objects, and were not handled during the rearing period. SC rats were pair-housed, lacked novel objects, and were only handled once a week during cage changes. After 30 days of rearing, all rats were implanted with an indwelling jugular catheter. Upon recovery, rats were placed into the operant chamber to undergo twelve, 6-hr FR-1 sessions. Active lever presses resulted in an infusion of amphetamine (0.1 mg/kg/infusion) which was followed by a 20 s time-out period signaled by the illumination of both cue lights. After twelve sessions, all animals were given daily treatments of NAC (100 mg/mL; ip) during the incubation period while remaining in their respective environments. After twelve NAC treatments, all animals were placed back into the operant chambers for a 2-hr long incubation test and brains were extracted immediately after for IHC staining of GFAP.
Preliminary results indicate an escalation of AMP SA across sessions and that enrichment did not

protect against escalation. During the incubation test, NAC did not appear to decrease active lever responding, but enrichment may protect against relapse as it reduced active lever responses when compared to IC or SC rats. In addition, active lever pressing was greater for females than for males. IHC results will help to determine the effect of rearing on glial cell density and whether this can modulate the effectiveness of NAC treatment.

Disclosures: T.D. Fort: None. **T.J. Moser:** None. **J.T. Gomendoza:** None. **J.P. Rack:** None. **M. Allison:** None.

Poster

156. Neural and Behavioral Mechanisms of Addiction: Amphetamine

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 156.10/U11

Topic: G.08. Drugs of Abuse and Addiction

Support: Fondecyt 1191152

Title: LSD1 neuronal-specific splicing variant modulates acute and chronic responses to amphetamine in mice

Authors: *G. B. MERELLO[1], M. OLIVARES COSTA[3], M. P. GONZALEZ[2], E. BATTAGLIOLI[4], F. S. RUSCONI[5], M. E. ANDRES[6];
[1]Cell. and Mol. Biol., [2]Pontificia Univ. Catolica De Chile, Santiago, Chile; [3]Cell. and Mol. Biol., Univ. Católica, Santiago, Chile; [4]Dept Mol. Biotech. and Translational Med., [5]Med. Biotech. and Traslational Med., Univ. of Milan, Segrate, Italy; [6]Cell. and Mol. Biol., Pontificia Univ. Catolica de Chile, Santiago, Chile

Abstract: Lysine-specific demethylase 1 (LSD1) is an epigenetic modifier with H3K4me1/2 and H3K9me1/2 demethylase activity. LSD1 has four splicing variants, classified either as ubiquitous LSD1 (uLSD1) or neuronal LSD1 (nLSD1). nLSD1, which is only expressed in neurons, differs from uLSD1 by the retention of a twelve nucleotide microexon. nLSD1 knock-out (KO) mice exhibit a low anxiety phenotype and are unable to induce immediate early gene (IEG) expression in the brain. Considering the relationship between anxious behavior and drug addiction, we studied behavioral and molecular changes in nLSD1 KO mice subjected to acute and chronic amphetamine (AMPH) treatments. Both heterozygous (HT) and KO mice responded to an acute dose of AMPH, by increasing locomotor activity, with HT and KO displaying higher locomotor activity than WT. Also, chronic amphetamine treatments resulted in a significant increase in neuroLSD1/uLSD1 transcript ratios and decreased total LSD1 transcript levels in WT mice, while LSD1 transcript levels did not change in nLSD1 KO mice. H3K4me1/2 and H3Ac levels were both increased in nLSD1 KO mice striatum in response to chronic AMPH, whereas WT mice showed no significant augmentation. Finally, locomotor sensitization experiments

showed genotype associated differences in development and expression of behavioral sensitization induced by AMPH. These data suggest the involvement of LSD1 and nLSD1 in the neurobiological changes that take place after repetitive psychostimulant consumption, which could be useful in the search for pharmacological tools to treat addiction and other compulsive behaviors.

Disclosures: G.B. Merello: None. **M. Olivares Costa:** None. **M.P. Gonzalez:** None. **E. Battaglioli:** None. **F.S. Rusconi:** None. **M.E. Andres:** None.

Poster

156. Neural and Behavioral Mechanisms of Addiction: Amphetamine

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 156.11/U12

Topic: G.08. Drugs of Abuse and Addiction

Title: Antagonizing serotonin 2A (5-HT_{2A}) receptors with M100907 prevents methamphetamine-induced reward

Authors: *J. T. MADDEN, N. C. REYNA, C. M. MAGCALAS, N. S. PENTKOWSKI; Psychology, Univ. of New Mexico, Albuquerque, NM

Abstract: Methamphetamine (METH) is a psychostimulant that is highly addictive and widely abused, imposing a significant burden on society. Currently, there is no medical treatment for psychostimulant dependence, highlighting the need for research examining the neural mechanisms underlying psychostimulant-induced behaviors. Serotonin (5-HT) plays a critical role in mediating psychostimulant addiction-related behaviors, including those of cocaine and METH. Previous research indicates that the 5-HT_{2A} receptor antagonist M100907 attenuates several cocaine-induced behavioral effects, including conditioned place preference (CPP) and hyperlocomotor activity. However, these findings have not yet been extended to METH. The aim of the present study was to investigate whether pretreatment with M100907 can attenuate METH-induced CPP and stereotypy. Adult male rats (n = 10 per group, N = 60) were tested for CPP using an unbiased two-chamber apparatus across eight consecutive days. Prior to METH administration (0 or 1 mg/kg, i.p.), rats were pretreated with their assigned dose of M100907 (0, .025 or .25 mg/kg, i.p.) and then were immediately placed into their initially non-preferred chamber. Following four conditioning sessions, the effects of M100907 on METH-induced changes in place preference were assessed. Pretreatment with M100907 prevented METH-induced CPP without producing any observable effects when administered alone. These results suggest that M100907 attenuates the rewarding effects of METH, and importantly does not produce any intrinsic rewarding or aversive effects when administered alone. These results add to a growing body of work suggesting that targeting 5-HT_{2A} receptors represents a novel pharmacological approach for treating psychostimulant addiction.

Disclosures: **J.T. Madden:** A. Employment/Salary (full or part-time):; Graduate Researcher, University of New Mexico. **N.C. Reyna:** None. **C.M. Magcalas:** A. Employment/Salary (full or part-time):; Graduate Researcher, University of New Mexico. **N.S. Pentkowski:** A. Employment/Salary (full or part-time):; UNM Assistant Professor, Principal Investigator. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; M100907 purchased from ACADIA Pharmaceuticals, METH purchased from Sigma Aldrich.

Poster

156. Neural and Behavioral Mechanisms of Addiction: Amphetamine

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 156.12/U13

Topic: G.08. Drugs of Abuse and Addiction

Title: Central ghrelin 1a and glucagon-like peptide-1 receptor activation modulates d-amphetamine conditioned place preference and operant responding for palatable food

Authors: *D. P. DUNN, L. ENGEL, O. DAO, M. HALPERIN, K. HAYWORTH, M. KANTER, K. KESSLER, H. KILLEN, J. KOHN, E. LEIF, M. O'KELLEY-BANGSBERG, C. WALWORTH, D. WONG, P. J. CURRIE;
Dept. of Psychology, Reed Col., Portland, OR

Abstract: Increasing evidence implicates neuropeptides such as ghrelin and glucagon-like peptide-1 in augmenting incentive salience of reinforcers, hedonic behavior, and cue-induced reward seeking. To further investigate these effects, the present study investigated the ability of ghrelin or the glucagon-like peptide-1 analogue exendin-4 (Ex-4), administered into the ventral tegmental area (VTA), to modulate dextro-amphetamine-induced conditioned place preference (CPP) or operant responding for palatable food. Adult male Sprague-Dawley rats were given access to both sides of a two-chamber apparatus during the CPP pre-conditioning period to determine initial chamber preference. The rats were then restricted to their least preferred chamber (CS+) or their preferred chamber (CS-) over the conditioning period which lasted six days. On CS+ days rats were confined to their least preferred chamber and d-amphetamine (1.0 mg/kg IP) was administered prior to the conditioning period. On CS- days rats were treated with vehicle and placed in their initially preferred chamber. During the post-conditioning period, rats were allowed access to both chambers in order to reassess preference and pre-treated with VTA microinjection of either vehicle, ghrelin (300 pmol), JMV 2959 (10 µg) paired with ghrelin (300 pmol), or Ex-4 (0.025-0.05 µg) to assess the modulatory effect on d-amphetamine-induced CPP expression. To further demonstrate the role of neuropeptides in reward acquisition, in a separate study, rats were administered VTA ghrelin (300 pmol) or Ex-4 (0.025-0.05 µg) prior to operant

responding for palatable food. Our results demonstrated that d-amphetamine evoked a place preference and that Ex-4 attenuated CPP expression, and, as expected, Ex-4 decreased operant responding for palatable food. Additionally, VTA ghrelin treatment potentiated d-amphetamine-induced CPP, as well as operant responding for palatable food, while ghrelin paired with JMV 2959 suppressed CPP expression and reduced operant responses. Overall, these findings provide further support for the role of mesolimbic peptidergic signaling and its role in psychostimulant and food reward.

Disclosures: **D.P. Dunn:** None. **L. Engel:** None. **O. Dao:** None. **M. Halperin:** None. **K. Hayworth:** None. **M. Kanter:** None. **K. Kessler:** None. **H. Killen:** None. **J. Kohn:** None. **E. Leif:** None. **M. O'Kelley-Bangsberg:** None. **C. Walworth:** None. **D. Wong:** None. **P.J. Currie:** None.

Poster

156. Neural and Behavioral Mechanisms of Addiction: Amphetamine

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 156.13/U14

Topic: G.08. Drugs of Abuse and Addiction

Support: DGAPA-PAPIIT IA205218
DGAPA-PAPIIT IN215218
DGAPA-PAPIIT IN217918

Title: Effects of pre-weaning on amphetamine conditioned place preference development and dopaminergic system

Authors: *M. MENDEZ-DIAZ[1], O. AMANCIO-BELMONT[3], Y. A. ALVARADO RAMÍREZ[2], A. E. RUIZ-CONTRERAS[5], O. PROSPERO-GARCIA[4];
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Abstract: A considerable amount of experimental studies have shown that optimal maternal care is associated with an optimal brain development and less seeking of drugs of abuse. Correspondingly, lack of proper maternal care increases vulnerability to drug abuse and drug dependence. The aim of the study is to evaluate if pre-weaning or post-weaning affects the development of conditioned place preference (CPP) for AMPH, AMPH-sensitization and the dopaminergic system in nucleus accumbens (NAcc) and prefrontal cortex (PFC) in adult rats.Pregnant Wistar rats were obtained at gestational day 14-17 from our facilities (Facultad de Medicina, UNAM). Rats were weaned either on postnatal day (PND) 15, 21 or 30. Once the rats became adults, all groups (n=10 each group) were submitted to CPP for AMPH (2 mg/kg, ip).

Additional rats (n=10 each group) were submitted to the AMPH-sensitization protocol for 5 days. At the end of the protocol, rats were sacrificed to dissect PFC and NAcc to analyzed the expression of DR1, DR2, DAT.Results. Although all groups developed CPP, those belonging to the post-weaning group exhibited 32% more time in the CPP than the control group (PND21).

Disclosures: M. Mendez-Diaz: None. **O. Amancio-Belmont:** None. **Y.A. Alvarado Ramírez:** None. **A.E. Ruiz-Contreras:** None. **O. Prospero-Garcia:** None.

Poster

156. Neural and Behavioral Mechanisms of Addiction: Amphetamine

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 156.14/U15

Topic: G.08. Drugs of Abuse and Addiction

Title: Characterization of second order methamphetamine self-administration in female rats

Authors: *G. F. GUERIN, V. Y. HEBERT, N. E. GOEDERS;
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Abstract: Second-order schedules of reinforcement can be used to measure drug-seeking behavior and reinforcement by generating large amounts of behavior while limiting the amount of drug self-administered during the experiment. This avoids the potential confounds produced by the non-specific pharmacological effects of the drug itself. Adult female Wistar rats were implanted with jugular catheters and trained to self-administer methamphetamine (0.06 mg/kg/infusion) under a fixed-ratio 1 (FR 1) schedule of reinforcement, whereby one response produced an infusion of the drug that was paired with a tone and houselight conditioned stimulus (cs), which was followed by a 20-second time-out period. The response requirement was subsequently increased to FR 2 and then FR 4 over several days. Once responding was stable under the FR 4 schedule, a 30-second fixed-interval was added so that any fixed-ratio completed before the end of the interval was only reinforced by presentation of the cs. The first fixed-ratio completed after the interval elapsed was then reinforced by an infusion of the drug and the presentation of the cs. This interval was increased by 30-second increments over several weeks for a final fixed-interval of 600 seconds. Under this schedule, multiple fixed-ratios could be completed before the end of each fixed-interval. While an average of 10 infusions were administered over the course of the 2-hour session, an average of 75 cs presentations and 563 total active lever responses also occurred at the training dose. These data demonstrated that a significant amount of behavior could be maintained in rats with a relatively small amount of drug delivered (i.e., essentially 56 responses per infusion of methamphetamine). A methamphetamine dose-response curve was also generated by randomly substituting other doses of the drug (0, 0.015, 0.03, 0.12, 0.24 mg/kg/inf) for one week each. While the number of infusions was essentially unchanged, the number of cs presentations varied with the dose (i.e., 20, 36, 56, 87,

and 89, respectively), as did the total number of active lever responses (i.e., 149, 259, 373, 610, 688). Potential pharmacological treatments for methamphetamine substance use disorder can be tested using this second order schedule of reinforcement maintained under conditions of drug seeking that are less influenced by the intoxicating effects of the drug.

Disclosures: G.F. Guerin: None. **V.Y. Hebert:** None. **N.E. Goeders:** None.

Poster

156. Neural and Behavioral Mechanisms of Addiction: Amphetamine

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 156.15/U16

Topic: G.08. Drugs of Abuse and Addiction

Support: GM060665
MH09779
G12MD007599

Title: Removal of the 5-HT_{1B} receptor leads to enhanced voluntary consumption of methamphetamine in males and protects against the long-lasting effects of methamphetamine in females

Authors: *N. K. MEMOS[1,2], J. A. AVILA[1,2], E. RODRIGUEZ[1], N. S. BURGHARDT[1,2], P. A. SERRANO[1,2];
[1]Hunter College, CUNY, New York, NY; [2]The Grad. Center, CUNY, New York, NY

Abstract: Methamphetamine (MA) is an addictive psychostimulant that targets dopamine and serotonin neurotransmission and impairs prefrontal, hippocampal, and striatal-dependent cognition. Previous studies indicate that removal of the serotonin 1B receptor (5-HT_{1B} KO) from male mice increases the motivation to self-administer the psychostimulant cocaine, implicating a role for this receptor in addictive behavior. However, it is not known if removal of the 5-HT_{1B} receptor similarly affects consumption of methamphetamine or if there are sex difference in this response. We tested 5-HT_{1B} KO and wild-type (WT) littermates of both sexes in a long-access (28d) voluntary oral MA administration (VOMA) model, which we have previously used to model MA consumption patterns observed in humans. In this model, mice are given 10 days of escalating MA doses followed by 18 days of voluntary MA consumption, during which mice are presented with1mg/kg/bait (dissolved in 6µl Ensure) 16 times/day. A separate group of mice (No Drug) were never exposed to MA and instead received equivalent presentations of vehicle (Ensure only). After two weeks of forced abstinence from MA, mice were tested drug-free in the radial-8 arm maze (RAM) and the tail suspension test. Consistent with what has previously been shown with cocaine, we found that male 5-HT_{1B} KO mice consumed more MA than WT mice of the same sex. However, this was not found in females, in which case KO and WT mice

voluntarily consumed a similar amount of MA. Surprisingly, we found no effect of previous MA exposure on short-term working memory in the RAM in males or females of either genotype. In contrast, long-term MA exposure did increase mobility in the tail suspension test in both male and female WT mice when compared to controls (No Drug) of the same sex and genotype. Interestingly, this effected was attenuated by removal of the 5-HT_{1B} receptor in females only. Based on evidence indicating that changes in extracellular levels of serotonin affect mobility in the tail suspension test, we hypothesize that chronic administration of MA followed by a two-week period of abstinence produces a long-lasting increase in extracellular levels of serotonin, which is blocked by removal of the 5-HT_{1B} receptor in females. Analysis of tryptophan hydroxylase expression levels in the hippocampus of male and female KO and WT mice following 28 days of MA exposure is currently being assessed.

Disclosures: N.K. Memos: None. **J.A. Avila:** None. **E. Rodriguez:** None. **N.S. Burghardt:** None. **P.A. Serrano:** None.

Poster

156. Neural and Behavioral Mechanisms of Addiction: Amphetamine

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 156.16/U17

Topic: G.08. Drugs of Abuse and Addiction

Support: Institutional Pilot Funds

Title: Conditioned neural responses to methamphetamine-paired contexts

Authors: *H. YANG[1], J. WEAFER[3], E. CHILDS[2];
[2]Psychiatry, [1]Univ. of Illinois At Chicago, Chicago, IL; [3]Univ. of Chicago, Chicago, IL

Abstract: AIM: Human laboratory drug conditioning studies provide the opportunity to study the processes by which drug cue associations are formed and how the cues come to powerfully influence behavior. An understanding of these processes in humans, including the underlying neural circuitry, will guide new cue-focused treatment strategies for addiction. In this study, we examined conditioned neural responses to contexts paired with methamphetamine (MA) using conditioned place preference (CPP) procedures.METHODS: Volunteers (N=10) completed six conditioning sessions, three each with 20mg MA and placebo (PL), in pseudorandomized order. Participants received MA in one room and PL in another. We assessed acquisition of conditioning by comparing pre- to post-conditioning changes in preference for the MA-room. At a separate session, participants underwent fMRI scanning with a cue-reactivity task using MA-room, PL-room, and neutral images.RESULTS: Participants acquired CPP, shown by a significant pre- to post-conditioning increase in preference for the MA-room ($p<0.01$). In comparison to PL-room images, the MA-room produced greater activation in several regions of

interest, including the anterior cingulate/ventromedial prefrontal cortex, hippocampus, caudate, putamen, amygdala, and insula (ps<0.05). No ROIs showed greater activation to the PL-room. Comparison of MA- and PL-room activations to neutral images showed that the MA-room elicited greater activation than neutral images in the anterior cingulate and hippocampus (ps<0.05), whereas the PL-room did not differ to neutral.CONCLUSIONS: These data provide initial evidence that MA conditioning involves brain regions important to reward/motivation, salience, habitual learning, and cue memory formation. Patterns of activation to the CPP rooms are similar to those produced by generic drug images used in cue reactivity studies. Thus, the findings also support the notion that human CPP models the conditioning processes occurring during real world drug experiences. Supported by UIC Department of Psychiatry funds for pilot studies (Dr. Childs) and K01AA024519 (Dr. Weafer)

Disclosures: H. Yang: None. **J. Weafer:** None. **E. Childs:** None.

Poster

156. Neural and Behavioral Mechanisms of Addiction: Amphetamine

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 156.17/U18

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA Grant DA011064

Title: Fos expression associated with 5-HT_{1B} receptor agonist inhibition of methamphetamine seeking in the conditioned place preference model

Authors: T. S. DER-GHAZARIAN, D. CHARMCHI, S. N. NOUDALI, S. N. SCOTT, M. C. HOLTER, J. M. NEWBERN, *J. L. NEISEWANDER;
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Abstract: 5-HT_{1B} receptors (5-HT_{1B}Rs) modulate psychostimulant reward and incentive motivation in rodents. Here we investigated the effects of the 5-HT_{1B}R agonist CP94253 (10 mg/kg, IP) on the acquisition and expression of methamphetamine (Meth) conditioned place preference (CPP) in C57BL/6 male mice. We subsequently examined the potential brain regions involved in CP94253 effects using FOS as a marker of neural activity. In the acquisition experiment, mice received the agonist 30 min before each Meth injection given during conditioning. In the expression experiment, mice that had acquired Meth-CPP were given either saline or CP94253 and were tested for CPP 30 min later. We found that CP94253 attenuated the expression of Meth-CPP, but had no effect on acquisition. Mice expressing Meth-CPP had elevated numbers of FOS^+ cells in the ventral tegmental area (VTA) and basolateral amygdala (BlA) and reduced FOS^+ cells in the central amygdala (CeA) compared to saline controls. CP94253 given before the expression test, but not acutely in drug-naive mice, enhanced FOS^+

cells in the VTA, nucleus accumbens (NAc) shell and core, and the dorsomedial striatum, and reversed the Meth-conditioned changes in FOS in the BlA and CeA. Approximately 50-70% of FOS^+ cells in the NAc and VTA were GABAergic regardless of group. By contrast, we did not observe FOS-labeling in dopamine neurons in the VTA. The findings suggest that CP94253 attenuates the motivational effects of the Meth-associated environment and highlight the amygdala, VTA, NAc, and dorsomedial striatum as potential regions involved in this effect.

Disclosures: T.S. Der-Ghazarian: None. **J.L. Neisewander:** None. **D. Charmchi:** None. **S.N. Noudali:** None. **S.N. Scott:** None. **M.C. Holter:** None. **J.M. Newbern:** None.

Poster

156. Neural and Behavioral Mechanisms of Addiction: Amphetamine

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 156.18/U19

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA 027222

Title: Glutamine and dopamine in the VTA participate differently in the acute and chronic effect of methylphenidate

Authors: *S. FLOREN, N. KING, A. CARRASO, *P. DASH, N. DAFNY;
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Abstract: Psychostimulants such as methylphenidate (MPD) have long been the treatment of choice in behavioral disorders such as attention deficit/hyperactivity disorder (ADHD) and narcolepsy in both children and adults; however, its abuse by healthy children and young adults as a “study drug” and “recreational” drug is on the rise. This raises concern for dependence and brain chemistry alteration during a period of neuroplasticity and brain development. Many areas of the brain play a role in the response to psychostimulants; one of these areas is the ventral tegmental area (VTA). The VTA is known to be one of the major source of dopamine (DA) to the brain and MPD is considered an indirect DA agonist. The role of the VTA in acute and chronic MPD exposure have not been fully elucidated. In order to explore this, five groups of rats with different VTA lesions were used: intact, sham surgery, nonspecific electrolytic lesion, glutamatergic specific chemical lesion, and dopaminergic specific chemical lesion. After several days of adaptation, baseline locomotor activity was recorded, then the surgeries were performed, followed by several days of recovery and reestablishment of baseline post surgery. With the lesions in place, the rats then underwent acute and chronic MPD exposure followed by three washout days, then the rats were administered a re-challenge of MPD to assess expression of chronic neural changes. The results indicate that the ventral tegmental area and its glutaminergic and dopaminergic synapses play significant and different roles in the effect of MPD.

Disclosures: **S. Floren:** None. **N. King:** None. **A. Carraso:** None. **P. Dash:** None. **N. Dafny:** None.

Poster

156. Neural and Behavioral Mechanisms of Addiction: Amphetamine

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 156.19/U20

Topic: G.08. Drugs of Abuse and Addiction

Support: GM 64783
GM 81069

Title: Does sensitization to the stimulant effects of methamphetamine develop following repeated administration in laboratory rats?

Authors: *C. P. CERVANTES ALDANA[1], K. A. TRUJILLO[2];
[1]Psychology, CSUSM, San Marcos, CA; [2]Dept. of Psychology, California State Univ. San Marcos, San Marcos, CA

Abstract: Methamphetamine (METH), a powerful psychostimulant, is popular among young adults. Behavioral sensitization refers to the phenomenon in which repeated administration of a drug produces an increasingly greater behavioral response. According to prominent theories, sensitization contributes significantly to the development of addiction. Previous work from our laboratory raises questions as to whether repeated administration of METH can induce behavioral sensitization. The inclusion of a "challenge" test was essential to these studies. Although increases in METH response did occur with repeated administration, when animals were later challenged with METH, saline-pretreated and METH-pretreated animals showed similar increases in locomotor behavior. The current work further examined METH sensitization using a low dose of METH (0.3 mg/kg). A low dose is useful as it allows greater room for the development of sensitization due to avoidance of a potential ceiling effect. The objective of the present studies was to investigate if repeated administration of METH can induce sensitization in laboratory rats. In two identical experiments adult male Sprague-Dawley rats (n=12/group) were assigned to receive repeated administration of saline or METH (0.3 mg/kg). Treatment was administered once daily for 5 days with a cagemate. Two days following the last treatment day, all animals were challenged with METH and their activity recorded. A Kinder Scientific Motor Monitor was used to assess locomotor behavior. In both studies, we observed similar sensitization, which was small but statistically significant, and depended on the specific locomotor measure. Specifically, we found significant increases in ambulation (forward movement), fine movements (typically associated with stereotypy) and time active, but not rearing. Moreover, increases were generally restricted to the first 10 minutes following drug

exposure. These results demonstrate the development of modest sensitization to a low dose of METH. We are currently investigating other behaviors to further examine METH sensitization.

Disclosures: C.P. Cervantes Aldana: None. **K.A. Trujillo:** None.

Poster

156. Neural and Behavioral Mechanisms of Addiction: Amphetamine

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 156.20/U21

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH GM 64783
NIH GM 81069

Title: Reward-related ultrasonic vocalizations in response to methamphetamine are greater in a comfortable environment

Authors: *E. P. ROBERTS[1], C. P. CERVANTES ALDANA[1], K. A. TRUJILLO[2];
[2]Dept. of Psychology, [1]California State Univ. San Marcos, San Marcos, CA

Abstract: Methamphetamine (METH) is a powerful psychomotor stimulant that has a high potential for abuse due to its rewarding effects. Ultrasonic vocalizations (USVs) reflect affect in rodents and can be used to assess the rewarding effects of drugs and other stimuli. Specifically, 50kHz vocalizations represent positive affect and are produced in a number of rewarding situations, including in response to METH. Previous research has demonstrated that rats emit more 50 kHz USVs when tested in cages with bedding than when tested without bedding (Natusch and Schwarting, 2010). The present studies expanded on this work and examined the effects of METH in the presence and absence of bedding. USVs and locomotor behavior were tested concurrently in response to saline and two doses of methamphetamine (0.3 mg/kg and 1.0 mg/kg) either in the presence of clean bedding (Sani Chips, the same bedding used in their home cages) or in the absence of bedding (bare cage). Locomotor activity was assessed by a Kinder Scientific Motor Monitor and USVs were analyzed by an Avisoft Ultrasoundgate 416H recording device and Avisoft SASLabpro software. The presence of bedding in a test cage increased 50kHz vocalizations in male Sprague-Dawley rats administered either saline or methamphetamine (0.3 mg/kg and 1.0mg/kg). The effects of bedding were more prominent at the higher dose of METH than at the lower dose. Rearing behavior was also higher in the presence of bedding at 0.3 mg/kg, but no change was seen in rearing behavior at 1.0 mg/kg of METH. Horizontal locomotion was not affected by bedding. The increase in USVs in saline-treated rats points to rewarding effects of bedding alone; the increase in METH-treated rats suggests that METH reward is influenced by the environment. The lack of change in horizontal locomotion demonstrates a dissociation between METH-induced USVs and METH-induced locomotor

stimulation. These results are applicable to drug abuse in humans and highlights that the perceived rewarding effects of a drug may depend on environmental conditions.

Disclosures: E.P. Roberts: None. **C.P. Cervantes Aldana:** None. **K.A. Trujillo:** None.

Poster

156. Neural and Behavioral Mechanisms of Addiction: Amphetamine

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 156.21/U22

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH GM 64783
NIH GM 81069

Title: Inhibition of methamphetamine reward by the NMDA receptor antagonist MK-801

Authors: *C. A. CHAVEZ[1], K. A. TRUJILLO[2];
[2]Dept. of Psychology, [1]California State Univ. San Marcos, San Marcos, CA

Abstract: Methamphetamine (METH) is a popular substance of abuse that is traditionally thought to produce rewarding effects primarily through the dopaminergic system. However, it has been shown that other neurotransmitter systems contribute to the behavioral effects of METH. For example, growing evidence suggests that N-Methyl-D-aspartate (NMDA) receptors may be involved in METH reward. This study investigated the role of these receptors in METH-induced reward in laboratory rats using locomotor behavior and ultrasonic vocalizations (USVs). Locomotor behavior has long been used to assess drugs of abuse and USVs represent a relatively new approach to measuring reward and aversion in rats. For this experiment we investigated three doses of MK-801 (0.03 mg/kg, 0.05 mg/kg and 0.10 mg/kg) with two doses of METH (0.3 mg/kg and 1.0 mg/kg) in adult male Sprague-Dawley rats (n=8/group). MK-801 was administered 1 hour before METH, and locomotor behavior and USVs were recorded concurrently. A Kinder Scientific Motor Monitor was used to assess locomotor activity and an Avisoft Ultrasoundgate 416H recording device and Avisoft SASLabpro software were used to assess USVs. MK-801 facilitated METH-induced horizontal locomotion, but attenuated METH-induced USVs and produced complex effects on rearing behavior. Since USVs are thought to be a direct measure of psychomotor stimulant reward, the results suggest that NMDA receptors are involved in the rewarding effects of METH. The results point toward potential therapeutic use of NMDA receptor blockers in psychomotor stimulant abuse and addiction.

Disclosures: C.A. Chavez: None. **K.A. Trujillo:** None.

Poster

156. Neural and Behavioral Mechanisms of Addiction: Amphetamine

Location: Hall A

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Program #/Poster #: 156.22/U23

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH GM 64783
NIH GM 81069
NIH GM 08807
NIH GM 122659

Title: A supervised deep learning classification approach to quantifying ultrasonic vocalizations in rodents

Authors: A. ARANGO, *K. A. TRUJILLO, X. ZHANG;
California State Univ. San Marcos, San Marcos, CA

Abstract: Ultrasonic vocalizations (USVs) are a means by which rodents communicate. Recent research has demonstrated that USVs reflect affective states, with 50 kHz USVs reflecting positive affect and reward. USVs are therefore important tool to better understand the emotional state of rats and provide insight into the rewarding effects of drugs of abuse and other stimuli. Reward-related USVs in the 50 kHz range can be classified in different types of calls, which may reflect different aspects of reward. Identification and classification of USV calls is a time-consuming process. Recently, different software classification tools have emerged, which provide automated alternatives to classification of USVs. Some currently available tools are based on MATLAB which can be expensive due to commercial MATLAB licenses. Advances in deep learning have allowed for successful classifications of simple hand-written characters and complex images. The purpose of the current work was to develop a software application for accurate classification of rat ultrasonic vocalizations using deep learning. To develop this application (which we are calling USVSpectrum) we chose Python, a free and open source programming language that offers and array of machine learning frameworks. One of these is PyTorch, which offers fast, easily trainable, and retrainable models. Training a new model takes a large amount of data and transfer learning can be used to fine tune the model for rat USVs. USVSpectrum extracts single USV calls into images which are used to create the desired categories. These images are manually curated by trained researchers into the desired categories (in the present case, Fixed Frequency, Frequency Modulated, and Trills USVs). USVSpectrum then retrains and selects models to achieve the new classification scheme. Once the model is finalized, USVSpectrum uses the model to classify and count USV calls extracted from wave files for a group of rats. In our latest iteration USVSpectrum has achieved a 96 percent accuracy rate when compared to trained researchers. This is similar to some studies that show inter-rater

reliability between researchers averaging 95 percent. USVSpectrum is still in the development phase and will be released as open source software.

Disclosures: **A. Arango:** None. **K.A. Trujillo:** None. **X. Zhang:** None.

Poster

156. Neural and Behavioral Mechanisms of Addiction: Amphetamine

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 156.23/U24

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH GM 64783
NIH GM 08807

Title: Sensitization to the rewarding effects of D-amphetamine following repeated administration to laboratory rats

Authors: *L. INGRAM, K. TRUJILLO;
California State Univ. San Marcos, San Marcos, CA

Abstract: Dextroamphetamine (D-AMPH) is a popular psychomotor stimulant taken by many teens and adults for its rewarding effects. Repeated use of D-AMPH leads to an increase in its psychomotor effects, known as behavioral sensitization. According to prominent theories, sensitization is responsible for increases in incentive motivation that are key to drug addiction. Ultrasonic vocalizations (USVs) have been validated as a measure of reward in rodents. In particular, 50 kHz USVs are increased by rewarding stimuli, including social interaction, sexual activity and drugs of abuse. Strong increases in 50 kHz USVs are seen following administration of D-AMPH to laboratory rats reflecting its potent rewarding properties. The current study investigated changes in locomotor behavior and USVs following repeated administration of D-AMPH to adult, male Sprague-Dawley rats (n=6-7/group). Locomotor behavior was assessed by a Kinder Scientific Motor Monitor and USVs were recorded by an Avisoft Ultrasoundgate 416H recording device and Avisoft SASLabpro software. It was hypothesized that increases in D-AMPH-induced locomotor stimulation and USVs would develop with repeated administration, reflecting the development of sensitization. Saline or D-AMPH (0.3 or 1.0 mg/kg) was administered every other day for 7 treatment days. Locomotor activity and USVs were assessed concurrently during each test session. Following a one-week washout period, all animals (including the saline-pretreated group) received a challenge injection of D-AMPH (0.3 mg/kg) and the behavioral responses were again assessed. Although, the data analysis is not yet complete, the results currently support the development of sensitization to D-AMPH-induced USVs and locomotor behavior. The results suggest that sensitization develops not only to

amphetamine-induced psychomotor stimulation but also amphetamine reward. The results have implications for D-AMPH abuse and addiction.

Disclosures: L. Ingram: None. **K. Trujillo:** None.

Poster

156. Neural and Behavioral Mechanisms of Addiction: Amphetamine

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 156.24/U25

Topic: G.08. Drugs of Abuse and Addiction

Support: Valley Research Partnership Grant - Phoenix, AZ

Title: Mesolimbic dynamics underlying estrous cycle-dependent differences in social stress-induced amphetamine cross-sensitization in female rats

Authors: *M. L. RUDOLPH[1,2], A. F. AZUMA[2], R. L. NEVE[3], R. P. HAMMER, Jr.[1,2], E. M. NIKULINA[1];
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Abstract: Clinical studies reveal sex differences in drug use; women progress more rapidly from casual drug use to dependence with shorter periods of abstinence and symptoms that are exacerbated by stress. Moreover, ovarian hormones are thought to cause enhanced sensitivity to psychostimulants. Our recent data suggest that AMPA receptor subunit GluA1 in VTA dopamine neurons plays a critical role in the induction of stress-induced amphetamine (AMPH) cross-sensitization in *male* rats, and that females are more sensitive to psychostimulants in proestrus/estrus than in metestrus/diestrus. In this study, female TH-Cre rats were subjected to 20 min of threat and aggression by lactating female Long-Evans rats four times over a 10-day period; control rats were handled concurrently. Vaginal smears were taken daily to determine estrous stages. Behavioral testing was performed 10-14 days after stress termination. Control females in proestrus/ estrus had significantly higher FosB/ΔFosB labeling in nucleus accumbens (NAc) compared to those in metestrus/diestrus ($p<0.05$), while social stress exposure resulted in a trend toward enhanced FosB/ΔFosB labeling in the NAc during proestrus/estrus compared to metestrus/diestrus. Furthermore, social stress increased BDNF receptor TrkB expression in VTA dopamine neurons, with significantly more labeling in females during proestrus/estrus compared to metestrus/diestrus ($p<0.05$), and compared to stressed male rats ($p<0.01$). In addition, social stress induced GluA1 expression in VTA dopamine neurons, as evidenced by a significantly greater density of GluA1/TH double-labeling in VTA neurons of stressed compared to handling females, independent of estrous stage ($p<0.05$). To determine whether GluA1 is necessary for the effects of social stress in female rats, a Cre-dependent pore-dead GluA1 construct packaged in

AAV (AAV-pd-GluA1) was used to reduce GluA1 function in TH neurons. AAV-pd-GluA1 or control AAV-GFP viral constructs were bilaterally infused into the VTA three weeks prior to social stress exposure. Social stress induced cross-sensitization to AMPH challenge (0.5 mg/kg, i.p.) in rats with AAV-GFP, demonstrated by a significant augmentation of locomotion. Conversely, AAV-pd-GluA1 prevented cross-sensitization. Taken together, these results suggest that GluA1 and TrkB receptor expression in VTA dopamine neurons act concomitantly to cause enhanced AMPH cross-sensitization in female rats. Elucidating the reproductive cycle-dependent effects on drug use may provide insight into potential novel pharmacotherapies to prevent stress-induced substance abuse vulnerability in women.

Disclosures: M.L. Rudolph: None. **A.F. Azuma:** None. **R.L. Neve:** None. **R.P. Hammer:** None. **E.M. Nikulina:** None.

Poster

156. Neural and Behavioral Mechanisms of Addiction: Amphetamine

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 156.25/U26

Topic: G.08. Drugs of Abuse and Addiction

Title: Compulsive methamphetamine intake under punishment and striatal ATF2 p53 signaling

Authors: *J. L. CADET, R. SUBU, S. JAYANTHI, M. T. MCCOY, B. LADENHEIM; Nih/nida/Intramural Res., Baltimore, MD

Abstract: Addiction to methamphetamine (METH) is very prevalent. Our laboratory is deciphering the molecular neurobiology of compulsive METH taking behaviors by using a METH self-administration (SA) model. Towards that end, we have been employing a reproducible METH SA model that we developed. This approach uses footshock punishment to dichotomize METH self-administering rats into two phenotypes. Some animals continue to self-administer METH compulsively (shock-resistant, SR) whereas others decrease their consumption (shock-sensitive, SS) in the presence of punishment. This model thus includes two DSM criteria of addiction, namely escalated drug intake and continuous use in the presence of adverse consequences. In the present study, we have found that compulsive METH taking (resistant) rats exhibited increased striatal abundance of phosphorylated activating transcription factor 2 (ATF2) in comparison to shock-sensitive (sensitive) rats. ATF2 is a histone acetyltransferase that add acetyl groups to histones H2B and H4. Once active via phosphorylation, ATF2 can homodimerize or heterodimerize with other AP1 transcription factors in responses to a diverse set of cellular responses that are involved in stress regulation and cell death. Because of the known toxic effects of METH in the striatum, we measured the expression of the apoptotic protein, p53, and found significant increases in p53 protein levels in the dorsal striatum of compulsive METH taking rats in comparison to other groups. Increased p53 was accompanied

by decreased Bcl-2 protein levels. These results indicate that, similar to the observations in post-mortem human basal ganglia, compulsive METH taking is associated with the existence of pathological changes in the brain. Acknowledgement: This work is supported DHHS/ NIH/ NIDA/ IRP.

Disclosures: J.L. Cadet: None. **R. Subu:** None. **S. Jayanthi:** None. **M.T. McCoy:** None. **B. Ladenheim:** None.

Poster

157. Cocaine Relapse

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 157.01/U27

Topic: G.08. Drugs of Abuse and Addiction

Support: RO1DA033479
MUSC 23571

Title: Inhibition of a prelimbic to the posterior paraventricular thalamus pathway reduces anxiety-like behaviors during early withdrawal from cocaine

Authors: *R. A. R. REICHARD, D. M. COSSIO, J. F. MCGINTY;
Med. Univ. of South Carolina, Charleston, SC

Abstract: The high propensity of relapse despite rehabilitation continues to hinder recovery from addiction to cocaine. Environmental and drug-related cues associated with cocaine use as well as stress during early withdrawal from cocaine induce long-lasting changes in brain circuitry. Elucidating the alterations in brain circuitry induced by cocaine abuse is required to develop efficacious treatment strategies. We have shown that during early withdrawal from cocaine, there are dichotomous effects on two distinct projections from the prelimbic (PL) cortex. The projection from PL cortex to nucleus accumbens core (NA core) is suppressed while the projection to the posterior paraventricular thalamus (pPVT), a brain structure implicated in anxiety, is activated. Accordingly, inhibition of the PL-> NA core pathway during early withdrawal has no effect on reinstatement of cocaine seeking. Conversely, inhibition of the PL-> pPVT pathway attenuates context and cue-induced cocaine seeking following abstinence and extinction respectively. However, the mechanism by which inhibition of PL->pPVT neurons during early withdrawal alters subsequent cue-induced relapse is not known and is investigated in this study. We hypothesized that anxiety levels during early withdrawal from cocaine are positively correlated with subsequent relapse and that inhibition of PL-> pPVT neurons reduces relapse by alleviating anxiety during early withdrawal. Rats received combined infusions of the retrogradely transported cre-AAVrg into the pPVT with either AAV-DIO-hM4Di-mCherry or AAV-DIO-mCherry into the PL cortex. Following cocaine self-administration, all rats were

injected with the DREADD ligand, CNO (10 mg/kg) immediately after the last SA session and were zero maze tested two hours later. The number of open arm entries and time were statistically compared between groups. The correlation between anxiety level and relapse of cocaine seeking was assessed following one week of forced abstinence during a two-hour post-abstinence cue test. Early withdrawal from cocaine induced an anxiogenic behavioral state indicated by decreased open arm time and entries compared to yoked saline controls and inhibition of the PL-> pPVT pathway alleviated anxiety associated with cocaine withdrawal. This reduction in anxiety-like behaviors during early withdrawal corresponded to reduced relapse of cocaine seeking during the cue test. These results indicate that relapse is directly correlated to increased anxiety during early withdrawal and that the cocaine-induced anxiogenic state is mediated by activation of the PL-> pPVT pathway.
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Disclosures: R.A.R. Reichard: None. **D.M. Cossio:** None. **J.F. McGinty:** None.

Poster

157. Cocaine Relapse

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 157.02/U28

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA 2 R01 DA025646
F31 DA045430

Title: Distinct roles of basolateral amygdala anandamide and 2-arachidonoylglycerol signaling on cocaine-memory reconsolidation in male and female rats

Authors: *J. L. WALTERS, J. R. WANG, J. L. RITCHIE, J. A. HIGGINBOTHAM, T. A. BROWN, C. K. IBARRA, J. N. ROLAND-MCGOWAN, R. A. FUCHS;
Washington State Univ., Pullman, WA

Abstract: Exposure to an environmental context previously paired with the motivational effects of cocaine can induce intense drug cravings and trigger relapse. This phenomenon depends on the retrieval of long-term memories of contextual-drug associations. Following retrieval, these memories become labile and must undergo protein-synthesis dependent reconsolidation into long-term memory stores in order to be retained. Disrupting drug-memory reconsolidation can reduce subsequent drug-seeking-behavior in rodent models of drug relapse. Endocannabinoids (eCBs) are key regulators of synaptic transmission and neuroplasticity, and eCBs within extra-basolateral amygdala (BLA) brain regions regulate aversive and appetitive memory reconsolidation. Nonetheless, the contribution of BLA eCBs to cocaine-memory reconsolidation

(MR) has not been investigated. Our laboratory has recently shown that microinfusions of the cannabinoid type 1 receptor (CB1R) inverse agonist, AM251, in the BLA enhance MR. This suggests that eCBs may regulate MR, yet the impact individual eCBs have on MR remains unclear. Here, we explored the role anandamide (AEA) and 2-arachidonoylglycerol (2-AG) signaling in the BLA plays during cocaine MR in a rodent model of drug context-induced relapse. Male and female Sprague-Dawley rats were trained to lever press for cocaine infusions (i.v.) in a distinct context. Once acquisition criteria were reached, this behavior was extinguished in an alternative context. Rats were then re-exposed to the drug-paired context for 15 min to reactivate contextual drug memories and trigger MR. Immediately after MR or 6 h later (controls), rats received bilateral intra-BLA microinfusions of the fatty acid amide hydrolase (AEA degradative enzyme) inhibitor, URB597 (1 µg/side), the diacylglycerol lipase (2-AG synthetic enzyme) inhibitor, DO34 (1.7µg/side), the monoacylglycerol lipase (2-AG degradative enzyme) inhibitor, JZL184 (1, 0.1 µg/side), or their vehicles. Rats then received additional extinction sessions until extinction criteria were reached and were subsequently placed into the drug-paired context for a 2-h test session during which lever presses were not reinforced. URB597 failed to alter, whereas JZL184 attenuated and DO34 enhanced, MR in male rats. Preliminary results suggest sex differences in these effects. These findings indicate that alterations in BLA 2-AG, but not AEA, signaling during reconsolidation bidirectionally regulate cocaine memory strength and/or subsequent drug context-induced motivation for cocaine in male rats.

Disclosures: **J.L. Walters:** None. **J.R. Wang:** None. **J.L. Ritchie:** None. **J.A. Higginbotham:** None. **T.A. Brown:** None. **C.K. Ibarra:** None. **R.A. Fuchs:** None. **J.N. Roland-McGowan:** None.

Poster

157. Cocaine Relapse

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 157.03/U29

Topic: G.08. Drugs of Abuse and Addiction

Support: PSI2013-44901-P
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Universidad de Málaga
CD12/00455
FPU13/04819
FPU14-01610

Title: More adult-born dentate gyrus neurons to weaken cocaine-related retrograde memories: An *in vivo* strategy employing exogenous lysophosphatidic acid

Authors: D. LADRÓN DE GUEVARA-MIRANDA[1], R. MORENO-FERNÁNDEZ[1], S. GIL-RODRÍGUEZ[1], C. ROSELL-VALLE[2], G. ESTIVILL-TORRÚS[2], A. SERRANO[2], F. PAVÓN[2], F. RODRÍGUEZ DE FONSECA[2], *L. J. SANTÍN[1], E. CASTILLA-ORTEGA[2];
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Abstract: The post-training enhancement of adult hippocampal neurogenesis (AHN) has been receiving growing interest as a potential method to manipulate retrograde memories. Recent hypothesis suggest that the addition of adult-born dentate granule cells might promotes remodeling of pre-existing hippocampal circuits, which might both clear cocaine-related memories and facilitate the learning of new adaptive information. Here, we study the effect of stimulating AHN in vivo with exogenous lysophosphatidic acid (LPA, a biolipid with neurogenic properties) on the maintenance of retrograde cocaine-contextual associative memories. For this purpose, male C57BL/6J mice (N = 28) trained in a cocaine-induced Conditioned Place Preference (CPP) model were later submitted to repeated intracerebroventricular (i.c.v.) injections of LPA, Ki16425 (a selective LPA_1 receptor antagonist) or vehicle solution (bovine serum albumin, BSA) during withdrawal. Afterwards, the long-term persistence of the cocaine-CPP was assessed and the mediational role of AHN in this process was evaluated statistically. In an additional experiment, wild-type (n = 14) and mice lacking the LPA_1 receptor (maLPA_1-null; n = 14) received a single i.c.v. injection of LPA, Ki16425 or BSA in order to assess the implication of the LPA_1 receptor in the LPA-induced increase of AHN in vivo. Our results revealed that the chronic administration of exogenous LPA during withdrawal decreased the retention of a previously acquired cocaine-induced CPP. Furthermore, this effect was mediated by an LPA-induced increase in the number of adult-born dentate granule cells. In contrast, mice repeatedly treated with Ki16425 showed reduced cocaine-CPP retention, but they abnormally increased their preference for the cocaine-paired compartment throughout CPP extinction. Besides, no effects of Ki16425 on AHN were found. Immunohistochemical studies suggested that LPA stimulated cell proliferation and promoted neuronal maturation with a key role of the LPA_1 receptor. Taken together, these findings emphasize the relevance of LPA and its LPA_1 receptor as an in vivo modulator of AHN and the utility of the post-training increase of adult-born hippocampal neurons to weaken cocaine-context associations. This strategy could contribute to addiction therapy by promoting the maintenance of abstinence from cocaine and improve therapeutic success.

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Poster

157. Cocaine Relapse

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 157.04/U30

Topic: G.08. Drugs of Abuse and Addiction

Support: T32 DA007244
DHHS R01DA041455

Title: The effect of PARP-1 inhibition on cue-primed reinstatement to cocaine seeking

Authors: *E. A. WILLIAMS[1], K. J. REISSNER[2];
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Abstract: Interventions to effectively treat relapse vulnerability associated with psychostimulant use disorders are still needed. Previous studies in our lab found that systemic i.p. administration of nicotinamide (NAM), the main niacin-derived NAD+ precursor, PARP-1 inhibitor, and free radical scavenger, reduces cue-primed but not cocaine-primed reinstatement in male but not female Sprague-Dawley rats; however, the mechanism(s) responsible for this effect is unclear. Evidence suggests that increasing available NAM may oppose cellular adaptations that mediate cocaine seeking in a preclinical rodent model of addiction. As mentioned, NAM functions as an inhibitor of poly(ADP-ribose) polymerase 1 (PARP-1). PARP-1 activity and expression is upregulated by cocaine self-administration and is implicated in behaviors associated with cocaine reward. For example, overexpression of PARP-1 increases cocaine conditioned place preference (CPP) and cocaine self-administration (Scobie et al., 2014) while delivery of a PARP-1 inhibitor, PJ34, to the central nucleus of the amygdala (CeA) reduces cocaine CPP (Lax et al., 2017). Given the specific contribution of the CeA in cue-primed reinstatement as well as NAM's function as a PARP-1 inhibitor, the goal of this project was to test the hypothesis that the PARP-1 inhibitor, PJ34, would inhibit cue-primed reinstatement to cocaine seeking. In addition, given the sex-dependent effects of NAM on reinstatement as well as evidence in the literature suggesting sex-dependent differences in PARP-1 activity, we sought to investigate the impact of PJ34 on reinstatement in both male and female rats. Sprague-Dawley rats were trained to self-administer i.v. cocaine for 2 hrs per day for 12 days followed by 14-17 days of extinction. Rats were then tested on cue-primed reinstatement as well as locomotor activity. Following the final extinction session, as well as 30 minutes prior to the reinstatement session and locomotor activity sessions, animals were given bilateral microinjections of either PJ34 or vehicle to the CeA. Additional studies designed to investigate parallel mechanisms by which chronic NAM administration may oppose responsiveness to drug paired cues are underway.

Disclosures: E.A. Williams: None. **K.J. Reissner:** None.

Poster

157. Cocaine Relapse

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 157.05/U31

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH and DA 040965

Title: Pre- and post-reactivation perineuronal net removal differentially alters cue-reinstatement response in cocaine self-administering rats

Authors: *J. WINGERT[1], J. H. HARKNESS[2], A. E. GONZALEZ[3], R. P. TODD[3], B. A. SORG[4];
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Abstract: Repeated exposure to cocaine can lead to the formation of persistent drug memories. Activation of these drug memories are a motivating force behind drug seeking behavior. One of the important brain structures for cocaine-induced drug seeking behavior and memory is the medial prefrontal cortex (mPFC). Here we investigated the effects of perineuronal net (PNN) removal in the mPFC with chondroitinase-ABC (Ch-ABC) injected pre- or post-memory reactivation session on the reconsolidation of a cocaine-associated memory in self-administering rats. Male rats were trained to lever press for cocaine on a fixed ratio 1 (FR1) schedule of reinforcement for 10 days. One cohort of animals received intracranial Ch-ABC 3 days prior to the reactivation session (pre-reactivation) on the last day of training while the other received Ch-ABC by 90 min after the reactivation session (post-reactivation). Rats were given a 30 min memory reactivation session on either an FR1 or a novel variable ratio 5 (VR5) schedule of reinforcement for pre-reactivation-treated animals and on a VR5 schedule for post-reactivation animals. The next day, rats were tested for memory reconsolidation by measuring lever-pressing behavior for 30 min under extinction and then 30 min during cue-reinstatement conditions. We hypothesized that a novel reactivation session was necessary to induce updating of habituated self-administration drug memories and, in turn, make the memory susceptible to weakening in the absence of PNNs. It was also hypothesized that Ch-ABC given either pre- or post-reactivation would not impact its ability to block reconsolidation. Ch-ABC did not affect the extinction in any conditions; however, Ch-ABC reduced cue reinstatement in the pre-reactivation Ch-ABC cohort when memory was reactivated by the VR5 (but not the FR1) session, indicating that memory is reconsolidated only when a novel reactivation session is used. However, Ch-ABC significantly increased cue-reinstatement in the post-reactivation Ch-ABC cohort. Our

results suggest that PNNs in the mPFC may be a target for novel therapies in cocaine addiction, but further exploration into the time-dependent differences in behavioral outcome are necessary.

Disclosures: J. Wingert: None. **J.H. Harkness:** None. **A.E. Gonzalez:** None. **R.P. Todd:** None. **B.A. Sorg:** None.

Poster

157. Cocaine Relapse

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA Grant R00DA037271

Title: Effect of adolescent formed context-drug-associations on drug-seeking behavior: Use of abbreviated self-administration paradigm

Authors: *J. GERENA, A. BAL, D. I. OLEKANMA, B. R. CHO, A. A. ARGUELLO; Psychology, Michigan State Univ., East Lansing, MI

Abstract: Drug-use disorders are characterized by the risk of repeated relapse following periods of abstinence. Exposure to previously drug-paired cues and environmental stimuli can precipitate a return to drug-taking and -seeking behaviors. Adolescent initiation of drug use is associated with the continued risk of abuse and relapse throughout the lifespan. Previous research has shown increased drug-primed and stress-induced reinstatement of drug-seeking behavior in rats that initiated cocaine self-administration during adolescence (compared to adult onset). However, the role of drug-context-associations formed during adolescence to elicit drug-taking and reinstatement behavior during the adolescent period is underexplored. Previously we determined that adolescent drug-context-associations led to an increase in drug-seeking behavior during adulthood, however, to understand how the reinstatement process may differ during adolescence, we developed an abbreviated self-administration paradigm. Additionally, we examined the role of single- vs. pair-housed conditions on drug-taking and -seeking behaviors during adolescence. Male, sprague-dawley rats (postnatal day 25 (P25) upon arrival), received jugular catheterization surgery at P33-34. Following surgery and recovery, rats were either single- or pair-housed. At P39, rats began self-administration training (distinct contextual environment, FR1 schedule of reinforcement, 2-hr session, 2x/day, minimum of 10 sessions) followed by extinction training (alternate distinct context, 1-hr session, 4x/day, minimum of 8 sessions). Drug-seeking behavior (i.e. active lever responses) was examined during a 2-hr reinstatement test within the original drug-paired context. Adolescent rats (P48) displayed an increase in drug-seeking behavior upon re-exposure to the drug-paired context with no differences between single- and pair-housed conditions.

Future directions will determine whether an increase in contextually-induced, drug-seeking behavior during adolescence correlates with increased activation of prefrontal cortical regions (i.e. quantification of cfos protein via immunohistochemistry). An additional group of rats will undergo reinstatement testing in a novel context, to test whether increased drug-seeking behavior is specific to the drug-paired context.

Disclosures: J. Gerena: None. **A. Bal:** None. **D.I. Olekanma:** None. **B.R. Cho:** None. **A.A. Arguello:** None.

Poster

157. Cocaine Relapse

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 157.07/U33

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA Grant R00DA037271

Title: Cfos protein induction following cue-induced reinstatement of cocaine-seeking behavior: Automated analysis with SimpylCellCounter (SCC)

Authors: *A. BAL, J. GERENA, D. I. OLEKANMA, A. A. ARGUELLO;
Psychology Dept., Michigan State Univ., East Lansing, MI

Abstract: Cfos protein is an immediate early gene product that is upregulated in response to various stimuli. The analysis of Cfos-immunoreactive (IR) cells can provide valuable information about specific brain regions and cell types that are differentially activated. While Cfos-IR cell quantification has been performed extensively, data analysis is often time-intensive, requiring manual curation of microscopic images which can result in increased variability. Currently, the most widely-used, open-sourced, automated cell quantification methods include: OpenColonyFormingUnit (OpenCFU), ImageJ Edge Detection Macro, and CellProfiler, which are ideal for microbiological analyses such as quantification of cell colony number. However, in brain tissue, it is a challenge to utilize current automated quantification methods given that Cfos-IR cells can vary in both shape and stain intensity. Therefore, we developed a pipeline called SimpylCellCounter (SCC) to facilitate the automated analysis of Cfos-IR cells and other immunohistochemically-stained cells. SCC is a highly-flexible, time-optimized algorithm that is built on the computer vision package Open Source Computer Vision Library (OpenCV) and follows a processing chain of three functions: 1) thresholding, 2) morphology alteration and 3) pixel dilation. To test the feasibility of using SCC to quantify Cfos-IR cells in brain tissue, we analyzed a data set from rats that underwent a self-administration-extinction-reinstatement paradigm where cue-induced drug-seeking behaviors resulted in increased Cfos-IR cells in the orbitofrontal cortex. Tissue sections that had been colorimetrically stained for Cfos protein were

imaged at 20X magnification and over 600 images were quantified manually. The same images were then run through the SCC processing chain and automated counts were obtained. Statistical analysis revealed no differences between manual and SCC counts, indicating that SCC is an accurate, time-saving automated method to analyze Cfos-IR cells. Future directions will determine whether SCC can be used to analyze cell counts at variable magnifications and with images resulting from viral expression of fluorescent reporter proteins.

Disclosures: **A. Bal:** None. **J. Gerena:** None. **D.I. Olekanma:** None. **A.A. Arguello:** None.

Poster

157. Cocaine Relapse

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 157.08/U34

Topic: G.08. Drugs of Abuse and Addiction

Support: R00DA042895
T32DA007234

Title: Hierarchical cue control of cocaine seeking in the face of cost

Authors: *A. L. COLLINS, B. T. SAUNDERS;
Dept. of Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Drug addiction is characterized by intermittent, persistent drug seeking despite rising costs. Drug-associated cues are a powerful trigger of this behavior, capable of inciting relapse in recovering addicts. We set out to model three key aspects of human drug use in rats: the intermittent, binge-and-stop nature of drug intake, the motivational conflict of drug seeking in the face of escalating negative costs, and the ability of different types of drug cues to modulate seeking and spur relapse. We utilized an intermittent access paradigm, wherein rats were trained to self-administer cocaine during brief drug available periods that are interspersed with longer epochs of no drug availability, within the same session. The drug available periods were signaled by a change in “global” cues comprising the context of the chamber. During these periods, transient “proximal” cues were presented contingent with a drug seeking response and coincident with each cocaine infusion. Following this, rats underwent a “conflict” phase, wherein they had to overcome a cost (crossing an electrified floor barrier) in order to continue to use cocaine. This cost escalated between sessions until drug seeking was suppressed. By comparing intermittent access and conflict behavior, we found that greater cocaine binging history predicted persistence in the face of higher drug seeking cost. Following two weeks of abstinence, we next assessed the ability of the proximal cues to trigger relapse despite cost. Proximal cues were presented noncontingently in the presence or absence of global cues that had signaled drug availability during intermittent access. Critically, we found that the ability of proximal cues to trigger relapse

was gated by the presence of global drug available cues, suggesting that hierarchical cue interactions exert an important modulating influence on drug-seeking motivation.
Dopamine release within the nucleus accumbens core (NAc) has been implicated in cue-induced relapse of drug seeking. It is less clear, however, if dopamine signaling may encode hierarchical drug-related learning states where drug cues interact to guide seeking. To address this, we measured changes in dopamine receptor activity within the NAc core with fiber photometry, using the genetically encoded dopamine sensor dLight. Our preliminary data suggests that the dopaminergic signaling profile in the NAc core changed throughout intermittent access, as rats learned to pattern their intake in response to global signals of drug availability. Together these results demonstrate hierarchical cue control of drug seeking despite cost, and point to a role for NAc core dopamine in this process.

Disclosures: A.L. Collins: None. **B.T. Saunders:** None.

Poster

157. Cocaine Relapse

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 157.09/U35

Topic: G.08. Drugs of Abuse and Addiction

Title: How reinforcing is bupropion? Comparison with methylphenidate and cocaine by intravenous self-administration in rats

Authors: *S. L. SMITH, Z. TURNBULL, H. DAY, D. HEAL;
Renasci Ltd, Nottingham, United Kingdom

Abstract: Bupropion is a dopamine reuptake inhibitor that is used clinically to treat nicotine dependence. Bupropion is not a controlled drug (CD), but there are recent reports that this compound is occasionally subjected to human abuse (Stassinos & Klein-Schwartz, 2016, J Addict Med 10: 357-62). There are very few published studies describing the reinforcing effects of bupropion in animals and none to our knowledge that have systematically explored its reinforcing properties in rats. Therefore, we have investigated the reinforcing properties of bupropion compared with methylphenidate and cocaine (both C-II CDs) in an intravenous self-administration (IVSA) experiment.
Mildly food-restricted, male, Sprague-Dawley rats were initially trained to lever-press for food rewards before being surgically implanted with in-dwelling jugular vein catheters. Rats were allowed to selfadminister cocaine (360µg/kg/inj) on a fixed ratio (FR5) schedule of reinforcement in 2hr training sessions. After establishment of consistent cocaine selfadministration, the rats were subjected to saline extinction. The reinforcing effects of bupropion (30, 100 or 300µg/kg/inj [Group 1]) and methylphenidate (10, 30 or 100µg/kg/inj [Group 2]) were then evaluated on a FR5 reinforcement schedule in 2hr sessions. Intake was

limited during acquisition, extinction and testing to 20inj/session. Results are mean±SEM. Cocaine maintained selfadministration in rats (19.8±0.1inj/session, n = 10 [Group 1]; 20.0±0.1inj/session, n = 10 [Group 2]) at levels significantly greater ($p<0.001$) than saline (4.5±0.4 inj/session [Group 1]; 4.4±0.5inj/session, [Group 2]). Bupropion (30, 100 or 300µg/kg/inj) dose-dependently maintained self-administration (number of inj/session) at levels significantly greater than saline (30µg/kg/inj = 11.1±2.4; 100µg/kg/inj = 20.0±0.0; 300µg/kg/inj = 20.0±0.0, n=6/dose, all $p<0.001$). Selfadministration of bupropion was essentially identical to that of methylphenidate (10, 30 or 100µg/kg/inj), i.e. 10µg/kg/inj = 9.0±2.5, $p<0.05$; 30 µg/kg/inj=19.3±1.4, $p<0.001$; 100 µg/kg/inj = 20.0±0.0, $p<0.001$; all n=6/dose.
Bupropion served as a surprisingly strong, positive reinforcer in cocaine-maintained rats across a 10-fold dose range. Bupropion maintained self-administration levels on FR5 identical to the C-II stimulants, methylphenidate and cocaine. This result warrants further investigation of bupropion's relative reinforcing effect by break-point determination on a progressive ratio schedule.

Disclosures: **S.L. Smith:** None. **Z. Turnbull:** None. **H. Day:** None. **D. Heal:** None.

Poster

157. Cocaine Relapse

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 157.10/U36

Topic: G.08. Drugs of Abuse and Addiction

Title: Differences in the relative contributions of drug re-exposure / salient cues in relapse tococaine and heroin seeking

Authors: *D. J. HEAL, S. SMITH;
RenaSci Ltd, Nottingham, United Kingdom

Abstract: Relapse is a major problem in treating substance use disorders. It is known that reinstatement of drug seeking can be initiated by salient cues associated with abuse, drug re-exposure, or a combination of both. What is less clear is (i) the relative contribution of these triggers to initiating relapse, and (ii) whether their contributions are the same for different substances of abuse.
Two groups of mildly food-restricted, male Sprague-Dawley rats with jugular vein catheters were allowed to leverpress either for cocaine (0.36mg/kg/injection [inj]) or heroin (0.015mg/kg/inj) on a FR5 schedule of reinforcement in daily 2hr sessions. Each drug infusion was paired with contingent tone+light cues. After acquisition of stable cocaine and heroin self-administration, the active lever-press responses of the rats were extinguished on saline (FR5 without cues). In a 2hr reinstatement session, rats were presented with (i) salient cues+drug re-exposure (cocaine, 1.0mg/kg iv or heroin 0.25 mg/kg sc), (ii) salient cues alone, and (iii) drug re-

exposure alone. Presses on the active (drug-paired) lever were measured with rats receiving intravenous infusions of saline on a FR5 schedule. Results are reported as mean active lever-presses±SEM.
Cocaine and heroin both served as powerful reinforcers: cocaine (142.4±7.7, n=18) $p<0.001$ versus saline (16.7±1.2, n=18); heroin (240.6±50.1, n=12) $p<0.001$ versus saline (25.7±2.4, n=12). Relapse to drug-seeking was initiated by cues+drug re-exposure (cocaine: 104.4±15.1, n=8; heroin: 166.6±25.7, n=6; both $p<0.001$ versus respective saline extinction value), drug re-exposure alone (cocaine: 59.7±11.8, n=9; heroin: 131.2±39.6, n=6; $p<0.001$ and $p<0.01$ versus saline, respectively). However, cues alone induced reinstatement to cocaine, but not heroin seeking (cocaine: 60.0±14.9, n=10; heroin: 39.3±7.1, n=6; $p<0.001$ and non-significant versus saline, respectively). The effects of the contingent cues and drug re-exposure were approximately additive in the reinstatement of cocaine and heroin seeking. However, their relative contributions were very different. Thus, the effects of drug re-exposure and salient cues were equal in the reinstatement of cocaine seeking, but in the case of relapse to heroin seeking the effect of drug re-exposure was 3x greater than that of the salient cues ($p<0.05$).
These findings reveal that although cocaine and heroin both serve as powerful reinforcers which induce a high degree of psychological dependence, re-exposure to the reinforcing effect of the drug is a much more important factor in heroin relapse than it is for cocaine.

Disclosures: D.J. Heal: None. **S. Smith:** None.

Poster

157. Cocaine Relapse

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 157.11/U37

Topic: G.08. Drugs of Abuse and Addiction

Support: Prestamo BID PICT 2015-1622
Prestamo BID PICT 2012-1867
CONICET
SECyT

Title: CB1 receptor antagonism inhibits stress-induced enhancement of extracellular glutamate in nucleus accumbens core after extinction of cocaine-conditioned place preference

Authors: *A. S. GUZMAN, M. P. AVALOS, P. V. EULIARTE, M. A. SANCHEZ, D. RIGONI, F. A. BOLLATI, M. B. VIRGOLINI, L. M. CANCELA;
Dept. de Farmacologia. Facultad de Ciencias Quimicas. UNC, IFEC - CONICET, Cordoba, Argentina

Abstract: Stress is considered an important factor that induces relapse in human addicts and in animal models of addiction. Findings from our lab have demonstrated pharmacologically the role of the cannabinoid CB1 receptors within Core, but not Shell, subregion of nucleus accumbens (NAc) in restraint stress-induced reinstatement of extinguished cocaine- conditioned place preference (CPP). Given the well-established role of glutamatergic transmission within NAc Core in reinstatement of cocaine seeking, we evaluated the effects of AM251, a highly selective CB1 receptor antagonist, on stress-induced changes in extracellular glutamate levels within NAc Core under reinstatement conditions. *In vivo* microdialysis experiment in male Wistar rats, combined with high-performance liquid chromatography and electrochemical detection was used. Firstly, our results demonstrated that a reinstating stress session (30 min of restraint) induced an increase in extracellular glutamate levels within NAc Core in animals that were re-exposed to the drug-paired compartment after extinction of cocaine-CPP, while the 'unpaired group' and 'no stress group' of animals did not show such glutamate enhancement. Moreover, we found that microinjection of AM251 (10 ug/ul) directly into NAc Core, inhibited the observed stress-induced increase of glutamate in the same area. These data suggest that accumbal microinjection of AM251 prevents stress-triggered reinstatement of cocaine-CPP by inhibiting the context-specific enhancement of NAc glutamate after restraint stress. This study provides neurochemical basis to investigate the *in vivo* mechanisms underpinning the involvement of CB1 receptors within NAc Core in stress-induced reinstatement.

Disclosures: A.S. Guzman: None. **M.P. Avalos:** None. **P.V. Euliarte:** None. **M.A. Sanchez:** None. **D. Rigoni:** None. **F.A. Bollati:** None. **M.B. Virgolini:** None. **L.M. Cancela:** None.

Poster

157. Cocaine Relapse

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 157.12/U38

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA033436

Title: Ceftriaxone attenuates activity in the BLA and VTA during cue-primed seeking of cocaine after abstinence

Authors: *J. R. MESA[1], V. HODGES[2], C. N. LOGAN[2], A. R. BECHARD[3], L. A. KNACKSTEDT[1];
[1]Psychology, [2]Univ. of Florida, Gainesville, FL; [3]SUNY Geneseo, Geneseo, NY

Abstract: Cocaine use disorder is a chronic condition marked by a strong tendency for relapse. In rodent models of self-administration and relapse, the beta-lactam antibiotic ceftriaxone attenuates cue- and cocaine-induced reinstatement to cocaine seeking. Ceftriaxone restores

glutamate homeostasis in the nucleus accumbens core (NAc) by normalizing cysteine-glutamate exchange and upregulating the transporter GLT-1. The upregulation of GLT-1 was previously believed to account for the ability of ceftriaxone to prevent glutamate efflux during cocaine relapse. However, our lab has demonstrated that GLT-1 overexpression in the NAc is not sufficient to prevent the reinstatement of cocaine-seeking. Thus, we are exploring other mechanisms by which ceftriaxone attenuates glutamate efflux in the NAc. Here, we investigate the ability of ceftriaxone to reduce neuronal activity in regions sending glutamate projections to the NAc. Male Sprague Dawley rats underwent cocaine self-administration for 12 days followed by cue-induced reinstatement to cocaine seeking after 21 days of abstinence. Rats were perfused immediately following the reinstatement testing and Fos immunohistochemistry conducted on regions that project to the NAc. A second set of rats received Cholera Toxin b (CTb) infusions in the NAc. Thereafter, they underwent 12 days of cocaine self-administration, followed by 21 days of abstinence, and a cue-primed relapse test. Rats were perfused and the number of Fos+ CTb-labeled cells were quantified. We found that ceftriaxone reduced Fos expression in the VTA, BLA, and NA shell, but not the infralimbic or prelimbic cortices. Thus, in addition to altering glutamate uptake and reuptake in the NAcore, ceftriaxone also attenuates neuronal activity in regions that send glutamate projections to the NAcore.

Disclosures: **J.R. Mesa:** None. **V. Hodges:** None. **C.N. Logan:** None. **A.R. Bechard:** None. **L.A. Knackstedt:** None.

Poster

157. Cocaine Relapse

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 157.13/U39

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R00DA039991

Title: Identification of brain regions selectively activated during psychosocial stress-induced cocaine seeking in rats

Authors: I. D. WOJTAS, ***D. F. MANVICH**;
Cell Biol. and Neurosci., Rowan Univ. Sch. of Osteo. Med., Stratford, NJ

Abstract: A prominent feature of cocaine abuse is the high frequency of relapse events that occur even following prolonged periods of abstinence. Stress-induced relapse is frequently modeled in experimental animals using reinstatement procedures which employ physical or pharmacological stressors, however it has been suggested that psychosocial stressors are the predominant stressor modality that elicits drug craving and relapse in human substance abusers. This study aims to determine whether psychosocial stressors produce drug seeking via similar or

distinct neural circuits as compared to a physical stressor, footshock. Three groups of adult Long-Evans rats (n=6/group) were trained to self-administer 0.5 mg/kg/inf cocaine i.v. under a fixed-ratio 1 schedule of reinforcement in daily 2 hr sessions for 20 d. On days 11, 14, 17, and 20, subjects were removed from the chamber immediately following the session and subjected to one of three events: group 1, social defeat stress (SDS) using a resident-intruder procedure; group 2, intermittent footshock (FS; 15 min, 0.5 mA); group 3, a no-stressor control condition. On these days, discrete environmental stimuli presented within the chamber during self-administration signaled the impending event after the session. For all subjects, extinction training began on day 21. Once responding was extinguished, rats were re-exposed to the discrete cues that previously signaled their respective impending event and were allowed to lever-press under extinction conditions to assess cocaine-seeking behavior for 2 hr. Immediately after this test session, subjects were sacrificed and brains extracted and processed for c-Fos immunohistochemistry as a marker of neuronal activation. Re-exposure to SDS-predictive cues or FS-predictive cues induced significant reinstatement of cocaine seeking, whereas cues predictive of the no-stress control condition did not. Preliminary results of c-Fos expression analyses indicate that while components of canonical reward and stress circuits exhibited activation during stress-induced cocaine seeking in groups 1 and 2, SDS-exposed animals selectively showed activation of specific components of a hypothalamic “defensive” circuit that has not previously been associated with addiction processes. Our results are in agreement with previous findings that different stressors activate distinct neural stress-coping circuits and suggest the possible involvement of brain areas associated with social stress reactivity in drug-seeking behaviors. Assessment of a causal role for these regions in mediating psychosocial stress-induced cocaine seeking is currently underway.

Disclosures: D.F. Manvich: None. **I.D. Wojtas:** None.

Poster

157. Cocaine Relapse

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 157.14/U40

Topic: G.08. Drugs of Abuse and Addiction

Support: R00 DA040004
T32 DA007288

Title: Cue-induced reinstatement of cocaine seeking elevates glutamate and nitric oxide in the nucleus accumbens core in a pathway-specific manner

Authors: *B. M. SIEMSEN, J. MCFADDIN, M. LEATH, K. HAIGH, M. D. SCOFIELD; Med. Univ. of South Carolina, Charleston, SC

Abstract: Drug addiction is an insidious neuropsychiatric disorder characterized by high relapse rates to drug use, despite extended periods of abstinence. Preclinical findings indicate a role for dysfunctional glutamate transmission between the prelimbic (PL) cortex and nucleus accumbens core (NAcore) in cue-induced craving that drives relapse. Specifically, cue-induced reinstatement of cocaine seeking in rats, a model for craving following presentation of drug-associated stimuli, relies on increased extracellular glutamate in the NAcore. Recently, a role for cue-induced glutamate overflow and the activation of nNOS interneurons and, presumably, the subsequent release of nitric oxide (NO) in the NAcore has been shown to be critical for the induction of NAcore synaptic plasticity mediating relapse. However, it has yet to be determined whether cue-induced cocaine seeking in rats elevates extracellular NO, and how elevated NO is related temporally to cue-induced glutamate release. Here we used second-by-second measurements of glutamate and NO in the NAcore of freely-moving rats during cue-induced reinstatement of cocaine seeking using a wireless amperometry strategy, in conjunction with pathway-specific inhibitory DREADDs. Glutamate-oxidase (Glu-Ox)-coated electrodes show a high sensitivity for glutamate relative to common interferents such as ascorbic acid, whereas electrodes lacking Glu-Ox show high sensitivity and selectivity to NO relative to dopamine, glutamate, and ascorbic acid. We found that cue-induced reinstatement elevates glutamate and NO in a time-dependent manner. When comparing glutamate and NO in cocaine SA rats, we found that cue-induced glutamate precedes the induction of NO release. Preliminary data indicates that inhibition of PL cortex-NAcore neurons prevents cue-induced glutamate and cocaine seeking, and experiments are underway to examine pathway-specific regulation of cue-induced NO release. Our data indicate that cue-induced NO release occurs likely as a consequence of increased glutamate transmission in the NAcore, and that the PL cortex is likely the primary driver of this effect.

Disclosures: B.M. Siemsen: None. **J. McFaddin:** None. **M. Leath:** None. **K. Haigh:** None. **M.D. Scofield:** None.

Poster

157. Cocaine Relapse

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 157.15/V1

Topic: G.08. Drugs of Abuse and Addiction

Support: MICINN Grant SAF2013-49076-P
MICINN Grant SAF2017-85679-R

Title: Dopamine D3 receptor antagonism blocked the mTORC1 pathway downregulation in the dentate gyrus after the reinstatement of cocaine-induced CPP evoked by social stress

Authors: *C. NÚÑEZ, R. GUERRERO-BAUTISTA, J. M. HIDALGO, M. MILANÉS; Pharmacol., Univ. De Murcia, Murcia, Spain

Abstract: The high risk of relapse even after long periods of abstinence is a key feature of substance use disorder. It is well-known that aversive stressful experiences can trigger drug-seeking and drug-taking behaviors. Drugs of abuse, such as cocaine, induce long-term modifications in brain systems mediating reward, motivation, and memory processes through dysregulation of different molecular mechanisms. On the other hand, recent studies indicated that dopamine (DA) D3 receptor (DAD3R) is involved in stress-induced cocaine self-administration and relapse. Lately it has been described that DAD3R can exert some of their biological effects through alternative cAMP-independent signaling pathways, such as Akt-mTORC1 [mammalian (or mechanistic) target of rapamycin (mTOR) complex 1]. A number of studies point out that mTORC1 mediates synaptic plasticity through protein synthesis regulation. Since stress can modulate memory processing, in the present study we evaluated the influence of a selective DAD3R antagonist, SB-277011A (SB), in the possible modifications in mTORC1 pathway in the dentate gyrus (DG) induced by reinstatement of cocaine-induced place preference (CPP) evoked by social stress. For that, C57BL/6 male mice were conditioned with cocaine (25 mg/kg i.p.). All the animals were tested for extinction of CPP twice a week. After suppression of place preference was observed, we evaluated whether administration of SB (12 and 24 mg/kg i.p.) prevented relapse into drug-seeking behavior induced by social stress. Then, brain samples were analyzed by means of western blot and immunofluorescence to determine the expression of phospho (p)-mTOR and the mTORC1 target p-S6 ribosomal protein, respectively. Our results showed that reinstatement of cocaine-induced CPP evoked by social stress was accompanied by a significant decrease in p-mTOR levels in the DG, that was reversed by the DAD3R antagonist administration. In parallel, we observed lower number of p-S6 positive glutamatergic neurons in DG after the reinstatement of cocaine-induced CPP evoked by social stress, and that the administration of SB 30 min before social stress episode prevented that diminution. Our data are in concordance with recent findings revealing that inhibition of S6 phosphorylation boosts neurite outgrowth *in vitro*, which has been related to consolidation of new memories. Further work is needed to unravel the mechanisms underlying drug-associated learning and memory processing in the addicted brain.
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Disclosures: C. Núñez: None. **R. Guerrero-Bautista:** None. **J.M. Hidalgo:** None. **M. Milanés:** None.

Poster

157. Cocaine Relapse

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 157.16/V2

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH grant DA038663 to Mantsch and Hillard

Title: Corticosterone potentiates reinstatement through endocannabinoid-dependent activation of the cortico-accumbens pathway

Authors: *B. J. CONWAY[1], J. R. MCREYNOLDS[1], P. J. GOTTSHALL[1], E. VAN NEWENHIZEN[1], X. LIU[3], Q.-S. LIU[4], C. J. HILLARD[5], J. R. MANTSCH[2];
[1]Biomed. Sci., [2]Marquette Univ., Milwaukee, WI; [4]Dept. of Pharmacol. and Toxicology, [3]Med. Col. of Wisconsin, Milwaukee, WI; [5]Med. Col. Wisconsin, Milwaukee, WI

Abstract: Stress can act as a trigger for relapse but can also increase responsivity to other triggers for drug use. Under certain self-administration (SA) conditions in rats, a stressor administered alone does not reinstate cocaine seeking but potentiates reinstatement to a sub-threshold dose of cocaine. This effect is both corticosterone (CORT)-dependent and CORT is sufficient to reproduce the effects of stress. We have identified the prelimbic subregion (PL) of the medial prefrontal cortex as a key site for CORT actions. Furthermore, we have shown that CORT exerts its actions in the PL through endocannabinoid (eCB) signaling. The cannabinoid type-1 receptors (CB1R) are located on GABAergic interneurons in the PL and bath application of CORT to nucleus accumbens (NAc) core-projecting pyramidal neurons attenuates inhibitory neurotransmission in a CB1R-dependent manner. Thus, we hypothesize that CORT potentiates reinstatement through eCB-dependent increased activation of the cortico-accumbens pathway. To assess the functional necessity of this pathway for CORT-potentiated reinstatement, we used an intersectional DREADD viral approach. The Cre recombinase-dependent Gi-, Gq-DREADD, or control fluorophore was infused into the PL and a retrograde AAV containing Cre recombinase was infused into the NAc core. Rats underwent cocaine SA (0.5/mg/inf; 2 h/day x 14 days) followed by extinction/reinstatement. CNO (1, 2 mg/kg, i.p.) blocked CORT-potentiated reinstatement in rats expressing the Gi-DREADD, but not the control fluorophore, in the cortico-accumbens pathway. Preliminary data suggest that CNO-induced (5 mg/kg, i.p.) activation of the Gq-DREADD in the cortico-accumbens pathway is sufficient to potentiate reinstatement to low-dose cocaine. This it appears that activation of the cortico-accumbens pathway is both necessary and sufficient for potentiated reinstatement. To determine whether CORT-potentiated reinstatement increasesd activation of the cortico-accumbens pathway we used a double label immunohistochemical approach in the PL to quantify co-expression of a retrograde tracer, CTb, that was injected into the NAc core, and the activity marker Fos following CORT-potentiated reinstatement. Our data suggests increased activation of the cortico-accumbens pathway occurs following CORT-potentiated reinstatement, and the contribution of the eCB system to this effect is currently being examined. These findings support the hypothesis that CORT acts in the PL through CB1R-mediated inhibition of GABA to facilitate activation of the cortico-accumbens pathway and potentiate cocaine-seeking reinstatement.

Disclosures: B.J. Conway: None. **J.R. McReynolds:** None. **P.J. Gottshall:** None. **E. Van Newenhizen:** None. **X. Liu:** None. **Q. Liu:** None. **C.J. Hillard:** None. **J.R. Mantsch:** None.

Poster

157. Cocaine Relapse

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 157.17/V3

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA15758
NIH Grant DA038663
NIH Grant DA038663-S1

Title: Prelimbic cortical sex and stress hormone-potentiated reinstatement of cocaine seeking

Authors: *E. M. DONCHECK[1], G. T. LIDDIARD[1], C. D. KONRATH[1], E. M. ANDERSON[1], L. A. URBANIK[1], M. C. DEBAKER[1], X. LIU[2], M. R. HERBST[1], N. J. RADDATZ[1], E. C. VAN NEWENHIZEN[1], J. C. MATHY[1], L. M. BARRON[1], O. VRANJKOVIC[1], E. N. GRAF[1], J. R. MCREYNOLDS[1], J. J. TUSCHER[3], K. M. FRICK[3], M. R. GILMARTIN[1], M. C. HEARING[1], Q.-S. LIU[2], C. J. HILLARD[2], J. R. MANTSCH[1];
[1]Biomed. Sci., Marquette Univ., Milwaukee, WI; [2]Pharmacol. and Toxicology, Med. Col. of Wisconsin, Milwaukee, WI; [3]Psychology, Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: Clinical observations imply that females diagnosed with substance use disorder experience enhanced relapse vulnerability compared to males, particularly within the contexts of stress or peak levels of the primary estrogen 17β-estradiol (E2). We previously demonstrated that stress can potentiate cocaine seeking in male rats and E2 can potentiate cocaine seeking in female rats through prelimbic prefrontal cortical (PrL-PFC) cannabinoid type-1 receptor (CB1R)-dependent mechanisms. The present studies investigated the influence of biological sex on stress-potentiated cocaine seeking, sex and stress hormone interactions, further mechanisms underlying potentiated reinstatement, and sex and stress hormone effects on PrL-PFC synaptic physiology. Initial investigations revealed that, despite comparable self-administration and extinction, females display a lower threshold for cocaine-primed reinstatement than males. Unlike males, footshock stress (15-min) failed to potentiate reinstatement to subthreshold cocaine in females, while restraint stress (15-min) potentiated reinstatement in both sexes. Divergent footshock responding corresponded to sex differences in ultrasonic vocalizations, but not plasma corticosterone (CORT) or defensive behaviors. Systemic stress-level CORT administration (2 mg/kg, ip) reproduced stress-potentiated reinstatement in both sexes, but CORT-potentiation was only observed in females during diestrus and proestrus. As in males, CORT-potentiating effects were localized to the PrL-PFC (50 ng/0.3 µL) and found to be CB1R-dependent (300 ng/0.3 µL). In parallel investigations, proestrus-level E2-potentiated reinstatement required PrL-PFC estrogen receptor-β (ERβ) and g-protein coupled estrogen receptor (GPER) activation. *Ex vivo* whole-cell electrophysiological recording from female layer

V/VI PrL-PFC pyramidal neurons revealed both CORT and E2 suppress inhibitory synaptic activity in a CB1R-dependent manner, and E2 effects additionally required ERβ and GPER activation. In summary, stress and peak physiological E2 superimpose upon the inherently greater relapse vulnerability in females to potentiate reactivity to ordinarily weak triggers in females. Despite sex divergence in stressor responsivity, stress-level CORT reproduces potentiation in an estrous cycle-dependent manner. CORT and E2 regulate PrL-PFC synaptic activity and cocaine seeking in a CB1R-dependent manner, and E2 furthermore acts through PrL-PFC ERβ and GPER. These studies implicate the PrL-PFC as an integration site for hormonal regulation of behavior and highlight the nuanced influence of sex as a biological variable.

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Poster

157. Cocaine Relapse

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 157.18/V4

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant K99DA044331

Title: The rostromedial tegmental nucleus regulates reinstatement of cocaine seeking via input from the prefrontal cortex

Authors: *P. J. VENTO, M. EID, T. C. JHOU;
Neurosci., Med. Univ. of South Carolina, Charleston, SC

Abstract: A major obstacle to recovery from addiction is long lasting and persistent cravings induced by exposure to drug-associated cues. While a variety of abused drugs have been shown to cause plasticity in frontostriatal circuits leading to cue-induced hyperexcitability and pro-drug seeking behavior, relatively less is known regarding neural pathways that suppress cue-induced reward seeking. We have recently shown that the prelimbic prefrontal cortex (PL) contributes to encoding of aversive cues through projections to the rostromedial tegmental nucleus (RMTg), and inactivation of this PL→RMTg pathway causes persistent reward seeking in the face of punishment. Given this role for PL inputs to the RMTg in cue encoding and behavioral inhibition, we hypothesized that stimulation of the PL→RMTg circuit may be particularly

effective in reducing drug seeking. To test this, rats received bilateral PL injections of virus encoding excitatory (Gq) *designer receptors exclusively activated by designer drugs* (DREADDs) and were trained to lever press for intrajugular infusions of cocaine. During once-daily 2 hour sessions, a single lever press on the active lever yielded infusion of cocaine (0.75 mg/kg) and delivery of light and tone cues. After 8-10 days with at least 10 cocaine infusions, rats underwent extinction training during which time lever pressing yielded no programmed consequences. After at least 10 days of extinction training, rats were tested for reinstatement of drug seeking in a 2 hour test in which light and tone cues were restored after active lever presses, but no drug was delivered. Thirty minutes prior to reinstatement testing, rats received bilateral intracranial infusion of either vehicle (0.5% DMSO in saline) or the synthetic DREADDs ligand clozapine-N-oxide (CNO) into PL terminals in the RMTg. We found that stimulation of the PL→RMTg pathway via injection of CNO in rats expressing Gq DREADDs robustly suppressed cue-induced reinstatement. Further, this effect was not better explained by general impairments in locomotion or non-specific suppression of behavior. These findings suggest that connections between the PL and RMTg may be a particularly useful target in suppressing drug seeking.

Disclosures: P.J. Vento: None. **M. Eid:** None. **T.C. Jhou:** None.

Poster

157. Cocaine Relapse

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 157.19/V5

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA037744

Title: Inactivation of prelimbic projections to rostromedial tegmental nucleus enhances cue-induced reinstatement of cocaine seeking

Authors: *A. M. CRUZ[1], H. F. SPENCER[1], T. C. JHOU[2], R. J. SMITH[1];
[1]Psychological and Brain Sciences, Inst. for Neurosci., Texas A & M Univ., College Station, TX;
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Abstract: The prelimbic cortex (PL) has been implicated in the regulation of drug-seeking behavior but has been shown to have a degree of functional flexibility that supports roles in both promoting and suppressing of drug seeking. Within PL we found distinct neuronal subpopulations that project to either the nucleus accumbens core (NAc core) or the rostromedial tegmental nucleus (RMTg). Previous studies have established PL projections to NAc core are necessary for reinstatement of cocaine seeking in rats. Given that RMTg has been implicated in behavioral inhibition, we hypothesized that PL projections to RMTg may suppress drug seeking, indicating that PL-NAc core and PL-RMTg pathways play opposing roles in regulating drug-

seeking. To test this hypothesis, we used a functional disconnection to temporarily disrupt the PL-RMTg pathway. Male Sprague Dawley rats self-administered cocaine during daily 2-hour sessions for 12-15 days, in which lever presses were reinforced by intravenous cocaine infusions (0.2 mg/infusion; fixed ratio 1) paired with light/tone cues. Rats then underwent extinction training for 7-14 days, during which cocaine and cues were no longer available. Reinstatement of drug-seeking behavior was elicited by tone/light cues or cocaine prime (10 mg/kg, i.p.). To temporarily disconnect the PL-RMTg pathway prior to reinstatement, rats received a unilateral microinjection of GABA agonists baclofen/muscimol in PL (1 mM/ 0.1 mM) and a contralateral microinjection of AMPA receptor antagonist NBQX in RMTg (1 mM). We found that functional disconnection of PL-RMTg increased cue-induced reinstatement, as compared to within-subject vehicle control, indicating that this circuit normally plays a suppressive role in cocaine seeking. In contrast, we found that functional disconnection of PL-RMTg had no effect on cocaine-induced reinstatement. Taken together with previous evidence supporting a role for PL projections to NAc core in driving drug seeking, these data indicate that PL projections to RMTg play an opposing role by suppressing drug seeking.

Disclosures: **A.M. Cruz:** None. **H.F. Spencer:** None. **T.C. Jhou:** None. **R.J. Smith:** None.

Poster

158. Hippocampal Function

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 158.01/V6

Topic: H.01. Animal Cognition and Behavior

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NIH R01AG049722
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Title: Advanced age and ketogenic diet have dissociable effects across hippocampal subregions

Authors: *A. HERNANDEZ[1], C. M. HERNANDEZ, III[2], L. M. TRUCKENBROD[1], K. CAMPOS[1], Q. FEDERICO[3], J. L. BIZON[5], S. N. BURKE[4];
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Abstract: As the number of individuals living beyond the age of 65 is rapidly increasing, so is the need to develop strategies to combat the age-related cognitive decline that may threaten independent living. Although the link between altered neuronal signaling and age-related cognitive impairments is not completely understood, it is evident that changes in behavioral function are at least partially due to synaptic dysfunction. Aging is accompanied by well-documented changes in both excitatory and inhibitory synaptic signaling across species. Age-related synaptic alterations, however, are not uniform across the brain with different regions showing unique patterns of vulnerability in advanced age. In the hippocampus, increased activity within the CA3 subregion has been observed across species (Wilson, 2005; Yassa et al., 2011), and this can be reversed with anti-epileptic medication (Bakker et al., 2012). In contrast to CA3, the dentate gyrus shows reduced activity with age and declining metabolic activity (Small et al., 2004). Ketogenic diets have been shown to decrease seizure incidence and severity in epilepsy (reviewed in Martin-McGill et al., 2018), improve metabolic function in diabetes type II (reviewed in Feinman et al., 2015), and to improve cognitive function in aged rats. This link between neuronal activity and metabolism suggests that metabolic interventions may be able to ameliorate synaptic signaling deficits accompanying advanced age. We therefore investigated the ability of a dietary regimen capable of inducing nutritional ketosis and improving metabolism to alter synapse-related gene expression across the dentate gyrus, CA3 and CA1 subregions of the hippocampus. Following 12 weeks of a ketogenic (KD) or calorie-matched standard diet (SD), RT-PCR was used to quantify levels of expression of excitatory and inhibitory synaptic signaling genes within CA1, CA3 and dentate gyrus. While there were no age or diet-related changes in CA1 gene expression, expression levels were significantly altered within CA3 by age and within the dentate gyrus by diet for several genes involved in presynaptic glutamate regulation and postsynaptic excitation and plasticity. These data demonstrate subregion specific alterations in synaptic signaling with age and the potential for a ketogenic diet to alter these processes within the brain in dissociable ways across different structures that are uniquely vulnerable in older animals.

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Poster

158. Hippocampal Function

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Topic: H.01. Animal Cognition and Behavior

Support: DARPA BTO Grant HR0011-17-2-0019

Title: Acute vagus nerve stimulation increases Arc expression in the dentate gyrus of the hippocampus

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Abstract: Vagus nerve stimulation (VNS) is currently an FDA-approved treatment for drug-resistant epilepsy, major depressive disorder, and migraine. Moreover, recent data have indicated that VNS may be beneficial for treating stroke (Kilgard et al., 2018), tinnitus (Tyler et al., 2017), and post-traumatic stress disorder (Noble et al., 2017), as well as for enhancing auditory discrimination (Engineer et al., 2017) and other cognitive functions (see Lamb et al., 2018). While the mechanisms of the clinical utility of VNS are not yet known, it is well established that VNS paired with sensory stimuli enhances cortical plasticity (e.g., Porter et al., 2012; Hulsey et al., 2016; Engineer et al., 2017; Borlan et al., 2018). Thus, it is likely that a potential mechanism of VNS efficacy is the ability of this treatment to modulate the expression of immediate-early genes, which are implicated in plasticity and known to be induced by behaviors associated with new learning. One such immediate-early gene is activity-regulated cytoskeletal (*Arc*) protein, which is involved in homeostatic synaptic scaling and hypothesized to be a nexus point for synaptic dysfunction in cognitive disorders (for review, see Shepherd and Bear, 2011). The current experiment investigated the extent to which VNS can modulate behaviorally-driven *Arc* transcription in the dentate gyrus of the hippocampus while rats explored novel objects in a novel environment. Previous work has shown that VNS modulates activity of locus coeruleus (LC) neurons. Because the LC sends strong projections to the dentate gyrus, which have been shown to augment plasticity (Harley et al., 1989; Harley 1991), we focused our analyses on *Arc* expression in the dentate gyrus. Seventeen male rats (4-7 months old) were implanted with a cuff electrode to stimulate the cervical branch of the vagus nerve. Twelve of these rats were given stimulation while five were sham controls. VNS during novel object exploration resulted in more *Arc* expression in the dentate gyrus compared to sham controls. These data suggest that VNS may promote plasticity in the dentate gyrus to increase the rate at which an animal learns novel information.

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Poster

158. Hippocampal Function

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 158.03/V8

Topic: H.01. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation

Title: Targeted hippocampal GABA neuron ablation produces hippocampal sclerosis, epilepsy, and dissociable effects on the Morris water maze and object-place paired association tasks

Authors: *L. M. TRUCKENBROD[1], A. V. BUMANGLAG[4], E. CHUN[5], A. HERNANDEZ[6], Q. P. FEDERICO[2], A. P. MAURER[3], R. S. SLOVITER[5], S. N. BURKE[2];
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Abstract: An epileptogenic role for hippocampal GABAergic dysfunction has recently been reported (Chun et al., 2019). Specifically, selective ablation of hippocampal GABA neurons by Stable Substance P-saporin (SSP-saporin) conjugate caused dorsal hippocampal sclerosis and chronic epilepsy, without involving convulsive status epilepticus or widespread brain injury. The current study assessed cognitive function in chronically epileptic SSP-saporin-treated rats and their vehicle-injected controls ~8 months following injection. First, rats completed the Morris Water Maze test of spatial learning and memory (Morris et al., 1982). Animals then underwent testing with the object-place paired association (OPPA) task, which requires the hippocampus as well as functional connectivity between the hippocampus and cortical areas (Jo and Lee, 2010; Hernandez et al., 2017), and then a simple object discrimination task. Interestingly, both controls and rats with dorsal hippocampal sclerosis and chronic epilepsy were able to learn the location of the hidden platform in the Morris Water Maze task and could also acquire a simple pair-wise object discrimination. However, epileptic rats with dorsal hippocampal sclerosis were significantly impaired on the OPPA task, which requires animals to integrate spatial location memory with a correct object choice and is a more sensitive measure of cognitive dysfunction (Hernandez et al., 2015). These data indicate that, similar to humans with medial temporal lobe epilepsy, selective hippocampal sclerosis and epilepsy in this model do not result in global cognitive decline. Rather, cognitive functions that require functional connectivity between the hippocampus and cortical areas are selectively affected.

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Poster

158. Hippocampal Function

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant MH109548
McKnight Brain Research Foundation

Title: Mesoscale collective action in the hippocampus: Turbulence and energy cascade

Authors: *A. SHEREMET[1], Y. QIN[2], A. P. MAURER[3];
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Abstract: Of the three scales of brain activity generally identified in LFP recordings (micro-, meso- and macro-scale), mesoscopic activity is the least studied. In the cortex, mesoscopic collective action (MCA) manifests as propagating waves (e.g., Muller et al., 2018). Dismissed often as marginally-significant neuron synchronization, MCA may in fact be the main function of the cortex, as suggested by the non-hierarchical (isotropic and homogeneous) structure of cortical layers, which favors MCA over hierarchical microcircuit activity. This is consistent with the conjecture that physical structures underlying cognition resemble biological systems, with no design and no a priori function (Edelman and Gally, 2001). As such, collective action might play an essential role in the integration of brain activity (e.g., Freeman 2010). Despite a few initial insights (Wilson and Cowan 1973, 1974; and others) a consistent theory for MCA dynamics is still lacking. Because the mesoscale is macroscopic with respect to microscopic processes, the wealth of knowledge accumulated about microscopic physics cannot be directly extended to mesoscopic processes. We propose that the weak turbulence theory (Zakharov, 1992) could provide the theoretical framework for studying self-sustained MCA dynamics. Turbulence describes the internal energy balance in nonlinear multi-scale systems with a large number of components. Nonlinear interaction between scales results in cross-scale flows of energy and other conserved quantities, known as the "turbulent cascade" (Richardson, 1922; Kolmogorov, 1941). We show that the observed evolution of MCA energy balance (LFP spectra and bispectra) in the hippocampus are consistent with mesoscopic weak turbulence. We derive the governing equations in a general conservation form, that generalize existing models (Wilson-Cowan, 1974, Wright and Liley 1995; and others). We derive dynamical equations for the evolution of the power spectral density, and investigate their averaged (kinetic) behavior. The turbulent model predictions of the theta-gamma phase coupling characteristics are consistent with observations. Turbulence holds the promise to provide a consistent theoretical framework for modeling hippocampal energy processes, including the persistent question about the significance of power law spectra and their slopes.

Disclosures: **A. Sheremet:** None. **Y. Qin:** None. **A.P. Maurer:** None.

Poster

158. Hippocampal Function

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant P30:GM122733

Title: Reductions in hippocampal IGF-I signaling in adulthood negatively regulates neuron structure and cognition

Authors: *C. A. HAYES[1], E. L. HODGES[1], J. MARSHALL[1], N. ASHPOLE[2];
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Abstract: Of the numerous growth factors that are reduced in advanced age, the loss of insulin-like growth factor-1 (IGF-1) is known to lead to cognitive impairment and contributes to Alzheimer's disease and other dementias. Studies have shown that IGF-1 peaks during puberty, but declines throughout adulthood. IGF-1 is critical for proper neuron development. Knockout of IGF-1 reduces cognitive function in young mice, but the exact mechanisms by which this happens remain poorly understood as these studies cannot differentiate whether the observed impairments are due to changes in neurons, astrocytes, other glial cells, or the vasculature. We hypothesize that IGF-1 regulation of neurons is essential to maintain proper cognition throughout adulthood. In order to test this hypothesis, we utilized post-pubertal (3 month old) IGFR flox mice and injected Adeno-Associated Virus 9-Synapsin-Cre recombinase (or GFP control) into the hippocampus to selectively delete Exon 3 of IGFR (the binding site for IGF-1) in neurons. Two months following injections, behavior was analyzed in the radial arm water maze and novel object/novel location tasks. In males, we found a significant difference in path length traveled and latency to find the platform within the radial arm water maze. However, there was not an observed difference in the novel object/location tasks, suggesting specificity of IGF-1 in regulating cognition. Behavioral tests in females are ongoing. Mechanistically, we have been examining neuron structure within these mice. Golgi-Cox staining was performed on cryosectioned brain slices and the number of boutons and overall synaptic structure were analyzed. Additionally, we performed gene and protein expression analysis for pathways regulating synaptic structure. We observe significant reductions in synapse number as well as alterations in cofilin and actin cytoskeleton remodeling when neuronal IGFR is reduced in the hippocampus. Together, these data indicate that IGF-1 continues to be an important promoter of neuron structure and function in adulthood.

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Poster

158. Hippocampal Function

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 158.06/V11

Topic: H.01. Animal Cognition and Behavior

Support: Sandia National Laboratories LDRD

Title: Context-modulation of hippocampal dynamics and deep convolutional networks: Using parallel pathways to limit network size

Authors: F. WANG, W. SEVERA, *J. B. AIMONE;
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Abstract: Complex architectures are a hallmark of biological neural circuits, particularly in contrast to conventional artificial neural networks. This architectural complexity, such as parallel processing pathways between two regions, has been behaviorally implicated in many cognitive studies; however the theoretical consequences of circuit complexity on neural computation have only been explored in limited cases. Here, we introduce a mechanism by which direct and indirect pathways from cortex to the CA3 region of the hippocampus can balance both contextual gating of memory formation and driving network activity. We show that these two inputs, one directly from layer 2 of the entorhinal cortex (EC2) and an indirect pathway from EC2 via the dentate gyrus (DG), influence the recurrent CA3 network in distinct and complementary ways. Specifically, the EC2 input has diffuse and relatively weak impact on CA3 neurons, enabling it to have a broad impact on the network with relatively setting the state of the system. In contrast, the DG input is very powerful but sparse both in connectivity and baseline activity, making it suitable for driving a small subset of CA3 neurons with minimal influence on the broader network. The context modulation we observe in CA3 has similarities to biases in artificial neural networks, so we then examined the computational potential of this approach within a deep convolutional network that uses context modulation instead of conventional biases in the training of the network. In a basic image processing dataset, when context information is used to modulate network context, the network performance is improved dramatically. We further observe that the use of context information in MNIST allows a considerably smaller network to achieve comparable performance.

Disclosures: **F. Wang:** None. **W. Severa:** None. **J.B. Aimone:** None.

Poster

158. Hippocampal Function

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Topic: H.01. Animal Cognition and Behavior

Support: NIMH Grant K99 MH118423

Title: Focal optogenetic stimulation reveals heterogeneous place field plasticity rules within and between hippocampal subregions

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Abstract: Synaptic plasticity is thought to underlie memory formation by changing how neurons integrate and respond to their presynaptic inputs. According to the rules of spike-timing dependent plasticity, changes in synaptic strength require depolarization of the postsynaptic neuron in temporal proximity to presynaptic drive. Recent *in vivo* studies have shown that depolarization of single neurons in a fixed position can drive new spatial firing fields around that location. We wondered how this spatial field plasticity affects the synchronization and coding properties of endogenous assemblies that code for the stimulated and non-stimulated locations. To test how artificial stimulation alters the pattern of network synchrony, and to compare the rules of coding plasticity across subregions of the hippocampus, we used fixed-place, focal optogenetic stimulation to depolarize small groups of neurons in CA1, CA3 and the dentate gyrus as mice (CaMKII-Cre::Ai32) ran laps on a linear track. In CA1, but not the dentate gyrus nor CA3, stimulation caused a subset of neurons to remap. Some CA1 neurons gained fields in the stimulated location or elsewhere, others lost fields, though the majority maintained their tuning despite repetitive, strong optogenetic drive. Of the neurons that remapped, most gained a field at the stimulation location. Surprisingly, non-light-driven CA1 neurons also showed remapping that coincided with the stimulation. To explain the remapping in the non-stimulated population, we are currently testing whether stimulation also induced changes in the excitatory drive to local CA1 interneurons, that would, in turn, alter the pattern of feedback (lateral) inhibition. Together these experiments show heterogeneous plasticity rules within and across hippocampal sub-regions.

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Poster

158. Hippocampal Function

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 158.08/V13

Topic: H.01. Animal Cognition and Behavior

Title: Cholinergic dynamic in hippocampus

Authors: *Y. Z. ZHANG[1], L. CAO[2], G. BUZSAKI[3];
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Abstract: Acetylcholine acts as a neurotransmitter by modulating neuronal excitability, presynaptic release, postsynaptic responsiveness and synaptic plasticity in central nervous system. Hippocampus has long been assumed to be a key structure in episodic memory and acetylcholine-dependent regulation of hippocampal function is crucial for learning and memory. However, no direct evidence has been provided to monitor real-time acetylcholine change and correlation with neural activities in freely behaving animals during different behavioral states. Here we used fiberphotometry in mice that express a bacterial recombinase for enabling the selective manipulation of cholinergic neurons combined with a sensitive fluorescent acetylcholine sensor capable of detecting milliseconds timescale fluctuation of acetylcholine levels (**CITE**).
Hippocampal network activity was monitored by high-density electrode arrays simultaneously from multiple regions and layers of the hippocampus. Animals were recorded during spontaneous behaviors. We found that acetylcholine signal significantly increased 5~10% during REM sleep and active wakefulness, especially locomotion. Optogeneticl activation of medial septum cholinergic neurons induced acetylcholine release during nonREM sleep but not during active wakeful behaviors. Signal dynamics was abolished by intraperitoneal injection of atropine or scopolamine. We will record from multiple hippocampal layers, while animals perform a hippocampus-dependent task, in an attempt to determine layer-specific contribution of cholinergic signaling to spatial learning.
Keywords: *acetylcholine, hippocampus, medial septum*

Disclosures: Y.Z. Zhang: None. **L. Cao:** None. **G. Buzsaki:** None.

Poster

158. Hippocampal Function

Location: Hall A

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ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: H.01. Animal Cognition and Behavior

Support: NSERC
CIHR

Title: Objects, context, memory and space: Neuronal representations in the hippocampus of virtually navigating primates

Authors: *R. A. GULLI[1], L. R. DUONG[3], B. W. CORRIGAN[4], G. DOUCET[5], S. WILLIAMS[6], S. FUSI[2], J. C. MARTINEZ-TRUJILLO[7];
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Abstract: The hippocampus is known to play a role in associative memory and spatial navigation. It is not clear how spatial and mnemonic activity are mixed in hippocampal neurons in primates. We considered two modes of interaction: linearly additive selectivity and nonlinear mixed selectivity for space and features of the environment. To disambiguate these possible coding schemes, neuronal activity must be recorded in a common space while features of the environment and cognitive demands are varied. We recorded activity of hippocampal neurons in monkeys navigating the same virtual maze during two different tasks: a foraging task requiring only cue guided navigation, and a memory task also requiring context-object association. During both tasks, single neurons encoded information about the animal's spatial position in the virtual maze, and a linear classifier could decode the animal's position across different maze segments. However, the population code for space did not generalize across tasks, particularly in maze segments where stimuli relevant to the associative memory task appeared. Closely examining single neuron activity revealed that cross-task changes were due to the emergence of sensory and mnemonic coding for non-spatial features and their associations exclusive to the associative memory task. Finally, using the activity of neuronal populations, a linear classifier could decode combinations of non-spatial elements in single trials, when they were visually available (perceptual coding) and following their disappearance (mnemonic coding). Our results show that neurons in primate hippocampus encode spatial, perceptual and mnemonic features in a

nonlinearly mixed manner. This creates a highly efficient code in single neurons and populations that flexibly represents information that is relevant to recent past and future behavior.

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Poster

158. Hippocampal Function

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Program #/Poster #: 158.10/V15

Topic: H.01. Animal Cognition and Behavior

Support: ERC StG 678790 NEWRON

Title: Neuronal discrimination of similar sensory inputs during hippocampal memory encoding

Authors: M. ALLEGRA, L. POSANI, C. SCHMIDT-HIEBER;
Inst. Pasteur, Paris, France

Abstract: Transforming similar sensory information from the outside world into distinct memory representations is a major challenge for the brain. The hippocampus has been suggested to play a key role in producing segregated memories of similar objects and events in a process commonly referred to as “pattern separation”. Where and how this function is accomplished in the hippocampal circuit is currently debated. To address this question, we perform in vivo 2-photon Ca^{2+} imaging from hippocampal subregions of head-fixed mice navigating in a linear virtual-reality environment while performing a visual discrimination task. We find that the input region of the hippocampus, the dentate gyrus, is sensitive to small changes in visual cues, producing decorrelated activity patterns in response to small differences in wall textures (decorrelation, i.e. relative reduction in pairwise spatial map correlations between track crossings in the same environments and different environments: 28 ± 5%, mean ± SEM, n = 41 recording sessions from 4 animals). By contrast, decorrelation is substantially lower in CA1 under the same conditions (16 ± 3%), where larger changes in the environment, including replacement of all spatial landmarks, are required to produce substantial decorrelation (41 ± 5%, n = 20 recording sessions from 4 animals). Thus, the degree of differences in the spatial environment differentially affects neuronal discrimination in the dentate gyrus and CA1. Our findings provide an understanding of how and where in the hippocampal circuit distinct memory representations of similar experiences are produced.

Disclosures: M. Allegra: None. **L. Posani:** None. **C. Schmidt-Hieber:** None.

Poster

159. Decision Making: Lateral Prefrontal Cortex

Location: Hall A

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Program #/Poster #: 159.01/V16

Topic: H.01. Animal Cognition and Behavior

Support: AMED

Title: Causal role for involving expected value in risky choice in macaque ventrolateral prefrontal cortex

Authors: *R. SASAKI[1], N. TAKAKUWA[3], T. ISA[2];
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Abstract: In our real life, one would need to flexibly take risky option efficiently depending on the returns, although it is well known that we do not prefer taking too much risk. Recent studies showed that many brain areas represent positive and/or negative reward-related parameters. However, little is known about where and how those parameters are integrated to make a decision. In this study, we investigated how the animals (Macaque monkey) handle their risky choice in the situation whether they might get reward with high risk and high return or low risk and low return condition. We defined the risks as the reward probability and the returns as the reward size. We also compared this in the conditions with different sizes of expected values to see how the expected value affects monkeys' risk seeking. Once the monkey fixated his gaze on the fixation point, two targets were presented simultaneously at horizontally symmetrical positions. The monkey was required to choose one of the two targets by a saccade to get reward in certain reward probability and expected value assigned to the color of the selected target. We found that the monkey preferred risky choice as default mode which is consistent with other previous studies. Interestingly, we also found that the monkey took more risk when the expected value was relatively small. These results suggest that the monkey makes a choice by integrating both risk and expected value. We next investigated the causal role of a variety of brain areas in the behavioral choices by reversible inactivation with microinjection of a $GABA_A$ receptor agonist, muscimol. We were careful to identify the target area and successfully localized it using MRI image injecting the gadolinium in advance. When muscimol was injected into bilateral ventrolateral prefrontal cortex (vlPFC) while the monkey was performing the task, the sensitivity to risky choice was gradually weakened over time, although the sensitivity to expected value was remained to be consistent. Interestingly, the interaction between risk and expected value was also weakened. Our results suggest that the integration of risk/return and expected value might be accomplished in vlPFC. We will also discuss about the role of the other brain areas such as Orbitofrontal cortex (OFC), Nucleus accumbens (NAc) and Anterior cingulate cortex (ACC).

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Poster

159. Decision Making: Lateral Prefrontal Cortex

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Support: NIH Grant R01MH110822

Title: The effect of delay to reward on probabilistic learning in non-human primates

Authors: *J. M. FREDERICKS, J. E. BOYCE, P. H. RUDEBECK;
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Abstract: Maladaptive reinforcement learning is a defining symptom of depression and anxiety disorders. Clinical studies have localized this maladaptive learning to the prefrontal cortex (PFC) and amygdala. In non-human primates both the amygdala and ventrolateral PFC are required for probabilistic reward learning. Despite this knowledge, the specific contribution of these areas to reward learning has not been deciphered. We hypothesize that within this circuit the ventrolateral PFC, but not amygdala, is specifically required to hold information about chosen options online until a reward is (or is not) delivered. To test our hypothesis, we developed a touch screen based dynamic two-choice probabilistic behavioral paradigm where the delay between the choice and the reward delivery was manipulated. In all sessions the probability of receiving a reward was set at 0.8 and 0.2 for the two options and the reward probabilities reversed halfway through the 150-reward session. The delay between choice and reward, either zero, one, or two seconds was fixed for the duration of a daily session. To ensure that effects on behavior were not simply a function of reduced reward value, the inter-trial interval was adjusted so that the temporal reward rate was the same across all sessions. Monkeys readily learned the task when there was no delay between choice and reward delivery, but in sessions where there was a delay, there was a progressive effect on behavior. As the delay increased, performance on the task was negatively impacted as measured by the number of choices of the option associated with the highest probability of reward (effect of delay, $F(1,2)=38.42$, $p=3.0456e^{-07}$). A reinforcement learning model fit to the data further confirmed the influence of the delay on learning. Importantly, while there was a change in learning, the rate at which monkeys initiated trials was unchanged (effect of delay, $F(1,2)=1.52$, $p=0.2255$). Overall we interpret these data as showing that the delay manipulation reduce the associative strength of the reward, not the value of the reward. To further test our hypothesis concerning the ventrolateral PFC in probabilistic learning we will utilize chemogenetic tools to influence activity in amygdala and ventrolateral PFC.

Disclosures: **J.M. Fredericks:** None. **J.E. Boyce:** None. **P.H. Rudebeck:** None.

Poster

159. Decision Making: Lateral Prefrontal Cortex

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 159.03/V18

Topic: H.01. Animal Cognition and Behavior

Support: Wellcome Trust Investigator Award to TWR (104631./Z/14/Z/)

Title: The role of ventrolateral prefrontal cortex in spatial self-ordered response sequencing in marmosets

Authors: *S. F. A. AXELSSON[1,2], N. K. HORST[2,3], N. HORIGUCHI[1,2], T. W. ROBBINS[1,2], A. C. ROBERTS[3,2];
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Abstract: Abnormalities in cortico-striatal pathways are thought to underlie much of the psychopathology of OCD. More specifically, lateral prefrontal-striatal projections have recently been shown to be underactive in the planning of response sequences and this impairment in goal-directed behaviour may be an endophenotype of OCD (Vaghi et al 2017 Biol Psychiatry: CNNI. 2(8), 655-663). This study investigated the causal role of the lateral PFC in marmosets in a self-ordered response sequencing task by inactivating this region via intra-cerebral infusions and by manipulating its chemical neuromodulation by infusions of dopamine and serotonin receptor antagonists.
Marmosets were trained on a touch screen task that required generation of spatial self-ordered sequences. In this task two or three circles were presented on a screen in each trial and subjects in a self-ordered fashion, had to respond once (and once only) to each stimulus in order to gain access to juice reward. Stimulus locations were varied over trials, thus requiring flexible, goal-directed responding. An error, defined as repetition of a response already made on that trial, resulted in termination of the current trial and initiation of the next trial. After reaching a high, stable level of performance at both 2- and 3-box levels, marmosets were implanted with permanent indwelling cannula targeting ventrolateral prefrontal cortex (vlPFC), Brodmann's area 47 (BA47).
Temporary inactivation of BA47 by infusion of the $GABA_a$/$GABA_b$ receptor agonists muscimol/baclofen (n=4) profoundly impaired accuracy of performance of the task, especially enhancing cumulative errors on incorrect trials. A milder but qualitatively similar deficit was seen after infusion of the $5HT_{2A}$-receptor antagonist M100907. Infusion of the D_2-receptor antagonist s-sulpiride caused a comparable, but more perseverative deficit.
This deficit in self-ordered sequencing by vlPFC inactivation will be compared with spatial response sequencing in which marmosets are overtrained on one specific array of stimuli,

inducing a preferred response sequence. This will determine whether the impairment was specific or not to the performance of goal-directed, flexible response sequences rather than automatic tendencies.

Disclosures: S.F.A. Axelsson: None. **N.K. Horst:** None. **N. Horiguchi:** None. **T.W. Robbins:** None. **A.C. Roberts:** None.

Poster

159. Decision Making: Lateral Prefrontal Cortex

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 159.04/V19

Topic: H.01. Animal Cognition and Behavior

Title: Pac-man for monkeys: A behavior paradigm for the investigation of the neural mechanisms of complex cognitive functions

Authors: *W. ZHANG, X. CHEN, J. LI, Y. BAI, R. SU, Z. LIN, T. YANG;
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Abstract: A complex network of brain areas, including the prefrontal and the posterior parietal cortices, is underlying cognitive control. Yet, its investigations using animal models are impeded by the typical behavior paradigms employed that are too simplistic to reflect the complex nature of both cognition and the corresponding neural circuitry. To address this problem, we trained two macaque monkeys to play a game that was adapted from but very similar to the original Pac-Man game. The monkeys used a joystick to control the Pac-Man to navigate the maze. The maze contained a number of randomly distributed dots and two ghosts of different colors. Eating a dot earned the monkeys a small amount of juice reward. Being eaten by the ghosts led to a timeout penalty. The goal of the game was to clear all the dots on the maze, which was rewarded with a big amount of juice. In addition, there were a number of energizers randomly placed in the maze. Eating an energizer turned the ghosts into the scared mode temporarily, in which they could be eaten by the Pac-Man for the monkeys to gain additional juice reward. All these elements made the game cognitively demanding. For comparison, we also tested three human players using exactly the same version of the game. After training, both monkeys understood the game rules and achieved satisfactory performance. They were able to clear a game with 4 attempts on average (3.96 for monkey O, 4.08 for monkey monkey P). The monkeys tended to move in the direction leading to larger rewards. They also distinguished the ghosts of different modes: they were more likely to chase the scared ghosts and run away from the ghosts in the normal mode. Their strategies were similar to those of human players. To further understand the monkey's behavior, we used a random forest algorithm to predict the monkeys' joystick movements with the game states, which included features such as the Pac-Man location, the Pac-Man direction, the reward distribution, the ghosts' locations, etc. The model predicted the monkeys' joystick

movement with over 90% accuracy over all (92.18% for monkey O, 90.82% for monkey P). Models trained with human players' behavior data predicted the monkeys' performance similarly well (83.53% for monkey O, 81.90% for monkey P), reflecting that the monkeys were using similar strategies to play the game. The results demonstrated the feasibility to train macaque monkeys to perform complex behavior tasks previously thought to be limited to humans. More importantly, both the task and the monkeys' behavior can be fully quantified to allow the investigations of the underlying neural mechanisms.

Disclosures: **W. Zhang:** None. **X. Chen:** None. **J. Li:** None. **Y. Bai:** None. **R. Su:** None. **Z. Lin:** None. **T. Yang:** None.

Poster

159. Decision Making: Lateral Prefrontal Cortex

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Topic: H.01. Animal Cognition and Behavior

Support: Ministry of Education Tier 2 Academic Research Fund MOE2016-T2-2-117
NUS-NUHS Memory Networks Program

Title: Action initiation decision signals in the lateral prefrontal cortex

Authors: ***C. LIBEDINSKY**, R. HERIKSTAD, S.-C. YEN;
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Abstract: Voluntary decisions involve deciding which actions to take out of the available options, and when to initiate them. Action initiation can be triggered by external factors (stimulus-triggered) or internal factors (internally-generated). For example, the sight of a glass of water may trigger the action of reaching for the glass (stimulus-triggered), while the recollection that you run out of milk may trigger the action of going to the store (internally-generated). Common experimental paradigms to study stimulus-triggered actions are delayed-movement tasks, where instructions for which actions to take (given by a target) and when to initiate them (given by a go-cue) are separated by a delay period, allowing for the clean separation of both decision components.Selective neural activity during the delay period, or "preparatory" activity, appears to be related to the decision of which action to take, while activity between the go-cue and action initiation, or "pre-movement" activity, appears to be related to the decision of when to initiate the action, as well as its execution. However, It is unclear whether pre-movement neural activity can be divided into separate decision and execution components, or whether the decision itself serves as an execution signal (e.g. after the signal crosses a threshold). Variability in the time between the go-cue and the initiation of the action (reaction time variability) allows for the dissociation of signals that correlate with the go-cue and those that correlate with the execution

of the action. Both types of signals are conspicuous in several brain regions, however, go-cue-related signals may simply reflect sensory processing, without necessarily reflecting a decision process. If an action-initiation decision signal exists, its timing should be correlated with the onset of the go-cue in stimulus-triggered tasks, but it should also be present prior to internally-generated actions, where it would presumably correlate with the onset of an internal signal that leads to the decision to initiate the action (such as the recollection of a memory). Here we tested whether an action-initiation decision signal exists in the prefrontal cortex. We trained monkeys to perform a visually-guided delay saccade task while we recorded the activity of neurons in the lateral prefrontal cortex. We found that the activity of a subset of neurons was aligned to the go-cue rather than the saccade execution. Importantly, some of these cells also increased their activities prior to internally-generated saccades. These results support the existence of an action-initiation signal in the prefrontal cortex, which is separate from the action-execution signal.

Disclosures: C. Libedinsky: None. **R. Herikstad:** None. **S. Yen:** None.

Poster

159. Decision Making: Lateral Prefrontal Cortex

Location: Hall A

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Program #/Poster #: 159.06/V21

Topic: H.01. Animal Cognition and Behavior

Title: Neural signals in the monkey prefrontal-striatal network reflect multiple cognitive strategies in category rule learning with trial and error feedback

Authors: *M.-Y. PARK[1], A. L. DENICOLA[1], D. A. CROWE[2], M. V. CHAFEE[3];
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[3]Dept Neurosci / Brain Sci., Univ. Minnesota, Minneapolis, MN

Abstract: Trial-and-error feedback is an important aspect in decision making for the maintenance or adjustment of behavioral strategies to increase reward under uncertain environments. To test how feedback affects categorical decision making and strategy development in the prefrontal cortex (PFC) and dorsal striatum (STR) we trained two monkeys to perform a rule-selection categorization task and recorded the two brain areas simultaneously while monkey performed the task. Two circular stimuli with different positions and sizes were presented in sequence and monkeys had to categorize the second stimulus as left or right under a SPACE rule or as larger or smaller under a SIZE rule in relation to the first stimulus. Monkeys made a GO/NOGO saccade response depending on the category and rule applied. The categorization rule was not explicitly instructed; Monkeys inferred the correct rule in each block using trial-and-error feedback. During one ensemble of recording each monkey saw two sets of trials, one for the size rule and one for the spatial rule in a random order.
Our first question was the cognitive strategy that monkeys employed to solve the task.

Surprisingly, we found behavioral evidences that monkeys formed categories by combining both SPACE and SIZE feature dimensions in the case that the resulting compound category required the same response under the two rules ('congruent' categories). For example, stimuli that were both left and larger always required a GO response regardless of the rule applied. Conversely, left and smaller stimuli required a GO response under one rule (SPACE) and NOGO response under another (SIZE).
We recorded from PFC and STR using multielectrode arrays concurrently to relate neural dynamics to the switch between rules with learning. In both PFC and STR, the dominant neural signals reflected the rules, SIZE or SPACE, that monkeys had to discover from feedback. By task design, these rule signals were abstract, in that they were dissociated from stimulus size, position, as well as the direction of the required response. The strength of the rule signal reflected learning in the PFC-STR network. In addition, we found a second populations of neurons that encoded compound categories (e.g. LEFT and LARGER), reflecting the cognitive strategy applied. Finally, we gradually varied the magnitude of the difference between the two circles along the SPACE and SIZE feature dimensions and found that neurons which encoded the relationship between stimuli (e.g. the SIZE relationship) were modulated by the difficulty of the visual discrimination required.

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Poster

159. Decision Making: Lateral Prefrontal Cortex

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Program #/Poster #: 159.07/V22

Topic: H.01. Animal Cognition and Behavior

Support: Marie Sklodowska-Curie grant agreement No 798255

Title: A cognitive signal controls speed-accuracy trade-off in a neural model of two-stage decision making in the frontal eye fields

Authors: *D. STANDAGE[1], G. BLOHM[2], D. HEINKE[1];
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Abstract: In decision making tasks, subjects are faster and less accurate when motivated to favour speed, and are slower and less accurate when motivated to favour accuracy. The speed-accuracy trade-off (SAT) is nearly ubiquitous across tasks and species, and provides a window on cognitive control. In experiments by Heitz and Schall (Neuron, 2012; Philos Trans Royal Soc B, 2013), single-neuron activity and local field potentials (LFP) were recorded from the frontal eye fields (FEF) of monkeys performing a visual search task, where a cue indicated SAT

condition and monkeys made their choices by foveating one of the stimuli. Monkeys were faster and less accurate in the speed condition (vice versa for accuracy), where neurons responsive to stimuli (visual neurons) and neurons that increase their firing rates prior to eye movements (movement neurons) showed elevated rates during a pre-stimulus interval following the cue. We hypothesise that this modulation reveals a cognitive signal projected diffusely to FEF, controlling the dynamics of a two-stage decision process. We tested this hypothesis with a neural model of FEF, in which a visual network projects to a movement network. Pyramidal neurons and inhibitory interneurons are simulated in both networks, connected by AMPA, NMDA and GABA conductance synapses. The visual network receives stimuli, but otherwise, the only differences between the networks are that excitatory recurrent synapses in the movement network are stronger and have faster vesicle replenishment. The simulated cognitive signal controls SAT, where a stronger signal produces higher response rates and earlier target selection in the visual network, and produces steeper ramping and higher peak rates in the movement network, as shown by Heitz and Schall (2012) in the speed condition. We further simulated LFPs by summing synaptic currents onto stimulus-selective pyramidal populations, accounting for the discrimination of SAT condition by single-neuron and LFP recordings, as shown by Heitz and Schall (2013). The model makes testable predictions for the time-frequency response of LFPs and electroencephalogram (EEG) recordings in different task conditions.

Disclosures: **D. Standage:** None. **G. Blohm:** None. **D. Heinke:** None.

Poster

159. Decision Making: Lateral Prefrontal Cortex

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant MH108629

Title: Learning the values of reward sequences

Authors: *Z. CHENG, D. LEE;
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Abstract: Although previous behavioral and neurobiological studies on decision-making have mostly focused on choices to obtain unitary rewards, many real-life actions lead to a series of outcomes. For example, accepting a job offer produces a series of monthly incomes. Similarly, animals in the wild also explore their territory for a sequence of food items. Nevertheless, how reward sequences are evaluated remains largely unknown. One possibility is that a reward sequence is evaluated by its total amount alone. Alternatively, the value of a later reward in a sequence might be attenuated by its delay. Here, we designed a reward sequence evaluation

(RSE) task and trained two rhesus monkeys to investigate how various reward sequences are evaluated.
During the RSE task, each trial began with a central fixation point and two columns of 3 gray circles presented 3 deg away from the vertical meridian. Following a 0.5-s fore-period, 1 to 3 green discs appeared inside each gray circle, indicating the amount of juice available after a variable delay (0.5~15.5 s) determined by the position of the gray circle. Then, the fixation point disappeared 1.0~1.7 s later and two choice targets were presented along the horizontal meridian 7.6 deg away from the center. The animal was allowed to shift its gaze to indicate its choice, and the unchosen target and its corresponding reward cues were extinguished. Next, the gray circle at the top or bottom on the chosen side turned green for 5 s, and the animal received one or more drops of juice reward immediately as the green discs changed to empty circles. This was repeated sequentially for the remaining gray circles. In some sessions, reward was dispensed from the top to bottom, whereas the order was reversed in the remaining sessions. We used a logistic regression model to estimate the weights the monkeys assigned to the number of discs delivered at each time step. Consistent with temporal discounting, we found that the weights decreased gradually for later time steps. When the direction of reward delivery was reversed, the weights for each time step were opposite to the prediction from temporal discounting initially and were gradually reversed over 10 to 20 sessions. The dynamical changes in weight after the reversal in reward direction rules out the contribution of a potential systematic spatial bias and further supports the role of temporal discounting in reward sequence evaluation. These results suggest that the animals evaluated a reward sequence by discounting delayed reward, and that the RSE task provides a good paradigm to study the neuronal mechanisms for evaluating reward sequences.

Disclosures: Z. Cheng: None. **D. Lee:** None.

Poster

159. Decision Making: Lateral Prefrontal Cortex

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant 5T32NS007292-33

Title: Context dependent computation by randomly connected attractor networks with synaptic depression

Authors: *S. QIU[1], P. MILLER[2];
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Abstract: The nature of computation underlying flexible, context-dependent behavior in animals is one of the most interesting fundamental questions in theoretical neuroscience. Mante et al. (2013) have explored the prefrontal cortex activity in macaque monkeys, which were trained to make a choice based on one of two distinct types of concurrent noisy sensory inputs (motion and color), with the relevant sensory input indicated by a prior context cue. Mante et al. found neural activity responded in an integration-like manner to both types of stimulus, irrespective of which was relevant for the task, but the prior context cue determined how the stimulus-dependent information impacted the final choice. Here, we explore how such context-dependent computations can arise in biologically inspired randomly-connected attractor networks with synaptic depression, with minimal defined structure. In simulations of the model, we presented one of two possible context cues followed by two motion cues (one for each direction) and two color cues (representing red and green) as distinct sets of random inputs. Motion cues and color cues appeared at different levels of coherence, each in a manner such that total motion input and total color input was fixed. Network output was indicated by a two-alternative winner-take-all circuit, with the correct choice indicating which color input was the larger in the color context, or which motion input was the larger in the motion context. Reinforcement learning was instantiated via Hebbian plasticity following correct choices. Making use of linear regression analysis and QR-decomposition in the same manner as Mante et al, we projected the activity onto axes representing the 4 different task variables (context, motion, color, choice). We found network neural responses to be similar to those found in the experiment, supporting the hypothesis that itinerancy of neural activity through attractor states can underlie neural processing in cognitive tasks.

Disclosures: **S. Qiu:** A. Employment/Salary (full or part-time):; Brandeis university. **P. Miller:** None.

Poster

159. Decision Making: Lateral Prefrontal Cortex

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Program #/Poster #: 159.10/V25

Topic: H.01. Animal Cognition and Behavior

Support: ONR

Title: Top-down and bottom-up expectations in the prefrontal and auditory cortices

Authors: *L. SURIYA-ARUNROJ[1], J. I. GOLD[2], Y. E. COHEN[3];
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Abstract: Auditory perceptual decision-making is affected by expectations that can be established via both bottom-up and top-down processes. For example, bottom-up sensory processing can reflect regularity violations in the temporal sequencing of auditory stimuli, whereas top-down processing can encode learned task statistics. However, it is not well understood how these different processes interact in the brain to shape the interpretation of auditory signals that form perceptual decisions. The goal of this study was to investigate the interaction between bottom-up and top-down processing in the auditory cortex and prefrontal cortex. These brain regions were selected because they are part of the ventral auditory pathway, which has a key role in auditory perception and decision-making.

To test these interactions, two rhesus monkeys listened to a sequence of high-frequency or low-frequency tone bursts that were embedded in background of broadband noise. The monkeys reported whether the last tone in each sequence (the "test tone") was low or high frequency by pulling or pushing a joystick, respectively. We titrated task difficulty by varying the sound level of the test tone relative to the noisy background. We manipulated bottom-up expectations by presenting three identical low or high frequency tone bursts ("pre-tones") to establish a sequence regularity that either was or was not violated by the immediately succeeding test tone. We manipulated top-down processing by presenting, before sequence onset, a visual cue that indicated the prior probability ("pre-cue") that the test tone would be high or low frequency within a given block of trials (corresponding to ratios of low- versus high-frequency test tones of 3:1, 1:1, or 1:3).

The monkeys' behavioral choices and response times were affected by both the pre-tones and the pre-cue. Ongoing analyses of neural data are assessing the interactions between top-down and bottom-up processes in the auditory cortex and prefrontal cortex that are responsible for these effects of different sources of expectations on auditory decision-making.

Disclosures: L. Suriya-Arunroj: None. **J.I. Gold:** None. **Y.E. Cohen:** None.

Poster

159. Decision Making: Lateral Prefrontal Cortex

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Topic: H.01. Animal Cognition and Behavior

Support: NIH grant R01 EY017077
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Title: Rhythmicity of prefrontal cortical activity in impulsive and uncertain choices

Authors: *S. SUBRAMANIAM[1], A. B. PERSILY[1], B. SINGH[1], X. QI[1], M. L. PHILLIPS[2], C. CONSTANTINIDIS[1];

[1]Dept. of Neurobio. & Anat., Wake Forest Sch. of Med., Winston Salem, NC; [2]Dept. of Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The inability to delay gratification is a characteristic of maladaptive behaviors in adolescence and in clinical conditions such as bipolar disorder. To understand the neural basis of impulsive choices, we recorded single neuron activity and local field potentials from the prefrontal cortex of monkeys performing two tasks that imposed a delay between a choice and a reward, with a chronic array of 64 electrodes implanted over the prefrontal cortex. An inter-temporal decision making task required monkeys to choose between two different colored targets signifying a small reward delivered immediately, or a large reward delivered after a delay period (2-3s in most sessions). Data from a total of 19 sessions were analyzed. Neuronal activity was generally higher for larger than smaller rewards, and for the same reward delivered with a shorter delay than a longer delay. Choices for a smaller immediate reward vs. a larger delayed reward were also characterized by different patterns of LFP power in the beta-frequency range (13-30 Hz), which started to increase in the fixation interval that preceded an eventual choice of a large reward. An uncertain reward expectancy task required monkeys to select one of two color targets signifying high immediate reward, low immediate reward, or delay penalty. In some trials, two white targets appeared and the monkey selected one without knowing its value ahead of time. The monkey had to wait for a 2 s delay interval before the value of the targets and the choice outcome was revealed. Data from a total of 10 sessions were analyzed. Conditions differing in uncertainty in this task were also characterized by differences in LFP power. Beta-frequency power was greater during the target presentation interval when the value of the targets was known by virtue of their color compared to when it was not. This pattern reversed during the delay interval prior to reward, so that higher beta power characterized the delay interval when there was uncertainty about the reward. These results suggest that prefrontal neuronal activity and LFP beta-frequency power provide a signature of impulsive vs. deliberate choices in the face of uncertainty.
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Disclosures: S. Subramaniam: None. **A.B. Persily:** None. **B. Singh:** None. **X. Qi:** None. **M.L. Phillips:** None. **C. Constantinidis:** None.

Poster

159. Decision Making: Lateral Prefrontal Cortex

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Program #/Poster #: 159.12/V27

Topic: H.01. Animal Cognition and Behavior

Support: Gruber Fellowship
NIH Grant MH108629

Title: FEF signatures of saccade inhibition during perceptual decision-making

Authors: *M. SHINN[1], D. LEE[2], J. D. MURRAY[3], H. SEO[3];
[1]Interdepartmental Neurosci. Program, [2]Neurosci., [3]Dept. of Psychiatry, Yale Sch. of Med., New Haven, CT

Abstract: Perceptual decision-making tasks with dynamic noisy stimuli have been frequently used to investigate how the brain integrates sensory evidence over time. Previous neurophysiological studies using such tasks have often observed a dip in neural activity following a sudden stimulus onset. Although such a dip has been hypothesized to be due to a resetting of the decision variable to begin evidence accumulation, its function remains poorly understood.
We analyzed single-neuron activity recorded in the frontal eye fields (FEF) of rhesus monkeys during a perceptual decision-making task. Animals were trained to report via saccade the dominant color in a central square patch consisting of green and blue pixels rearranging randomly at 20Hz. During a short presample period which was 0, 400, or 800ms, this stimulus consisted of equal numbers of green and blue pixels. Then, at the onset of a sample stimulus, the ratio of blue to green pixels changed abruptly from 1:1 to some fixed value (color coherence) which varied randomly across trials. The onset of the sample stimulus can be detected easily in high coherence trials but not in low coherence trials. Animals were rewarded if they directed their gaze to the flanking target with the same color as the dominant color of the sample. Consistent with previous findings, we observed a dip in single-neuron FEF activity at the onset of the presample. We also observed a dip with similar duration and latency after sample onset. Activity during the dip was reduced more strongly for high-coherence samples following the sample onset, but only during the trials with a 400 or 800ms presample period. This dip in FEF activity was associated with a temporary inhibition of choice saccades as well as ongoing microsaccades. Therefore, the dip in FEF activity might underlie saccade inhibition, which is triggered when the animal detects the transition from presample to sample and serves to extend the period of evidence accumulation. This suggests that the dip might be closely related to saccade inhibition.

Disclosures: **M. Shinn:** None. **D. Lee:** None. **J.D. Murray:** None. **H. Seo:** None.

Poster

159. Decision Making: Lateral Prefrontal Cortex

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 159.13/V28

Topic: H.01. Animal Cognition and Behavior

Title: The maintenance and comparison of numerical information in the primate prefrontal cortex

Authors: *P. VISWANATHAN[1,2], A. M. STEIN[2], A. NIEDER[2];
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Abstract: Many of our everyday decisions rely on our ability to assess "how many" of something we sense, from putting food on our dinner plate to shopping for the dinner we plan to cook. An important node in the brain network that supports this behavior is the prefrontal cortex. The prefrontal cortex processes numerical information, maintains this information in working memory and flexibly uses this information in conjunction with rules to guide behavior. Evidence for this are prefrontal cortex neurons selective to the number of items in a dot array during stimulus presentation and during a subsequent delay period, as well as neurons selective to quantitative rules. However, a crucial element of decision-making involves comparing sensory information presented to us at different time points. To uncover the role of prefrontal cortex neurons in this aspect, we employed an abstract numerical comparison task during which rhesus monkeys judged whether a reference number and test number were the same or different. A rule cue then instructed them to make an appropriate response mapped to the same/different decision. We quantified numerical information in the responses of single neurons in prefrontal cortex during this task. We found that prefrontal neurons maintained information about the reference number in a stable manner well until the test number is displayed. At this point, neurons began to code for the test number and the decision derived from comparison of the reference and test. Our data suggest that the comparison of numerical information presented at different times engages prefrontal cortex neurons in a dynamic fashion.

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Poster

159. Decision Making: Lateral Prefrontal Cortex

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Program #/Poster #: 159.14/V29

Topic: H.01. Animal Cognition and Behavior

Title: Neural substrate underlying the selection of behavioral tactics to transform sensory information into action: A comparative study of primate medial frontal areas

Authors: *M. H. AWAN[1], H. MUSHIAKE[1], Y. MATSUZAKA[2];
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Abstract: Flexible, context dependent behavior necessitates the selection of diverse protocol to convert sensory information into valid action (i.e. behavioral tactics). Previous studies indicated implication of posterior medial prefrontal cortex (pmPFC) of primates in this process. In the

present study, we examined how the selected tactics is utilized to transform visual information into action and how the pmPFC and the downstream cortical motor areas contribute to this transformation. To address these issues, we devised a behavioral task that required monkeys to choose either reach to (pro-reach) or away from (anti-reach) a spatial cue. A trial started as the monkeys pressed a home button attached to a primate chair. Then a color cue briefly appeared to instruct the tactics (cyan and blue color instructed pro- and anti-reach, respectively) only but not action, then disappeared. After a variable length of delay period, a spatial cue (white LED appearing either on the left or the right) prompted the monkeys to perform an action (reach to either left or right button). The cued tactics (either pro- or anti-reach) determined how the spatial cue is converted into monkeys' action. In pro-reach, the monkeys were rewarded by reaching to the push button ipsilateral to the spatial cue whereas in anti-reach, they were rewarded by reaching to the contralateral button.While the monkeys were performing this task, neuronal activity was recorded from the posterior medial prefrontal cortex (pmPFC), the presupplementary motor area (preSMA) and the supplementary motor area (SMA) and their relation to the cued tactics, cue position and the monkeys' action was analyzed. We found both pmPFC and preSMA have tactics selective, action selective and dual coding (both tactics and action) neurons. Our findings also show that pmPFC has complete information about tactics, action and cue position but preSMA has information about the tactics and action only but not cue position. SMA is mainly action selective and has only action selective neurons. These results indicate that the three cortical areas play distinct roles in converting sensory information into action by multiple behavioral tactics.

Disclosures: **M.H. Awan:** None. **H. Mushiake:** None. **Y. Matsuzaka:** None.

Poster

159. Decision Making: Lateral Prefrontal Cortex

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 159.15/V30

Topic: H.01. Animal Cognition and Behavior

Support: WT100973AIA
203139/Z/16/Z

Title: Multiple memory traces of choice and reward in macaque frontal cortex

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Abstract: Prefrontal cortex signals relate to different aspects of the choices we are about to pursue [1]. Rewards increase the value of the choices with which they are associated, but the way rewards and choices influence decision making can be multifaceted. Choices are sometimes simply repeated regardless of whether they have been linked to reward receipt and rewards can reinforce choices to which they are not causally linked [2,3]. We investigate the brain networks underlying linked and unlinked choice and reward representations using reinforcement learning models and functional magnetic resonance imaging in macaque monkeys.
The history of reward paired with a choice guided decision making, but in addition unlinked effects of choice and reward drove behaviour. In particular, animals were more likely to repeat choices during phases of high average reward rates. We implemented memory traces of choice and reward in an RL model in addition to contingent learning. This improved model fit. Reward traces led to asymmetric and dynamic value updates consistent with the influence of the average reward rate on stay/switch choices.
Behavioural analyses and modelling suggest that choices are driven by an integration of different types of evidence. We found that, similarly, ventromedial prefrontal cortex (vmPFC) activity is better described by both value signals and choice memories and not value difference alone.
Based on the modelling, we identified unlinked memories of choice and reward in frontal cortex. Medial orbitofrontal cortex (mOFC) represented whether animals stayed with their previous choice stimulus or switched, while dorsal anterior cingulate (dACC) and agranular insula (AI) tracked the reward trace. Time course analyses of AI demonstrate a gradual change in coding the reward trace to coding of the current outcome, suggesting that this brain region integrates new reward into the reward trace.
Behavioural and RL modelling analyses suggest that memory traces of choice and reward persist to some degree independently and influence decision making. MOFC as well as dACC and AI carried such unlinked memory traces of choice and reward, respectively. Ventromedial prefrontal cortex signals reflected the general integration of choice evidence.
References 1. Murray EA, Rudebeck PH. (2018). *Specializations for reward-guided decision-making in the primate ventral prefrontal cortex.* Nat Rev Neurosci.
2. Akaishi R, Umeda K, Nagase A, Sakai K. (2014). *Autonomous mechanism of internal choice estimate underlies decision inertia.* Neuron.
3. Walton ME, et al. (2010). *Separable learning systems in the macaque brain and the role of orbitofrontal cortex in contingent learning.* Neuron.

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Poster

159. Decision Making: Lateral Prefrontal Cortex

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 159.16/V31

Topic: H.01. Animal Cognition and Behavior

Support: NIH NIDA DA041480
NIH NIDA DA043443

Title: Orbitofrontal circuits influence multiple reinforcement learning processes

Authors: *S. M. GROMAN[1], A. KEIP[1], R. J. DILEONE[2], C. J. PITTENGER[3], D. LEE[4], J. TAYLOR[5];
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Abstract: Adaptive decision-making in dynamic environments requires multiple reinforcement-learning steps that may be implemented by dissociable neural circuits. The orbitofrontal cortex (OFC) has been established to be critically involved in decision making, but the contribution of individual OFC circuits to decision making is unknown. Here we used a novel directionally-specific viral ablation approach to investigate the function of several anatomically-defined OFC circuits during adaptive, flexible decision-making in rats trained on a probabilistic reversal learning (PRL) task. Specific OFC circuits were targeted by combining a floxed diphtheria toxin (DT) receptor virus with a retrograde Cre virus to selectively express DT receptors either in OFC neurons projecting to the nucleus accumbens (N=20), OFC neurons projecting to the amygdala (N=20) or amygdala neurons projecting to the OFC (N=20). Once decision making on the PRL was reestablished, individual circuits were ablated by systemic administration of DT (30 ug/kg; i.p.) and decision making reassessed on the PRL. Ablation of OFC neurons projecting to the nucleus accumbens selectively disrupted performance following a reversal, by disrupting the use of *negative outcomes* to guide subsequent choices. Ablation of amygdala neurons projecting to the OFC also impaired reversal performance, but due to disruptions in the use of *positive outcomes* to guide subsequent choices. Ablation of OFC neurons projecting to the amygdala, by contrast, enhanced reversal performance by destabilizing action values. Our data are inconsistent with a unitary function of the OFC in decision making. Rather, distinct OFC-amygdala-striatal circuits mediate distinct components of the action-value updating and maintenance necessary for adaptive decision making.

Disclosures: S.M. Groman: None. **A. Keip:** None. **R.J. DiLeone:** None. **C.J. Pittenger:** None. **D. Lee:** None. **J. Taylor:** None.

Poster

159. Decision Making: Lateral Prefrontal Cortex

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 159.17/V32

Topic: H.01. Animal Cognition and Behavior

Support: NIH NIDA DA041480
NIH NIDA DA043443

Title: Ablation of orbitofrontal projections to the nucleus accumbens disrupts punishment-induced suppression of drug-taking behaviors in rats

Authors: *A. J. KEIP, J. R. TAYLOR, S. M. GROMAN;
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Abstract: Disruptions in the ability to engage in flexible, goal-directed behaviors, and adaption to changing environments may be involved in both the etiology and consequence of drug addiction. Chronic exposure to drugs of abuse alters neural circuits and brain regions that support adaptive decision-making systems, including the orbitofrontal cortex (OFC). We have recently demonstrated that OFC circuits are involved in multiple reinforcement learning mechanisms and, specifically, have found that projections from the OFC to the nucleus accumbens (OFCNAc) are critical in the integration of negative outcomes into choice. Given that exposure to drugs of abuse also disrupts the utilization of negative outcomes, we hypothesized that compulsive drug-taking behaviors may be due to disruptions in the OFCNAc circuit. To test this hypothesis, we used a directionally-specific, viral approach to ablate OFCNAc projecting neurons and examined how disruptions in the OFCNAc circuit impacted methamphetamine self-administration. Ablation of the OFCNAc circuit did not alter the number of drug infusions animals earned across the 30 day self-administration assessment paradigm, but did result in higher breaking-points on a progressive ratio schedule compared to rats with an intact OFCNAc circuit (control). We then examined the ability of rats to integrate negative outcomes into drug-taking behaviors by assessing methamphetamine self-administration in the presence of probabilistically delivered, cued foot shocks. Rats with OFCNAc ablations self-administered significantly more methamphetamine compared to controls during these punished sessions, suggesting that the OFCNAc circuit is critical for the suppression of maladaptive behaviors. These results suggest that drug-induced disruptions in the OFCNAc circuit may be a mechanism by which uncontrollable, compulsive drug-use persists in the face of adverse consequences. Our ongoing studies seek to extend on these findings by modulating OFCNAc neural activity as well as the activity of other circuits involved in decision-making processes in order to further characterize their involvement in multiple drug-taking and drug-seeking behaviors.

Disclosures: A.J. Keip: None. **J.R. Taylor:** None. **S.M. Groman:** None.

Poster

160. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 160.01/V33

Topic: H.01. Animal Cognition and Behavior

Title: Reconsolidation and retrieval deficits following prediction error induced reactivation of remote and recent appetitive odor discrimination memory in Long Evans rats

Authors: *G. HANSON GOTTHARD, A. BASHFORD, D. BSALES, H. GURA, J.-A. GOLBITZ, R. SHEAR;
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Abstract: Reconsolidation theory states that previously-consolidated memories become destabilized when reactivated; however, a variety of boundary conditions exist (e.g., memory strength, prediction error, and memory age). The current study examined age of memory in male, Long Evans rats (n=30). Rats were trained on an appetitive odor discrimination task that involved digging for buried cereal rewards in cups of scented sand. Memory was reactivated 1 day (Recent; n=19) or 72 days (Remote; n=11) post-training with a single non-reinforced trial, followed by an intraperitoneal injection of cycloheximide (CHX; 1 mg/kg) or saline (SAL; 1 mg/kg). One week later, rats received a single non-reinforced test trial. Latency to dig declined significantly from the first to the last training trial in all groups [$F(1,26)=36.89$, $p<.001$, partial $\eta^2=.59$], and no differences were seen between groups during acquisition [$F(1,26)=.137$, $p>.05$]. During reactivation, rats in the Remote condition took longer to dig than rats in the Recent condition [$F(1,26)=20.59$, $p<.001$, partial $\eta^2=.44$]; however, no significant differences were observed between CHX or SAL for latency to dig [$F(1,26)=.032$, $p>.05$] or preference index (preference for correct cup) [$F(1,21)=.827$, $p>.05$]. During testing, rats in the Remote condition took significantly longer to dig than rats in the Recent condition [$F(1,26)=18.14$, $p<.001$, partial $\eta^2=.41$]. Additionally, CHX rats took longer to dig than SAL rats in the Recent and Remote conditions [$F(1,26)=9.50$, $p=.005$, partial $\eta^2=.27$]. Simple effects analyses revealed significant differences between CHX and SAL for Recent memory [$F(1,26)=6.269$, $p=.019$; partial $\eta^2=.19$] and marginally significant differences for Remote memory [$F(1,26)=3.88$, $p=.06$; partial $\eta^2=.13$]. None of the Remote/CHX rats dug on the test trial [χ^2 (3, N=30)$=14.115$, $p=.003$]. For rats that did dig on the test trial, no differences in preference index were observed for drug condition (CHX or SAL) [$F(1,15)=.002$, $p>.05$] or age condition (Recent or Remote) [$F(1,15)=.37$, $p>.05$]. These results suggest that CHX was effective in disrupting retrieval of recently and remotely acquired appetitive memory. The present study expands the literature on memory reconsolidation by examining the boundary condition of memory age in an appetitive task, rather than an aversive task. Past research using an aversive task showed that older memories were less susceptible to destabilization (e.g., Suzuki et al., 2004); however, the current study supports the notion that recent and remote memories are both susceptible to the retrieval-disrupting effects of a protein synthesis inhibitor.

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Poster

160. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 160.02/V34

Topic: G.03. Emotion

Support: University of Michigan Start-up Fund

Title: Disruption of reconsolidation of conditioned threat memories in rats by electroconvulsive shock

Authors: X. AN[1,2,4], **A. G. GIBSON**[1,2,3], A. WHITE[1,2,3], B. SAVARD[1,2], *J. DEBIEC[1,2]; [1]Mol. & Behavioral Neurosci. Inst., [2]Dept. of Psychiatry, [3]Neurosci. Grad. Program, Univ. of Michigan, Ann Arbor, MI; [4]Sch. of Educational Sci., Yangzhou Univ., Yangzhou, China

Abstract: The memory reconsolidation hypothesis posits that well-established memories become labile and susceptible to interference following reactivation or retrieval. An early study suggests that electroconvulsive shock (ECS) may disrupt the reconsolidation of threat conditioning in rats measured by the conditioned cue-controlled changes in licking behavior. However, the effects of ECS on other conditioned threat responses remain unknown. Here, we examined in rats the effects of ECS on reconsolidation of conditioned freezing responses. Rats underwent an auditory threat conditioning procedure consisting of a single 30s 5 kHz tone conditioned stimulus (CS) that terminated with a 0.6 mA 1 s electric shock to the footpads. On the following day, a group of animals was re-exposed to the CS in order to reactivate the memory and trigger reconsolidation processes. Immediately after, animals were anesthetized with isoflurane and received 50 Hz, pulse width: 0.7 ms, duration: 1 s, 50 mA ECS (Retrieval-ECS) or not (Retrieval-Sham ECS). Another group of animals after a re-exposure to the CS received isoflurane anesthesia and ECS with a 4-hour delay (Retrieval-4hr delayed ECS). An additional group of animals, on the day following threat conditioning, received either ECS (No retrieval-ECS) or sham ESC (No retrieval-sham ECS) under isoflurane anesthesia without a prior re-exposure to the CS. On the following day, all animals received the memory retention test consisting of exposures to the CS. Analysis of immobility or freezing behavior during the presentations of the CS revealed that the Retrieval-ECS group displayed significantly less freezing than other experimental groups. This pattern of findings suggests that the ECS disrupted the reconsolidation of conditioned threat memories. Ongoing experiments are focused on determining brain site-specific effects of ECS on the reactivated threat conditioned memories.

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Poster

160. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 160.03/V35

Topic: H.01. Animal Cognition and Behavior

Support: Grant-in-Aids for Scientific Research (A) 15H02488
Grant-in-Aid for Scientific Research(A) 18H03944

Title: Hippocampal neuroinflammation cytokine TNFα negatively regulates retrieval and reconsolidation, but not encoding and formation, of contextual fear memory

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Abstract: Pavlovian fear conditioning generates fear memory, reflecting an association between the conditioned stimulus (CS) and unconditioned stimulus (US). This CS-US association is stabilized through gene expression-dependent memory consolidation, generating a long-term fear memory (LTM). Interestingly, retrieval of fear memory initiates gene expression-dependent process reconsolidation (Suzuki et al, 2004). On the other hand, brain microglia generates tumor necrosis factor α (TNFα), a key mediator of neuroinflammation. TNFα has been suggested to induce synapse/spine degeneration and inhibit long term potentiation (LTP). Interestingly, previous studies have suggested that hippocampal TNFα plays critical roles in regulation of contextual fear memory (Yu et al., 2016). However, roles of hippocampal TNFα in fear memory processes remain unclear. Here we examined effects of micro-infusion of TNFα into hippocampus on regulation of contextual fear memory. Mice were trained with a single footshock and 24 hours later, assessed freezing responses by the re-exposure to the training context (test). Mice showed normal LTM during the test when mice received micro-infusion of TNFα 6 hrs before the training, suggesting that mice micro-infused TNFα show normal encoding and consolidation of contextual fear memory. Interestingly, hippocampal TNFα micro-infusion 6 or 18 hrs, but not 2 or 24 hrs, before the test significantly reduced freezing responses during the test, compared with control group. These observations suggested that hippocampal TNFα micro-infusion impairs retrieval of contextual fear memory with critical time window affecting memory retrieval. Furthermore, mice micro-infused TNFα still showed reduced level of freezing when re-tested 24 hrs after the test, suggesting that TNFα impairs reconsolidation of contextual fear memory. To examine effects of TNFα-infusion on memory retrieval at the molecular level, we examined effects of this TNFα micro-infusion on c-fos expression that is regulated in a neural activity-dependent manner and induced in response to memory retrieval. We measured the number of c-fos positive cells at 90 min after the test. Consistent with behavioral observations,

mice micro-infused TNFα 6 hrs before test showed significantly less c-fos positive cells in the CA1 area of the hippocampus compared with control group, confirming that micro-infusion of TNFα blocks memory retrieval. Collectively, our findings suggest that hippocampal TNFα negatively regulates fear memory retrieval and reconsolidation.

Disclosures: S. Takahashi: None. **H. Fukushima:** None. **S. Kida:** None.

Poster

160. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 160.04/V36

Topic: H.01. Animal Cognition and Behavior

Support: NIH NS097750 (V.S.)
NJCBIR CBIR16IRG017 (V.S.)

Title: Resolving the interneuronal drivers of functionally and temporally diverse inhibitory regulation of dentate projection neuron

Authors: *M. AFRASIABI[1], V. SANTHAKUMAR[2];
[1]Rutgers, New Jersey Med. Sch., Newark, NJ; [2]Pharmacology, Physiol. & Neurosci., New Jersey Med. Sch. Dept. of Pharmacol. and Physiol., Newark, NJ

Abstract: The hippocampal dentate gyrus is notable for its sparse activity and tight inhibitory regulation essential for its role in memory processing. Compromises in dentate inhibition have been implicated in epilepsy. Dentate projection neurons, the classic granule cells (GCs) and the recently characterized semilunar granule cells (SGCs), are inhibited by diverse interneuronal subtypes including fast-spiking interneurons (PV-IN), neurons expressing cannabinoid receptor type 1 (CB1R) and somatostatin interneurons. Additionally, SGCs have been proposed to support sustained feedback inhibition of GCs. However, which interneuron populations inhibit GCs and SGCs during epochs of steady state, and afferent activation is not fully understood. Here we examined the contribution of PV-IN and CB1R sensitive interneurons to GC and SGC. Whole cell current and voltage clamp recordings were obtained from GCs and SGCs in hippocampal slices (350 µm) from Wistar rats (P20-25) and mice (C57BL/6, PV-ChR2, PV-NpHR2, 6-8 weeks). IPSCs were recorded using KCl-based internal solution in glutamate receptor antagonists or as outward currents using Cs-MeSO4. SGCs and GCs were identified based on post-hoc analysis of biocytin immunostaining. As reported in rats, SGCs in mice had wider dendritic arbors, inner molecular layer axon collaterals and lower spike frequency adaptation and received more steady-state sIPSCs than GCs. The CB1R agonist, WIN 55,212-2 (5µM) failed to reduce baseline sIPSCs frequency in both GCs and SGCs. Although optical activation of PV-IN expressing channelrhodopsin enhanced sIPSC frequency, optical suppression of PV-IN

expressing halorhodopsin failed to reduce sIPSC frequency in either cell type. Optical suppression of PV-INs reduced firing in YFP-expressing PV-INs confirming the experimental system. Surprisingly, optical suppression of PV-IN increased the peak amplitude of PP-evoked IPSC in both cell types and selectively reduced the frequency of sustained evoked inhibitory response in SGCs without reducing IPSC frequency in GCs. Our data indicate that CB1R sensitive interneurons and PV-IN have limited contribution to steady state sIPSCs in GCs and SGCs. The paradoxical increase in PP-evoked IPSC amplitude upon suppression of PV-INs suggests that circuit mechanisms may compensate for reduction in PV-IN inhibition. PV-INs contribute substantively to late sustained inhibitory activity in SGCs upon paired PP-stimulation. These differences in inhibitory inputs to GCs and SGCs could support distinct functional roles for the two cell types in the dentate network.

Disclosures: M. Afrasiabi: None. **V. Santhakumar:** None.

Poster

160. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 160.05/V37

Topic: H.01. Animal Cognition and Behavior

Support: CAPES
CNPq

Title: Role of prelimbic cortex protein kinases C and Mzeta in aversive memory reconsolidation and persistence

Authors: *T. R. DA SILVA[1], A. M. RAYMONDI[1], L. J. BERTOGLIO[2], R. ANDREATINI[1], C. A. J. STERN[1];
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Abstract: Investigating the mechanisms underlying aversive memory reconsolidation and persistence may improve the understanding of posttraumatic stress disorder development and treatment. Proteins kinase C (PKC) and kinase Mzeta (PKMζ) are necessary for the late stages of long-term memory formation and maintenance. Prelimbic cortex (PL) activity underlies fear memory reconsolidation and persistence. Here, we investigated the contribution of PL PKC and PKMζ to memory reconsolidation and persistence. Male Wistar rats with bilateral guide cannulas aimed at the PL underwent contextual fear conditioning, which consisted of familiarization to the Context A (day 1), conditioning (Context A-shock pairing; day 2), a brief Context A reexposure (reactivation; day 3), and subsequent Context A reexposures to estimate the drug effects on days 4, 11 and 25 (Tests A1, A2 and A3). Independent groups received vehicle (VEH), the unselective

PKC inhibitor chelerythrine (CHE; 1 or 3nmol/0.2μL/side), the selective PKMζ inhibitory peptide (ZIP; 10 nmol/0.2μL/side) or scrambled-ZIP (scr-ZIP; 10 nmol/0.2μL/side) immediately, 1, 6, 9, 12 or 18 hours after reactivation. A group had the memory reactivation prevented by the i.p. administration of nimodipine 16 mg/kg, and 6 hours later the animals received VEH or CHE 3 nmol. Freezing behavior was assessed. Data were analyzed by repeated measures ANOVA followed by Newman-Keuls test ($P \leq 0.05$). CHE-treated rats (1 or 3 nmol) immediately after memory reactivation presented significantly less freezing than controls during Tests A1 and A2, indicating a role for PKC in memory reconsolidation. The groups treated with CHE 3 nmol 6, 9 or 12 hours after memory reactivation presented less freezing than controls during Test A2, indicating a role for PKC in memory persistence. When CHE was given 18 hours after reactivation, it produced no changes in freezing expression. When memory reactivation was omitted, the infusion of CHE 6 hours later no longer reduced freezing time during Tests A1 and A2. Animals treated with ZIP 1 or 6 hours after memory reactivation showed less freezing time than controls (scr-ZIP) during Tests A2 and A3, indicating a role for PKMζ in the persistence of memory. No differences were observed in groups treated with ZIP immediately after reactivation of memory. The findings suggest that PKC activity in PL is necessary for both reconsolidation and persistence while PKMζ is necessary only for persistence of reactivated fear memory. Altogether, the present findings suggest that different mechanisms underlie fear memory reconsolidation and persistence in PL cortex.

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Poster

160. Memory Consolidation and Reconsolidation: Behavior

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Program #/Poster #: 160.06/V38

Topic: H.01. Animal Cognition and Behavior

Support: Consolidator Grant of the European Research Council (ERC), number 648176

Title: Recovery of generalized fear memory after experimentally induced amnesia challenges the reconsolidation blockade account of forgetting

Authors: *J. M. ALFEI PALLONI[1], D. DE BUNDEL[2], L. LUYTEN[1], T. BECKERS[3]; [1]KU Leuven, Leuven, Belgium; [2]Dept. of Pharmaceut. Sci., Vrije Univ. Brussel, Brussels, Belgium; [3]KU Leuven & Leuven Brain Inst., Leuven, Belgium

Abstract: Consolidated fear memories can be destabilized and rendered vulnerable to various pharmacological agents that disrupt the later expression of memory (i.e., amnesia). Such pharmacologically induced amnesia has particularly been studied in AAA experimental designs,

where memory is initially created for context (or stimulus) A (conditioned context; CX) and later reactivated and tested using the same CX. The present study aimed to gain more insight in the conditions that govern the induction and generalization of post-retrieval amnesia. In 10 experiments using contextual fear conditioning in rats and post-retrieval injection of Midazolam (MDZ), a positive allosteric modulator of the GABA-A receptor, as amnestic agent, we found that post-retrieval amnesia after re-exposure to training context A (AAA) generalizes readily to a similar but distinguishable context B (AAB). Amnesia was also observed when memory reactivation was conducted through exposure to generalization context B prior to MDZ administration and fear was later tested for that same context B (ABB), but fear recovered when animals were instead exposed to the original training context A (ABA) or an equally similar but novel context C (ABC) at test. Critically, we also demonstrated that MDZ-induced ABB amnesia exhibits critical features of a reconsolidation-dependent effect: (1) The amnestic potential of MDZ administration is restricted to a limited time window following memory reactivation: Amnesia was observed on a LTM test when MDZ was given shortly after memory reactivation but not when it was administered 6 h after reactivation. (2) MDZ-induced amnesia was expressed on a LTM test but not on a STM test after drug administration. (3) Administration of ifenprodil, a GluN2B-NMDA antagonist that prevents fear memory destabilization, prior to memory reactivation blocked the post-retrieval effects of MDZ. Overall, our data reveal a lack of generalization of MDZ-induced amnesia. Our results challenge the reconsolidation blockade account of pharmacologically induced selective retroactive amnesia and raise important questions about the viability of reconsolidation-based interventions for the treatment of emotional disorders.

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Poster

160. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 160.07/V39

Topic: H.01. Animal Cognition and Behavior

Support: NSERC Grant

Title: M_1 muscarinic receptor activity is necessary for retrieval-induced object memory updating: Evidence from a newly developed memory modification task for rats

Authors: *K. H. JARDINE, C. E. WIDEMAN, C. C. MACGREGOR, K. A. MITCHNICK, B. D. WINTERS;
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Abstract: Memory traces can be reactivated with retrieval, causing a protein degradation-dependent destabilization process that renders the memory labile. It has been found that destabilized memories can be erased or strengthened within a certain period of time following reactivation, but there is limited research characterizing the mechanisms underlying retrieval-induced qualitative updates (i.e., information integration), which are equally, if not more, important to daily life. We have previously implicated cholinergic transmission in object memory destabilization. Here we posit that this acetylcholine (ACh)-mediated mechanism is essential for information integration that results from new learning experiences following memory trace reactivation. We previously reported that activation of the M_1 muscarinic receptor subtype triggers a cellular cascade that could promote the protein breakdown implicated in destabilization, likely by mobilizing intracellular proteasomes that target post-synaptic density proteins, preceding retrieval-induced object memory "erasure". The involvement of this cascade in retrieval-induced information integration has not been empirically tested due to the lack of a robust rodent model of qualitative memory change. The present study addresses this gap with a newly developed spontaneous object memory modification task for rats. In this task, rats sample an object, the memory of which is destabilized by a brief re-exposure 24h later.
After reactivation, rats explore an alternate empty context. On test day, rats explore the sampled objects less when they are in the same alternate context as the reactivation phase compared to a different alternate context. Thus, the object-context configuration is treated as 'familiar', and this only occurs when the context is presented within 3h after object memory reactivation. Blockade of M_1 receptors by pirenzepine in perirhinal cortex prior to object memory reactivation prevented this apparent contextual updating of the original memory, supporting our hypothesis that M_1 receptor activation is required for object memory destabilization as the gateway to memory modification.
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Poster

160. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 160.08/V40

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant MH118754
NIH Grant MH104384

Title: Optogenetic stimulation of the pathway from the basolateral amygdala to the medial entorhinal cortex after spatial Barnes training increases activity-regulated cytoskeletal-associated protein (ARC) in the dorsal hippocampus

Authors: *K. L. WAHLSTROM[1], R. T. LALUMIERE[2];
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Abstract: Previous work suggests that hippocampus-based systems mediate spatial learning and that the basolateral amygdala (BLA) modulates the consolidation for this type of learning. The medial entorhinal cortex (mEC) is a critical region in the hippocampus-based system for processing spatial information and we have previously shown the pathway from the BLA to the mEC to be a critical mechanism by which the BLA influences spatial learning. Other studies have found that administration of memory-enhancing drugs into the BLA increases protein levels of activity-regulated cytoskeletal element-associated protein (ARC) in the dorsal hippocampus and that blocking ARC impairs spatial memory consolidation. However, whether altering activity in the pathway from the BLA to the mEC also influences ARC expression in the dorsal hippocampus in a manner dependent on learning is unknown. Therefore, to address this question, male Sprague-Dawley rats were given intra-BLA AAV injections containing the opsin ChR2(E123A) under the control of the CaMKIIα promoter and then optical fiber implants aimed at the mEC. The rats were then trained on a Barnes maze, using a spatial training version in which the rats had to use extra-maze cues in order to find the escape port. Rats underwent three consecutive trials of training and then, immediately after training, received optical stimulation of the BLA-mEC pathway, using parameters we have previously found to be effective at enhancing the consolidation of memory for this task. The rats were sacrificed 1 h after the start of training and brains were removed and flash-frozen. Tissue punches were collected for ARC protein analysis. Western blot was used to determine the density of ARC protein in the dorsal hippocampus as wells a control region in the sensorimotor cortex. Results revealed that there were significantly higher levels of ARC in the dorsal hippocampus of rats that received optical stimulation of the BLA-mEC pathway compared to sham control animals. There were no significant differences in ARC levels in the control sensorimotor region between groups, suggesting a region-specific increase in ARC levels. These findings suggest a mechanism by which BLA-mEC stimulation enhances spatial memory in rats.

Disclosures: K.L. Wahlstrom: None. **R.T. LaLumiere:** None.

Poster

160. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 160.09/V41

Topic: H.01. Animal Cognition and Behavior

Support: NSFC 81070881

Title: Selective deletion of Dnmt1 and Dnmt3a in a subpopulation of excitatory or inhibitory neurons affects different forms of learning and memory

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Abstract: Studies in recent years have indicated that DNA methylation participates in the processes of memory formation and consolidation by regulating the expression of memory-related genes. DNMT1 and DNMT3A are two major DNA methyltransferases expressed in mature neurons. To fully study the effect of DNA methylation on learning and memory, and to explore the underlying mechanisms,we established *αCaMKII-Cre; Dnmt(3a,1)$^{2flox/2flox}$*, *Dlx5/6-cre;Dnmt(3a.1)$^{2flox/2flox}$*,*Pv-cre ; Dnmt(3a.1)$^{2flox/2flox}$* and *Sst-cre ; Dnmt(3a.1)$^{2flox/2flox}$* mice lines by Cre-LoxP recombinase system. These mutant mice allow us to genetically knock out *Dnmt1* and *Dnmt3a* gene in αCaMKII^{+} excitatory neurons, DLX5/6^{+} , PV^{+} or SST^{+} inhibitory neurons. We found that *αCaMKII-Cre;Dnmt(3a,1)$^{2flox/2flox}$* mice displayed enhanced motor learning, and impaired spatial memory and recognition memory .*Dlx5/6-Cre;Dnmt(3a,1)$^{2flox/2flox}$* mice showed motor learning disabilities and spatial memory deficit,while *Pv-cre ; Dnmt(3a,1)$^{2flox/2flox}$* and *Sst-cre ; Dnmt(3a,1)$^{2flox/2flox}$* mice only showed spatial memory deficit similar as that observed in *Dlx5/6-Cre;Dnmt(3a,1)$^{2flox/2flox}$* mice. To explore the network mechanism of spatial memory deficits in these mutant mice, we are using fiber photometry to record population neuronal activity and head-mounted miniature microscope (miniscope) to record population calcium spikes in the CA1 region of the dorsal hippocampus.

Disclosures: W. Sun: None. **M. Yu:** None. **Y. Zhou:** None.

Poster

160. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 160.10/V42

Topic: H.01. Animal Cognition and Behavior

Support: NSERC Discovery Grant

Title: New spatial learning dynamics in mice differ by environmental familiarity

Authors: *E. G. FRASER, R. J. MCDONALD;
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Abstract: Encoding spatial information differs based on environment familiarity. For the initiation of LTP, while NMDA receptors in the hippocampus are necessary for rats to learn in unfamiliar environments, they are unnecessary for learning in familiar environments (Bye & McDonald, 2019). Cellular representations of space, the basis of spatial learning, also differ based on environment familiarity. As familiar environments are modulated either by morphing from one to another (Leutgeb et al., 2005), or by rotating local and distal cues (Knierim, 2002), neural place codes in rats can reflect these modulations. Furthermore, shifting reward locations in a continuous T alternation task is also represented in rat place cell activity (Lee, Griffin, Zilli, Eichenbaum, & Hasselmo, 2006). These data indicate that both degrees of spatial familiarity, and shifting goals are encoded for by rat hippocampal place cells. Recent technical advances allow imaging place cells in freely moving mice (Ghosh et al., 2011) but there is a dearth of spatial learning tasks developed for mice that measures learning based on environmental familiarity. These experiments describe a spatial learning task in which all external cues were under experimental control, and mice were assessed on rapid new learning with familiar versus new spatial information. 22 male C57BL/6J mice were trained to locate a water reward in an open field task over 5 days, 6 trials per day. On the 6^{th} day, they were split into three rapid learning groups, a same location group (n=7), a same cues-new location group (n=7), and a new cues-new location group (n=8). These groups underwent new learning for the location of the water reward over 10 trials in 1 hour. On the 7^{th} day, mice underwent a 20 second probe, still under water restriction, and on the 8^{th} day they underwent a subsequent 20 second probe after 24 hours of ad libitum access to water. During rapid learning, both new location groups showed increased path length and latency to retrieve the reward, and the new cues-new location group showed marked improvements after the first trial of new learning. During the first probe, the new location group showed a preference for the new reward location, while the same location group showed a preference for the initial reward location. During the second probe, there was no discernable effect. This task is rapidly acquired, easily manipulated, and shows a robust learning effect, making it ideal for experiments analyzing place cell activity and other learning mechanisms in freely moving mice.

Disclosures: **E.G. Fraser:** None. **R.J. McDonald:** None.

Poster

160. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 160.11/V43

Topic: H.01. Animal Cognition and Behavior

Support: UNAM, PAPIIT - IN300806

Title: Acute stress impairs memory in ETM in female and male rats and modifies serotoninergic and dopaminergic dorsal striatal systems

Authors: *E. A. RENDON-OCHOA[1], M. R. GONZALEZ LOPEZ[1], N. L. GARCIA SALDIVAR[1], J. MONROY[2], R. DOMINGUEZ[2], S. E. CRUZ-MORALES[1];
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Abstract: The striatum has been implicated in several cognitive processes, such as non-declarative procedural memory and stress regulation. Dopamine (DA) and serotonin (5-HT) are essential in memory formation. Nevertheless, it has been observed that exposure to stress impairs memory formation and retention and catecholamine synthesis and release in male rats, but the information in female rats is scarce and sometimes contradictory. Considering that ovarian hormones may influence memory formation and susceptibility to stress, the objective of this work was to study the sexual differences of 180 minutes of acute restriction stress (R) on elevated T-maze (ETM) performance and DA and 5 - HT concentrations in dorsal striatum. Rats were assigned to nine groups (n= 70) as follows: three intact groups [male (M), female in proestrus (P) and female in diestrus 2 (D2)] were trained in ETM (control group). Three groups were restricted for 180 minutes and trained in ETM (R+ETM group) and three groups were restricted only (R group). DA and 5-HT concentrations, metabolites and neuronal activity (based upon neurotransmitter and metabolite concentration) were measured for all groups. In the training sessions (day 1) all intact groups performed equally. In the testing session (day 2) rats in D2 showed detriment in memory retention suggesting that ovarian hormones affect memory retention even without stress exposure. R, on the other hand, impaired memory formation in all groups compared to their non-stressed counterparts. For memory retention, rats in P and M showed latencies significantly lower than non-stressed rats, indicating that this quantity and quality of stress, in fact, reduced memory formation and retention in ETM test independently of gender and ovarian hormones. The neurochemistry analysis showed that 5-HT concentrations are lower in intact M rats than in female (both P and D2). R and R+ETM decreased in D2 only. No differences were found in 5-HIAA concentrations. 5-HT activity was lower in D2 and higher in M with R. Finally, for DA, R and R+ETM decreased DA concentrations in D2 only. In M, R produced an increased in DA concentrations. These results indicate that R has an impact on memory formation in all rats and modifies 5 - HT and DA concentration in a gender-dependent manner.

Disclosures: E.A. Rendon-Ochoa: None. **M.R. Gonzalez Lopez:** None. **N.L. Garcia Saldivar:** None. **J. Monroy:** None. **R. Dominguez:** None. **S.E. Cruz-Morales:** None.

Poster

160. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 160.12/V44

Topic: H.01. Animal Cognition and Behavior

Title: Stress hormone effects on memory accuracy in an object-in-context task in mice

Authors: *C. GUO[1], M. TIMPLALEXI[1], P. COLUCCI[1,2], M. HENCKENS[1], B. ROOZENDAAL[1];
[1]Donders Inst. For Brain, Cognition and Behavior Radboud Univ. Med. Center,, Nijmegen, Netherlands; [2]Dept. of Physiol. and Pharmacol. . Sapienza Univ. of Rome, Rome, Italy

Abstract: Stress and emotional arousal induce the activation of two major stress hormones, norepinephrine and corticosteroids (CORT; corticosterone in rodents, cortisol in humans). Although it is now well accepted that these two stress hormones create stronger memories, it remains unclear, however, how this strengthening affects the quality of such memories. This experiment investigated the effects of norepinephrine and CORT on memory accuracy in an episodic-like object-in-context task. On the training session, male C57BL/6J mice could explore two identical objects in one context and immediately afterwards explored another set of two identical objects in a second context. The noradrenergic stimulant yohimbine (0.3, 1 or 3 mg/kg), CORT (1, 3 or 10 mg/kg) or their respective vehicle were administered systemically immediately after the training. On the 24-h retention test, mice were placed in one of these two training contexts (in counterbalanced order) with one of each of the different training objects. Accurate episodic-like memory was reflected by a preferred exploration of the object they had not seen previously in that particular context. We found that both yohimbine and CORT treatment induced dose-dependent enhancement of episodic-like memory. Currently, we are investigating whether norepinephrine and CORT increase neuronal activity within the hippocampus during the memory consolidation phase after training, as would be in line with this memory-enhancing effect. These findings indicate that both norepinephrine and CORT enhance the accuracy of episodic-like memory.

Disclosures: C. Guo: None. **M. Timplalexi:** None. **P. Colucci:** None. **M. Henckens:** A. Employment/Salary (full or part-time):; junior PI. **B. Roozendaal:** A. Employment/Salary (full or part-time):; Professor.

Poster

160. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 160.13/V45

Topic: H.01. Animal Cognition and Behavior

Title: Hippocampal $GABA_A$ α5 subunit and BDNF expression in a postnatal day 7 model of fetal alcohol syndrome: Role in memory impairment

Authors: *V. LOCCI[1], E. GATTA[2], S. C. PANDEY[2,3], D. R. GRAYSON[1,2];
[1]The Psychiatric Institute, Dept. of Psychiatry, Col. of Medicine, Univ. of Illinois at Chicago, Chicago, IL; [2]Ctr. for Alcohol Res. in Epigenetics, Dept. of Psychiatry, Col. of Medicine, Univ. of Illinois at Chicago, Chicago, IL; [3]Jesse Brown VA Med. Ctr., Chicago, IL

Abstract: Fetal Alcohol Syndrome is characterized by neurobehavioral impairments, such as attention deficit, mild to severe learning impairment in children, and psychopathologies in adulthood. Acute exposure to ethanol in mice during the first week of life triggers apoptotic neurodegeneration in many regions of the developing brain with subsequent neurobehavioral impairments. To better understand the consequences of a prenatal ethanol exposure, we used a post-natal day 7 (PND7) ethanol exposure mouse model that mimics the third trimester of gestation in humans. We focused primarily on learning and memory deficits in treated mice. Swiss albino ND4 male mice were injected twice with either saline or ethanol 20% on PND7 (2.5 g/kg, sc, 2h apart). Recognition memory was studied in males at PND65, using the Novel Object Recognition (NOR) test. In the memory test, PND65 mice showed a significant lack of discrimination between a familiar and the novel object, with no significant difference in the exploration index. Because the hippocampus is the main site of learning and memory, we assessed if this memory impairment could be due to changes in the expression of selected target genes using qRT-PCR in hippocampus. We studied the expression of the α5 subunit of the GABAA receptor and *Bdnf* gene, in adult male mice and in PND9-12 pups. Ethanol-treated pups showed a decrease in α5 receptor subunit expression at PND9, and no changes at PND12. They also showed no changes in mRNAs encoding Bdnf exons VI and IX expression. In contrast, adult mice showed a significant increase in expression of the α5 receptor subunit mRNA, as well as significant increases in Gad1 and Gad2 mRNAs. We also detected a large reduction in both Bdnf VI and XI expression in the hippocampus. In addition, we detected no changes in the expression of the Nr2a and Nr2b receptor mRNAs. The changes noted above were detected in the adult hippocampus and were not present in the PND9-12 pups, suggesting that they occurred after alcohol was no longer present in the mice. The observed memory impairment may be associated with alterations in synaptic and extra-synaptic GABA receptor signaling and an imbalance in excitatory/inhibitory neurotransmission.

Disclosures: **V. Locci:** None. **E. Gatta:** None. **S.C. Pandey:** None. **D.R. Grayson:** None.

Poster

160. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 160.14/V46

Topic: H.01. Animal Cognition and Behavior

Support: NIH R21 AA024983

Title: The effects of moderate prenatal alcohol exposure on the organization of exploratory behavior by adult rats

Authors: *M. GONCALVES GARCIA[1], L. E. BERKOWITZ[1], T. DONALDSON[1], R. E. HARVEY[1], J. L. WAGNER[2], S. DAVIES[2], D. D. SAVAGE II[3], B. J. CLARK[1];
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Abstract: A large body of research has indicated that moderate prenatal alcohol exposure (PAE - 60 mg/dl peak blood alcohol content) can produce subtle impairments on cognitive processes, such as spatial learning and memory (Savage et al., 2010). The neurobiological basis of these impairments is poorly understood but may be linked to alterations in hippocampal functioning. Although the hippocampus has a central role in learning and memory, damage to the hippocampus can also produce impairments in locomotor and exploratory behaviors by rodents (Thompson et al., 2018). Rodent exploratory behavior is organized around home bases, which serve as central points of attraction from which animals organize their exploratory excursions into the remaining environment. Although rats can express home bases in featureless environments and in darkness, they are typically established near prominent environmental cues. Given the evidence of impaired spatial memory in PAE rats, we sought to test the hypothesis that exploratory behaviors are disrupted after moderate prenatal alcohol exposure. In the present study, we utilized an established rat model of moderate prenatal alcohol exposure (Davies et al., 2018). Adult PAE and saccharin (SACC) control rats were allowed to freely explore a circular open field (5 ft dia) for ~30min. Animals were tested in two conditions: a dark test in which the room lights were turned off and a lighted condition in which the room was illuminated. We specifically predicted that PAE rats would express disrupted home base establishment in the absence of visual cues (i.e., the dark condition), similar to what is observed in hippocampal lesioned animals (Hines & Whishaw, 2005). Our results indicated that PAE rats were able to establish home bases within the first 5 minutes of exploration under both light and dark conditions. Furthermore, exploratory behaviors, such as excursions to and from the home base, stops within the home base, total distance traveled, and grooming were also observed to be

similar to that of SACC controls. These results indicate that rats exposed to moderate levels of alcohol prenatally are able to organize their exploratory behavior around home base locations. While this pattern of behaviors may indicate that moderate PAE does not impair the gathering of spatial information from a novel environment, future studies are needed to determine whether spatial information is retained after exploratory behavior.

Disclosures: **M. Goncalves Garcia:** None. **L.E. Berkowitz:** None. **T. Donaldson:** None. **R.E. Harvey:** None. **J.L. Wagner:** None. **S. Davies:** None. **D.D. Savage II:** None. **B.J. Clark:** None.

Poster

160. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 160.15/W1

Topic: H.01. Animal Cognition and Behavior

Support: AARG-17-531572

Title: Distributive home base behavior in the TgF344-AD rat model of Alzheimer's disease

Authors: L. E. BERKOWITZ[1], ***M. A. GABALDON-PARISH**[1], R. E. HARVEY[2], E. A. SNEDDON[4], B. J. CLARK[3];
[2]Dept. of Psychology, [3]Psychology Dept., [1]Univ. of New Mexico, Albuquerque, NM;
[4]Psychology - Brain, Cognition, and Develop. Program, Miami Univ., Oxford, OH

Abstract: Spatial disorientation is an important behavioral marker of Alzheimer's disease (AD). The TgF344-AD rat model of AD, which expresses the full spectrum of AD pathology observed in humans, serves as an ideal rodent model for evaluating behavioral features of AD. TgF344-AD rats have been shown to exhibit reference memory and reversal learning impairments that progressively worsen over time. These deficits may correspond to how rodents organize their movements and interact with environmental landmarks during exploration. Thus, we sought to evaluate the exploratory behaviors of TgF344-AD rats and F344 controls. One such behavior is the establishment of a "home base," or a place in the environment that the animal will use as a reference point for navigation. Research has shown that familiarity with an environment and environmental landmarks contribute to the establishment of a home base. Given TgF344-AD rats seem to become impaired at forming a reference memory, we sought to determine whether home base behavior was also impaired. TgF344-AD rats (n=16) and aged matched F344 controls (n=12) were evaluated in a landmark no-landmark open-field paradigm. Results indicated that the home bases of TgF344-AD rats were more distributed throughout the environment and that stops made during exploration were further from established home bases relative to controls. In addition, home base behavior in TgF344-AD rats was more distributed after a proximal cue was

removed, indicating that TgF344-AD rats may be more reliant on an egocentric (body-centered) reference frame when establishing spatial relationships. These findings correspond to how humans with early AD use egocentric cues more effectively than world-centered (map-like) cues during navigation. Overall, these findings support the importance of investigating how spatial cues influence spatial disorientation in Alzheimer's disease.

Disclosures: L.E. Berkowitz: None. **M.A. Gabaldon-Parish:** None. **R.E. Harvey:** None. **E.A. Sneddon:** None. **B.J. Clark:** None.

Poster

160. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 160.16/W2

Topic: H.01. Animal Cognition and Behavior

Support: VA RR&D Award #IK2 RX002488

Title: Chronic repetitive mild traumatic brain injury disrupts reference memory in adult male Sprague-Dawley rats

Authors: *T. E. WHITE[1,2], **P. T. JUZANG**[1,2], M. A. GRAHAM[2,1];
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Abstract: Background
The behavioral deficits due to repetitive mild traumatic brain injury (rmTBI) or multiple concussions has become a major concern for participants of contact sports and the military population. Even when minimal anatomical brain damage is detected, the physical and psychological deficits can be severe. Deficits in memory and cognition are often reported as some of the persisting symptoms after rmTBI. In this experiment, we probed for these deficits in an experimental model of rmTBI.
Methods
Adult male Sprague-Dawley rats received unilateral mild controlled cortical impacts (angle: 15° from vertical; velocity: 6 m/s; depth: 0.5 mm; dwell time: 100ms). Each rat received a total of 4 injuries, 5 days apart. Control rats received a craniotomy and 3 subsequent re-exposures, 5 days apart. 14 weeks after the last injury, the rats were evaluated for memory deficits using the Morris Water Maze. Over 4 days, the rats performed 4 training (day 1) and 20 experimental (8-day 2; 8-day 3; 4-day 4) platform trials with start positions randomly and evenly distributed across the four quadrants of the maze. The platform was fixed in the NW quadrant during all platform trials. A probe trial with the platform removed was performed on day 4 (after the last platform trial), starting in the SE quadrant, to assess reference and search strategy.
Results

Platform trials: While there was a small trend towards rmTBI rats showing greater overall latency to the platform than controls, the difference in this measure was only significant when the trial began in the NE quadrant (p=0.04). The rmTBI rats also spent more time than controls in the NE quadrant when the trial began in the NE quadrant (p=0.025). When the trial began in the SE quadrant, rmTBI rats spent more time in the NW and SW quadrants than controls (p=0.015; p=0.024).
Probe trials: The rmTBI rats spent more time than controls moving towards the platform zone during probe trials (p=0.050).
Search Strategy: While there were no significant differences in the search strategies employed by rmTBI rats and controls, there are strong trends towards the rmTBI rats employing a higher percentage nonspatial search strategies.
Conclusions
This data was gathered during the establishment of the chronic rmTBI model in our laboratory. Overall, the persistent symptomology for this model has been mild, showing trends towards expected outcomes. We are in the process of optimizing the injury model to obtain more significant deficits that will allow this model to be utilized for testing therapeutic strategies and improving diagnostic techniques.

Disclosures: T.E. White: None. **P.T. Juzang:** None. **M.A. Graham:** None.

Poster

160. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 160.17/W3

Topic: H.01. Animal Cognition and Behavior

Support: CSUN Thesis Support Program

Title: Examining cognitive deficits in IL-6 deficient mice in a high fat diet induced neuroinflammatory state

Authors: *T. SIMON, S. SHATELA, P. SONI, P. GHOTRA, L. R. BANNER;
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Abstract: Currently more than one-third of the population in the United States is obese and this percentage is also projected to increase in the future. Increases in the number of cases of insulin-resistance and type-2 diabetes have paralleled the obesity rates. Studies have shown that obesity and type-2 diabetes significantly increases the risk of dementia and Alzheimer's disease and changes in hippocampal plasticity and spatial learning have been documented. While the exact mechanism has yet to be fully understood, neuroinflammation is thought to be an important factor in impaired cognitive function as inflammation in the absence of obesity can cause

cognitive deficits. The process of inflammation is regulated at almost every stage by changes in the expression of small signaling proteins known as cytokines. We are interested in the cytokine interleukin-6 because levels are increased in the brains during obesity where it is associated with cognitive decline. Patients with a number of neurodegenerative diseases show elevated IL-6 in the hippocampus and other cortical areas which is associated with poorer cognitive function. Treatments that reduce neuroinflammation and lower levels of interleukin-6 result in improvements in cognition.
To address the role of IL-6 in obesity-induced cognitive decline, we are studying the behavioral changes in IL-6 knockout (KO) vs wildtype (WT) C57BI6/J mice following consumption of a high fat diet (HFD). Young adult mice at 12 weeks of age fed a HFD for 20 weeks developed obesity and elevated blood glucose levels. Animals were for tested for memory using Novel Object Recognition and Morris Water Maze and anxiety using Elevated Plus Maze. Results suggest that obese mice missing IL-6 display subtle differences in both memory and anxiety. Continued behavioral and molecular analysis will yield additional information on the role of this pleiotropic cytokine in learning and memory.

Disclosures: **T. Simon:** None. **S. Shatela:** None. **P. Soni:** None. **P. Ghotra:** None. **L.R. Banner:** None.

Poster

160. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 160.18/W4

Topic: H.01. Animal Cognition and Behavior

Title: Maternal perinatal fructose intake impairs learning and memory, and insulin receptor expression in hippocampus of adult male offspring

Authors: *F. A. TOBAR[1], D. X. CUEVAS[1], L. QUEVEDO[1], R. FÉLIX[2], S. R. ZAMUDIO[1]; [1]Fisiología, IPN, Ciudad de México, Mexico; [2]Biología Celular, Cinvestav, Ciudad de México, Mexico

Abstract: In less than three decades, fructose has become one of the sweeteners most used by the food industry. Multiple studies have evaluated the relationship in their high consumption with a set of metabolic disorders, as obesity, dyslipidemia, insulin resistance, fatty liver and type 2 diabetes. Additionally, experiments in adult rats have shown that fructose consumption induces metabolic changes and reduces cognitive performance and synaptic plasticity in hippocampus. Even, maternal perinatal ingestion of fructose has been associated with metabolic damage in male offspring, however, little is known about the repercussions of maternal fructose consumption during gestation and lactation in the later development of behavior of adult male offspring. For this purpose, Sprague Dawley rats were fed during the gestation and lactation

stages with a standard or high fructose diet and the effect of these diets on metabolism and learning and memory performance in adult male offspring was evaluated. Impairments of spatial and recognition learning and memory performance in male offspring of the fructose fed dams were observed. In addition, hippocampal insulin receptor expression were decreased in these rats. On the other hand, a significant increase in body weight, fat deposits, insulin resistance and serum concentrations of glucose, insulin, leptin and triglycerides were found, as well as decreased adiponectin levels. All these changes were shown by male offspring compared to their respective control. Taken together, these results suggest that high maternal fructose intake during the perinatal stages alters metabolism and impairs cognitive performance of adult male offspring, which can be explained by a decrease in insulin receptor expression in the hippocampus.

Disclosures: F.A. Tobar: None. **D.X. Cuevas:** None. **L. Quevedo:** None. **R. Félix:** None. **S.R. Zamudio:** None.

Poster

160. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 160.19/W5

Topic: H.01. Animal Cognition and Behavior

Title: Effects of circadian desynchronization by the T22 protocol on the rhythmicity and memory of adolescent Wistar rats: An ontogenetic perspective

Authors: *P. E. L. MARINHO, K. C. PUGLIANE, J. C. PEREIRA, K. K. B. C. ARAÚJO, V. S. DIAS, B. H. L. SANTOS, A. L. A. DIAS, R. V. C. B. MOURÃO, C. A. SILVA, F. F. BARBOSA;
Univ. Federal da Paraíba, João Pessoa, Brazil

Abstract: It was previously shown that circadian desynchronization impairs the cognitive performance of rodents, and these effects vary according to the test used and the age at which light exposure occurs. Adolescence is a critical period for the Circadian System maturation, and it is noticed that human adolescents are often submitted to weekly phase shifts due to their sleep routine. Therefore, our study aimed to characterize the influence of the T22 protocol on the rhythmicity and memory of adolescent rats and to investigate the persistence of these effects. We analyzed the rat´s locomotor activity to validate the T22 Protocol and applied memory tasks to verify the cognitive effects of desynchronization. The subjects underwent two classes of memory tasks: an amygdala-dependent task: the passive avoidance task and a hippocampus-dependent task: the associative spatial recognition task. We studied 59 Wistar rats, allocated into two experimental stages. In the first stage, the subjects were divided into: a control group, in which the rats were exposed to a circadian light-dark cycle, the T24 (LD 12h/12h), and a group in which the rats were exposed to the T22 protocol (LD 11h/11h). The second stage included a

control group of rats exposed to the T24 protocol and a group comprising rats initially exposed to the T22 protocol, but later resynchronized to the T24 protocol. The animals in the first stage underwent memory evaluations during the adolescence, and the animals in the second stage were tested in the adult phase, after being resynchronized. According to our results, all animals submitted to the T22 protocol presented two distinct rhythms of locomotor activity: one synchronized to the external LD cycle and another expressed in free running course, which implies that the decoupling of the two areas of the Suprachiasmatic Nucleus occur in the adolescence period. Regarding the memory tasks, we observed that all rats presented impairment in the hippocampal-dependent task, but not in the amygdala-dependent task. We emphasize that the same pattern of results were found for the rats desynchronized in adolescence and tested in the adult phase, after being resynchronized. Our findings indicate that circadian desynchronization during adolescence does not impair aversive memory, but poses negative short-term and long-term consequences in spatial memory.

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Poster

160. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 160.20/W6

Topic: H.01. Animal Cognition and Behavior

Support: FAPEMIG - Foundation for Research Support of the State of Minas Gerais

Title: Effect of different musical classes on the extinction of fear memory in female rats

Authors: *V. A. P. DE PAULA[1], N. C. DE OLIVEIRA[1], A. H. L. RESENDE[1], G. P. L. TEIXEIRA[1], P. H. M. OLIVEIRA[1], D. H. PIETROBON[1], J. C. PRETO[1], G. A. C. CAMANDUCAIA[1], I. C. MESQUITA[1], M. S. DE PAULA[1], R. S. DE FARIA[1], C. M. F. TRZESNIAK[1], C. R. SARTORI[2];
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Abstract: INTRODUCTION: Learning and memory are related cognitive factors, primarily involving the acquisition of information and later storage of acquired memories. Memory may also undergo an extinction process, inhibiting the expression of previously acquired memories. There are clear evidences in the literature regarding the influence of music in the process of learning and memory. However, there are no reports in the literature that expose the effect of music on the extinction of memory in female rats. OBJECTIVE: To analyze the influence of the

exposure to the Mozart's Sonata K448 and Classical Music on the extinction of fear memory in female rats. METHODS: Twenty pregnant Wistar rats were daily exposed to music or ambient sound, subdivided into the following groups: G1-Mozart; G2-Classical; G3-Ambient sound and G4-Control (ambient sound). After delivery, offspring female rats were separated into the respective groups: G1-Mozart (n=17); G2-Classical (n=18); G3-Ambient sound (n=17); G4-Control (n=16), remaining the musical exposure according to the gestational period. On the 39th day, a Fear Conditioning test was performed, in which the groups G1-Mozart, G2-Classical and G3-Ambient sound were submitted to 3 shocks. On the 67th-71th days, the Extinction Test was performed, in which all animals were individually placed in the same shock chamber for 5 consecutive days (D1, D2, D3, D4, D5), but not receiving shock, being collected freezing time data. Statistical analysis was performed with one-way ANOVA, followed by the Tukey test, being significant $p<0.05$. RESULTS: On the D1 day, G1-Mozart, G2-Classical and G3-Ambient sound groups presented higher freezing time compared to controls ($p<0.001$). On the D2-D3, G1-Mozart, G2-Classical and G3-Ambient sound groups maintained higher freezing time when compared to controls ($p<0,03$). However, on D4-D5 days, there was no freezing behavior time differences between the four experimental groups (all $p>0,05$). CONCLUSION: The results demonstrate that the method of aversive conditioning is efficient for the evaluation of memory extinction. However, no significant benefits of exposure to music were demonstrated for the process of aversive memory extinction compared to the positive effects on the early stages of memory formation.

Disclosures: V.A.P. de Paula: None. **N.C. de Oliveira:** None. **A.H.L. Resende:** None. **G.P.L. Teixeira:** None. **P.H.M. Oliveira:** None. **D.H. Pietrobon:** None. **J.C. Preto:** None. **G.A.C. Camanducaia:** None. **I.C. Mesquita:** None. **M.S. de Paula:** None. **R.S. de Faria:** None. **C.M.F. Trzesniak:** None. **C.R. Sartori:** None.

Poster

160. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 160.21/W7

Topic: H.01. Animal Cognition and Behavior

Support: FAPEMIG - Foundation for Research Support of the State of Minas Gerais

Title: Comparative analysis of the effect of different musical genres on the recognition memory of objects in female rats

Authors: *J. C. PRETO[1], M. S. DE PAULA[1], C. M. F. TRZESNIAK[1], C. R. SARTORI[2], R. S. DE FARIA[1], T. H. PEREIRA[1], P. A. J. MACIEL[1], G. A. C. CAMANDUCAIA[1], V. A. P. DE PAULA[1];

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Abstract: INTRODUCTION: It is known that music enhances learning ability and memory, including consolidation and information recovery. Studies have shown positive effects on rats´ memory when exposed to classical music. However, research has been restricted to this musical genre and only to one animal development stage. AIMS: To compare the memory effects of the exposure to different musical genres during animal gestation and adult rats. METHODS: We used 20 pregnant Wistar rats, whose female offspring was divided into 5 groups: G1-Classical Gestation (n=16); G2-Electronic Gestation (n=16); G3-Classical Adult (n=16); G4-Electronic Adult (n=16); G5-Control (n=16). The G1-G2 pregnant rats were exposed to music, and their offspring remained with this exposure until the behavioral tests. G3-G4 adult offspring was exposed to music only during the behavioral tests; G5 was not exposed to music. On the 38-39th days of the experiment, the animals were submitted to Habituation in the experimental box. On the 40th day, the Object Recognition Training was made. On the 41st day, after one hour of training, the rats went to the short-term memory test. Later, the animals returned to the vivarium, where they remained until the 82nd day, when the long-term memory test was performed. The data from the training test was analyzed with the one-way ANOVA test. The data related to the object exploratory behavior tests were presented as exploratory preference, and were analyzed with the Kruskal-Wallis test. Values of $p \leq 0.05$ were considered significant. RESULTS: No difference was found among groups on the training test (all $p>0.05$). G1-G2-G4 animals did not show any significant difference when compared to controls ($p>0,05$). However, rats exposed to classical music only during training and memory tests (G3) showed significant difference on the short-term memory test when compared to controls ($p=0,007$). No difference was seen on the long-term memory teste (all $p>0.05$). CONCLUSION: According to this study, rats exposed to music since gestation did not present alterations on short- and long-term memory; however, exposure to classical music during the learning period showed short-term memory benefit.

Disclosures: J.C. Preto: None. **M.S. de Paula:** None. **C.M.F. Trzesniak:** None. **C.R. Sartori:** None. **R.S. de Faria:** None. **T.H. Pereira:** None. **P.A.J. Maciel:** None. **G.A.C. Camanducaia:** None. **V.A.P. de Paula:** None.

Poster

160. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 160.22/W8

Topic: H.01. Animal Cognition and Behavior

Support: FAPEMIG - Foundation for Research Support of the State of Minas Gerais

Title: Comparative analysis of the effect of different musical genres on the acquisition of memory during breastfeeding and adult hood in female rats

Authors: *G. C. CAMANDUCAIA[1], I. C. MESQUITA[1], M. S. DE PAULA[1], M. P. P. FONTES[1], K. A. D. N. BRITO[1], V. A. P. DE PAULA[1], J. C. PRETO[1], R. S. DE FARIA[1], C. M. F. TRZESNIAK[1], C. R. SORTORI[2];
[1]Lab. of Neurophysiol. of Memory, Fac. of Medicina of Itajubá, Itajubá, Brazil; [2]Dept. of Structural and Functional Biology, Inst. of Biol., State Univ. of Campinas (UNICAMP), Campinas, Brazil

Abstract: Introduction: Learning is the process by which we acquire information, and memory is the storage of this information. Music can alter neuronal plasticity, leading to memory formation. However, the role of different musical genres on memory acquisition in distinct developmental phases has not yet been clearly explained. Objective: To compare the influence of different musical genres on female rats´memory during breastfeeding and adulthood. Methods: Twenty pregnant Wistar rats were used, of which the female offspring was divided into five groups: G1-Classical breastfeeding (n=16); G2-Electronic breastfeeding (n=16); G3-Classical adult phase (n=16); G4-Electronic adult phase (n=16), G5-control (n=16). The G1-G2 offspring was exposed to its respective musical genre from their birth onward. The G3-G4 adult offspring was exposed to music when from 28 days after birth onward. G5 was not exposed to music. On the 38th and 39th days of the experiment, the animals were submitted to the habituation of the arena. On the 40th day, the object recognition training was performed. On the 41st day, after one hour of training, the rats were submitted to the short-term memory test. Subsequently, the animals returned to the biotherium, where they remained until the 82nd day, when the long-term memory test was performed. The data from the training test was analyzed with the one-way ANOVA test. The data related to the object exploratory behavior tests were presented as exploratory preference and were analyzed with the Kruskal-Wallis test. Values of $p \leq 0.05$ were considered significant. Results: There was no statistical difference among groups neither related to the musical genre nor to the developmental stage (all $p > 0.05$). Conclusion: The present study showed that the rats exposed to music, both classical and electronic, during breastfeeding and adulthood did not have alterations in short and long-term memory.

Disclosures: G.C. Camanducaia: None. **I.C. Mesquita:** None. **M.S. de Paula:** None. **M.P.P. Fontes:** None. **K.A.D.N. Brito:** None. **V.A.P. de Paula:** None. **J.C. Preto:** None. **R.S. de Faria:** None. **C.M.F. Trzesniak:** None. **C.R. Sortori:** None.

Poster

160. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 160.23/W9

Topic: H.01. Animal Cognition and Behavior

Title: Chronic seizures cause lasting retrograde amnesia for a spatial memory in the Morris Water Task

Authors: *K. ROBERTS[1], L. BRANDT[1], N. M. FOURNIER[2], H. LEHMANN[3];
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Abstract: Research on the relationship between memory and epilepsy has found that seizure activity produces retrograde amnesia. For instance, seizures within hours after learning disrupt the memory trace. The studies examining the retrograde amnesic effects of seizures, however, have typically focused on memories being disrupted during the period of cellular consolidation. Few studies have addressed whether repeated seizures also impair memories that have completed the consolidation process and whether any deficit in memory following the seizures would be transient or long-lasting. To address these issues, rats were trained on the hidden platform version of the Morris Water Task. Two days after training, beyond the cellular consolidation window, the rats were given a two-week treatment with the chemoconvulsant pentylenetetrazole (PTZ) to induce chronic seizures. The rats were then assessed for retention 2 or 14 days after the treatment, with the longer retention interval assessing persistence of the possible memory impairment. The retention results showed that the PTZ-treated rats had longer latencies to find the platform location than the control rats. In addition, the PTZ rats, unlike the control rats, did not show increased swim time in the pool quadrant that originally contained the hidden platform. Thus, the onset of chronic seizures disrupted spatial memory and this retrograde amnesia persisted even 14 days after seizure termination. Following the retrograde amnesia test, the rats were retrained on the original hidden platform location followed the next day by training on a new location (reversal). The PTZ rats did not show any learning impairments, suggesting that the seizures did not cause anterograde amnesia for spatial memory. Histological examination is currently being completed to determine whether the retrograde amnesia is associated with the changes in the hippocampus, a critical structure for spatial memory. Overall, our findings suggest that memories currently believed to be more resistant to brain injury, those outside of the cellular consolidation period, remain sensitive to repeated seizures.

Disclosures: K. Roberts: None. **L. Brandt:** None. **N.M. Fournier:** None. **H. Lehmann:** None.

Poster

160. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 160.24/W10

Topic: H.01. Animal Cognition and Behavior

Title: Kindled seizures promote accelerated long-term forgetting of context fear memories in rats

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Abstract: Mesial temporal lobe epilepsy is one of most prevalent forms of drug refractory epilepsy, with over half of patients reporting some aspect of memory and learning problems. One type of memory deficit that is particularly common among these patients is accelerated long-term forgetting (ALF), which is characterized by an initially normal acquisition and retention of memories over short periods of up to 30 minutes, but abnormally fast forgetting over periods of days or weeks after the event. Despite the prevalence of memory deficits among epileptic patients, the neurobiological mechanisms contributing to these problems remain obscure. In the present study, we will examine the impact of repeated seizures on the long-term retention of a previously acquired contextual fear memory. Male Sprague Dawley rats will be trained using contextual fear learning paradigm, and following acquisition, they will undergo either 2 weeks of chemical kindling using pentylenetetrazole (PTZ) or will receive two weeks of saline injections (non-kindled controls). A series of follow-up memory retention tests will be conducted beginning 48 hrs after the last PTZ treatment by placing the rats into the conditioning chambers and measuring for freezing behaviour. Our preliminary data revealed that the PTZ kindled group showed significantly less freezing than non-kindled rats upon re-testing suggesting that seizures produced ALF and retrograde amnesia of the original fear memory. As previous work found that the removal of postsynaptic GluA2/AMPARs mediates normal long-term forgetting, we are currently expanding our study to examine whether chronic seizures alter levels of AMPA receptor trafficking within memory circuits and whether these changes contribute to long-term forgetting during epilepsy.

Disclosures: L.E. Brandt: None. **H. Lehmann:** None. **N.M. Fournier:** None.

Poster

160. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 160.25/W11

Topic: H.01. Animal Cognition and Behavior

Support: Research division of Universidad Iberoamericana, A. C.

Title: The effect of sexual behavior and social interaction in the memory of old male rats

Authors: *A. TAPIA DE JESUS, L. M. RODRIGUEZ-SERRANO, O. GALICIA-CASTILLO, L. F. S. HERNÁNDEZ-GONZÁLEZ, P. ESPINOSA-VILLAFRANCA, I. LÓPEZ-CORTINA, M. E. G. CHÁVEZ-HERNÁNDEZ, M. I. F. MATA-ESQUIVIAZ, G. LAGO, M. H. BUENROSTRO-JAURÉGUI;
Psychology, Univ. Iberoamericana, Mexico City, Mexico

Abstract: Several studies have shown that there is neurogenesis in the old brain, specifically in the hippocampus and olfactory bulb. This has been associated with better cognitive function. The hippocampus has a fundamental function in learning and memory. On the other hand, physical activity, sexual intercourse, and social interaction have been linked to enhanced cognitive function, while aging has been associated with cognitive impairment and decline. Thus, the aim of our study is to determine the effect of sexual intercourse and social interaction of old male rats in the performance of memory tasks. We divided our sample into 3 groups in terms of the conditions of aging: sexual interaction, social interaction, and control group. Sexual interaction group had sexual intercourse once a week until 8-months-old; the rats in the social interaction group were housed in groups of 3 rats until 8-months-old, and the rats of the control group were housed individually and left undisturbed until 8-months-old. All groups were tested in three memory tasks: inhibitory passive avoidance test, the novel object recognition test, and water maze test. The results showed better performance of the experimental groups when compared to control group. Also, there are differences in the performance of the memory tasks between both experimental groups. These findings indicate that sexual interaction and social interaction can improve the memory function of old male rats.

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Poster

161. Hippocampus: Intrinsic Hippocampal Circuits

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 161.01/W12

Topic: H.01. Animal Cognition and Behavior

Support: Ecole de Neurosciences de Paris (ENP)

Title: An examination of hippocampal interneurons underlying enkephalin release and their potential role in memory

Authors: *S. CASSIM[1,2], A. FAFOURI[1], L. THERREAU[3,1], V. CHEVALEYRE[1,2], R. PISKOROWSKI[1,2];
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Abstract: It has been recently demonstrated that synaptic plasticity in hippocampal area CA2 is important for social recognition memory. Specifically, a unique delta-opioid receptor (DOR)

mediated long-term depression of inhibitory transmission (iLTD) that has been shown to tightly control excitation of CA2 pyramidal neuron output has been implicated in this process. While it has been shown that this plasticity may be consequential for social recognition memory, the hippocampal source of enkephalin, the endogenous ligand for DORs, is currently unknown. We make use of a knock-in transgenic mouse line that expresses cre-recombinase in the promoter elements of the preproenkephalin locus (PENK-cre mice). Using cre-dependent viral vectors, we selectively label and record from PENK-expressing (PENK+) internuerons, characterizing neuronal morphology and physiological properties. We found that PENK+ neurons are located in area CA1, near the border with CA2. Their soma are small in diameter and are located predominantly in *stratum pyramidale* and their basal dendrites project into *stratum oriens*, whilst their apical dendrites extend and bifurcate into *stratum radiatum*. The biophysical properties of these neurons show that these neurons are interneurons that fire action potentials (APs) in the range of 10-50 Hz, suggesting they may be regular spiking interneurons. Immunochemistry characterization of these PENK-cre interneurons reveals a co-localization for the calcium-binding protein, calretinin. Upon examining synaptic inputs that drive AP firing in these interneurons, we show that inputs received from the Schaeffer collaterals can readily drive AP firing in these cells. We further show that these interneurons do not form direct synapses on to CA2 pyramidal neurons, but likely target interneurons. Expressing inhibitory Gi-DREADDs in these neurons prevents the induction of delta-opioid iLTD with Schaeffer collateral stimulation, showing enkephalin release from these PENK+ neurons play an important role in evoking plasticity in area CA2. These findings suggest that intra-hippocampal sources of enkephalin release are sufficient to evoke a DOR-mediated plasticity in hippocampal area CA2. Our next goal is to examine the contribution of PENK+ neurons in social recognition memory.

Disclosures: S. Cassim: None. **A. Fafouri:** None. **L. Therreau:** None. **V. Chevaleyre:** None. **R. Piskorowski:** None.

Poster

161. Hippocampus: Intrinsic Hippocampal Circuits

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 161.02/W13

Topic: H.01. Animal Cognition and Behavior

Support: Exploratory Research for Advanced Technology (JPMJER1801)
Precursory Research for Embryonic Science and Technology (JPMJPR1785)

Title: Prioritized experience replay for learning in the rat hippocampus

Authors: *H. IGATA[1], Y. IKEGAYA[1], T. SASAKI[1,2];
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Japan; [2]Precursory Res. for Embryonic Sci. and Technol., Japan Sci. and Technol. Agency, Kawaguchi, Saitama, Japan

Abstract: Hippocampal place cells are considered as a crucial neuronal substrate to construct a cognitive map in the brain. Recent findings have shown that neuronal ensemble reactivations followed by sharp-wave ripples, so-called replays, support the stabilization of place cell maps, indicating the involvement of hippocampal replays in learning process. Analogous to hippocampal replays, studies in artificial intelligence research field recently show that introducing prioritized experience replay, a strategy to replay episodes weighted by its importance, into a deep reinforcement learning algorithm leads to higher computational performance, suggesting that such characteristics of replays might be a common feature for some built-in strategies in learning processes of both the organisms and machines. To further reveal detailed physiological characteristics of hippocampal awake replay during learning, we designed a spatial task in which rats learn to take a specific route point to a fixed goal in a two-dimensional task field. In this task, a trial began when the animals performed an active nose poke in a start box where sucrose water was presented for 10 seconds. A start door was then opened so that the rats entered into the field. The rats could obtain reward at a goal box if they correctly stopped at a specific check point where a small amount of chocolate milk was placed on the way to the goal box. After the rats continuously performed the same task for 3-4 days, a check point was replaced to a different location. The rats first showed exploratory behavior throughout the field after the rewarding rule was changed but could learn a new optimal route within a single session. During this learning process, a multiunit recording was performed from hippocampal CA1 neurons. Some hippocampal neurons responded to behavioral task contexts rather than places and the frequency of replay events increased at a check point and a goal location during learning. Active neurons in synchronous events were highly correlated after the rats found an optimal route to a new choice point, demonstrating that specific sets of neuronal ensembles are more frequently recruited by synchronous events. Bayesian decoding analyses revealed that synchronous events over-represented the optimal trajectory to new check point during learning. Taken together, animal's internal motivation and/or prediction error changed both the frequency and neuronal contents of hippocampal replay events. These results provide evidence that active neuronal patterns of hippocampal awake replays are prioritized by internally evaluation of previous behavior, which might support the reinforcement of a specific behavior pattern.

Disclosures: H. Igata: None. **Y. Ikegaya:** None. **T. Sasaki:** None.

Poster

161. Hippocampus: Intrinsic Hippocampal Circuits

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 161.03/W14

Topic: H.01. Animal Cognition and Behavior

Title: Impairment of cognitive function under Hyper-Gravity in rats

Authors: *J. LEE[1], D. JANG[1], H. KANG[1], H. KIM[2], G.-S. KIM[2], S. YANG[1];
[1]Dept. of Bioengineering and Nano-Bioengineering, Incheon Natl. Univ., Incheon, Korea, Republic of; [2]Dept. of Otorhinolaryngology-Head & Neck Surgery, Inha University, Col. of Med., Incheon, Korea, Republic of

Abstract: The gravity is necessary for living organism to operate many biological events including hippocampus-related functions, learning and memory. There were only a few studies regarding how gravity alters hippocampus functions, mainly due to difficulties in conditioning an animal to considerable alteration of gravity. Here, we demonstrated the effects of Hyper-Gravity (HG), 4xG, for 4 weeks on rat hippocampus using electrophysiology and behaviors. Briefly, HG group showed impaired cognitive function such as decreased synaptic efficacy, long-term potentiation and behavioral learning performance, remaining no alteration of presynaptic release-ability. Interestingly, the short time conditioning protocol (24 ~ 48 hours) showed more intensified long-term potentiation compared to control group. Taken all together, our studies suggested that the cognitive function is sensitive to the level of gravity.

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Poster

161. Hippocampus: Intrinsic Hippocampal Circuits

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 161.04/W15

Topic: H.01. Animal Cognition and Behavior

Support: Grant No.91632303 to FX

Title: Whole brain mapping of the monosynaptic inputs and projections of dorsal hippocampus and ventral hippocmpus

Authors: *S. TAO[1,2], F. XU[3,1];
[1]Wuhan Natl. Lab. for Optoelectronics, Huazhong Univ. of Sci. and Technol., Wuhan, China; [2]Wuhan Inst. of Physics and Mathematics, Ctr. for Excellence in Brain Sci. and Intelligence, Wuhan, China; [3]Wuhan Inst. of Physics and Mathematics,Center for Excellence in Brain Sci. and Intelligence, Wuhan, China

Abstract: The hippocampus is a complex but highly organized cortical structure located in the mammalian medial temporal lobe, which is known to be divided into dorsal and ventral parts in rodents. Here, to understand more detailed differences between dorsal and ventral parts of hippocampus in the level of anatomical connectivity, we systematically identified the inputs to

the both dorsal and ventral parts of hippocampus by using rabies virus mediated monosynaptic retrograde trace tracing and their axonal projections by using adeno-associated virus. Our mapping revealed the input proportions and distributions of dorsal and ventral parts of hippocampus with quantitative differences in major brain regions (and subregions therein). For example the dorsal parts tend to project to dorsal SUB while the ventral hippocampus tend to project to ventral SUB. Meanwhile both dorsal and ventral parts get MS projections while dorsal parts make monosynaptic connections with ECT but ventral parts make monosynaptic connections with ENT. Together with brain-wide reconstruction of single neurons, we were able to present different single-neuron projectomes in the same part of dorsal or ventral hippocampus. Comparison of the afferents and efferents of these two parts of hippocampus can expand our knowledge about how they work separately in different behavior.

Disclosures: S. Tao: None. **F. Xu:** None.

Poster

161. Hippocampus: Intrinsic Hippocampal Circuits

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 161.05/W16

Topic: H.01. Animal Cognition and Behavior

Support: Office of Naval Research, In-house Laboratory Independent Research (ILIR) program

Title: Correlation of electrophysiological parameters with behavioral performance in an animal model using aluminum exposures

Authors: *J. G. ROHAN[1], T. ETHRIDGE[1,2], M. K. MIKLASEVICH[1,3], N. M. GARGAS[1,3], S. M. MCINTURF[1];
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Abstract: At Naval Medical Research Unit Dayton, we have incorporated electrophysiology as part of our neurotoxicity assessment repertoire. Specifically, we have used microelectrode arrays to effectively screen the effects of various environmental hazards and stressors on neuronal function, especially on hippocampal neurons. Electrophysiology provides quantitative assessments of synaptic transmission efficiency, synaptic plasticity, and spontaneous activity as measured by input-output relationship, short term or long term potentiation, and spontaneous non-evoked activity, respectively. However, the physiological significance of changes in these electrophysiological parameters remains unclear. The overall objective of our study is to establish a correlation analysis between electrophysiological recordings and behavioral performance. We are using aluminum chloride ($AlCl_3$) exposures because they are known to

induce memory loss and other cognitive deficits. We have completed our preliminary characterization of the acute toxicity of aluminum exposures. Male Sprague Dawley rats were exposed to $AlCl_3$ via intraperitoneal injection (IP) over 14 consecutive days at a concentration of 0 (control), 1 (low), 10 (medium), or 50 (high) mg/kg body weight. These concentrations have been shown by others to induce delayed cognitive dysfunction. There are fewer studies that characterize the acute effects of short term aluminum exposures. Here, animals were euthanized within 24 hours following exposures to obtain electrophysiological and biochemical measurements. We found a high degree of abdominal adhesion as well as visible systemic inflammation in various organs (liver, kidney) in animals exposed to the medium and high doses of $AlCl_3$. Interestingly, plasma inflammatory cytokine measurements revealed only mild increases in TNF-α from animals exposed to the highest $AlCl_3$ dose. We also saw an average decrease in long term potentiation induced by theta burst stimulation with increasing dose of $AlCl_3$. We will use these preliminary data to guide the next phase of this study in which we will compare the degree of systemic inflammation resulting from $AlCl_3$ exposure via IP injection versus oral gavage, and evaluate how changes in synaptic parameters and inflammatory markers correlate with behavioral performance in an open field arena to evaluate motor activity as well as novel object recognition and Morris water maze testing to assess cognitive function.

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Poster

161. Hippocampus: Intrinsic Hippocampal Circuits

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 161.06/W17

Topic: H.01. Animal Cognition and Behavior

Title: Characterizing resonant and synchronizing mechanisms in a hippocampal theta model

Authors: *T. BANKS[1], V. GUNTU[1], A. M. HUMMOS[2], S. S. NAIR[1,2];
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Abstract: Neuronal processing across multiple brain regions is thought to be subserved by oscillations. Many studies involving rhythms show participation of a number of rhythm generators, likely mediated through a diverse set of physiological mechanisms. Removal of circuit components can sometimes yield unintuitive effects on the oscillatory pattern and strength.
We use computational modeling of the CA3 region of a rodent hippocampus to characterize the interaction between the physiological mechanisms that might participate in generating theta rhythms. To further refine the interactions, we distinguish between resonant and synchronizing

mechanisms and show that such a categorization can help predict the mechanisms that would interfere or possibly substitute for another. Resonant mechanisms that produce rhythms through their intrinsic dynamics, including slow inhibition, spike-frequency adaptation, slow neuronal currents, and rhythmic external input. Synchronizing mechanisms support rhythm coordination include non-rhythmic external input, inhibitory feedback, and recurrent excitatory connections. Some of the mechanisms could participate in both functions. Through our computational models we found that robust rhythm generation requires at least one of each, resonant and synchronizing components. We found that pyramidal cell adaptation interferes with theta rhythms produced by slow inhibition and that fast inhibition can substitute for rhythm generation or interfere with it through slow inhibition, depending on the level of acetylcholine. These results shed light on the conflicting reports about effects on rhythm generation by the inactivation of specific circuit components. The model also predicts states where removing a component thought to participate in rhythm generation might augment rhythmic activity. Overall, our investigations reveal that effects of component removal can only be foreseen in the context of mechanisms present and on the neuromodulatory state of the system.

Disclosures: **T. Banks:** None. **V. Guntu:** None. **A.M. Hummos:** None. **S.S. Nair:** None.

Poster

161. Hippocampus: Intrinsic Hippocampal Circuits

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Program #/Poster #: 161.07/W18

Topic: H.01. Animal Cognition and Behavior

Support: NSF CBET-1848029
F31 NS 105420
DGE-1247312

Title: Distinct neuronal populations contribute to trace conditioning and extinction learning in the hippocampus

Authors: K. R. HANSEN[1], H. J. GRITTON[1], ***R. MOUNT**[1], S. SRIDHAR[1], A. I. MOHAMMED[1], M. ABDULKERIM[1], R. KESSEL[1], B. NAZER[2], X. HAN[1];
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Abstract: Trace conditioning and extinction learning are two learning processes that depend on the hippocampus. Previous studies have suggested that distinct neuronal populations contribute to fear conditioning and extinction in both the hippocampus and amygdala. In this study, we present the first evidence that two populations of cells in CA1 of the hippocampus contribute to trace eye-blink conditioned learning and extinction of that conditioning, and that neuronal extinction responses can be observed in the hippocampus in less than 6 consecutive extinction

presentations. Using trial-averaged neuronal responses, we observed neurons that consistently, but sparsely responded to a conditioned stimulus (CS) over multiple days of learning a trace conditioning task, and a different population of cells that responded to the CS during extinction learning. The ability of individual cells to encode CS presentations on a sparse number of trials suggests that network or population responses are critical for the encoding of learning and memory in CA1. To this end we developed a method to quantify network responses of co-active neurons, and found that subpopulations of neurons responded on significantly more trials with the correct behavioral response than with the incorrect behavioral response for both the last trace eye-blink conditioning session and extinction session. These results suggest an important role for distinct populations of neurons that encode information about the CS within the hippocampus.

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Poster

161. Hippocampus: Intrinsic Hippocampal Circuits

Location: Hall A

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Program #/Poster #: 161.08/W19

Topic: H.01. Animal Cognition and Behavior

Support: NSERC

Title: The role of the hippocampus in recognition memory in the common marmoset (Callithrix jacchus)

Authors: *W. J. ASSIS[1], L. PREMACHANDRAN[1], B. W. CORRIGAN[1], D. BUITRAGO-PIZA[1], Z. W. DAVIS[2], J. C. MARTINEZ-TRUJILLO[3];
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Abstract: The primate hippocampus (HPC) has been shown to play a key role in recognition memory, a form of memory that allows an animal to distinguish whether an object is novel or familiar. In this study, we aim to demonstrate neural activity in regions CA1 and CA3 of the common marmoset (*Callithrix jacchus*) HPC. Marmosets sit on a primate chair with their head-fixed, and eye movements are monitored using an EyeLink eye tracking system (SR Research Ltd., Canada). Neural recordings are conducted using a 32-channel brush array (Microprobes for Life Science Inc., USA) chronically implanted in the marmoset HPC. Marmosets are shown a series of image pairs comprised of a constant base image, and one of four sample images displayed at different relative frequencies (2, 4, 6, 8). Images include colony marmosets,

vegetation, and non-colony marmosets, with image sets changing daily. Pairs are shown at a relative frequency of 1, 2, 4, 6, and 8 (i.e. 4 being shown twice as often as 2), with 1 being the base image paired with itself, acting as a control for directional bias. Total gaze time per image or gaze fraction is recorded and analyzed as a fraction of sample image viewing time over constant base image viewing time (novel/base), currently with one marmoset (n=1). Sample images were viewed significantly longer than base images. Across relative frequencies, frequency of 2 (75.58) demonstrates a significantly longer gaze fraction in comparison to frequencies of 4, 6, and 8 (42.99, 43.72, and 42.71, respectively), which all show a relatively similar gaze fraction. These results show that past a certain threshold (frequency 2, 9.5%), the marmoset is indifferent to an increase in image frequency exposures. This suggests that the animal's recognition memory of an image's novelty has a threshold, above which the image is no longer novel. Neural recordings are being analyzed.

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Poster

161. Hippocampus: Intrinsic Hippocampal Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: NIH R01MH115304 to AF
NIH R01NS105472 to AF

Title: Temporal criteria for hippocampal maps and remapping

Authors: *J. L. KUBIE[1], E. R. LEVY[2], A. A. FENTON[2];
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Abstract: The discovery of place cells led to O'Keefe and Nadel's proposition that The Hippocampus (functions as) a Cognitive Map. Here we examine the nature of the hippocampal map and propose a time-based, rather than a location-based criterion for assessing an instance of the map and whether the map has changed.
The raw data are as follows. A neuron is called a "place cell" when it fires whenever the animal crosses a fixed location in the environment, the cell's "firing field". The initial and common measure of the hippocampal map is the set of place fields of an environment.
We propose temporal firing criteria to characterize the hippocampal map. Since an animal's movement speed is limited, the spatial relations transform to temporal relations: place fields with spatial overlap will, on average, fire together more than chance, while cell's with non-

overlapping firing fields will virtually never fire together. Now one can also describe the map as the set of all pair-wise spike-train correlations. As shorthand place-cell pairs can be dichotomized into pairs with high temporal co-firing and pairs with low temporal co-firing. This list describes the map. More formally, we use the momentary activity (firing rate) vector of the place cell population. Each vector is unique to a map and the set of activity vectors defines a map.
When the animal explores a second environment, the place fields of individual place cells can change, such that a place field can appear, disappear or change location with respect to a common reference frame — a phenomenon called remapping. A coherent shift of all firing fields is not remapping if it does not change the temporal relationships amongst the cells. Applying the temporal criterion, remapping is when the set of firing vectors change abruptly, or, perhaps more conveniently, when the set of pairwise cross-correlations changes.
There are multiple reasons for preferring temporal criteria. First, timing is fundamental; place cells directly respond to the timing of their inputs, not external space. Second, although the spatial and temporal descriptions of hippocampal maps are equivalent when the animal is continuously moving in an environment, the temporal criterion also describes hippocampal maps when the animal is not moving, such as during sleep, sharp-wave ripples, and running on a treadmill or ball. Finally, remapping can be observed without locomotion.
The relationship between the firing of place cells and physical locations in space is a separate issue we term “map registration.” By separating the concept of a map from map registration, the temporal framework broadens the concept of the hippocampal map and avoids a common confusion about remapping.

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Poster

161. Hippocampus: Intrinsic Hippocampal Circuits

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Support: EPSRC Grant 1815676
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Title: Learning alters hippocampal topology to nest new mnemonic representations without compromising previous ones

Authors: *G. GAVA[1], D. DUPRET[2], S. R. SCHULTZ[1];
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Abstract: The hippocampal circuit plays a central role in learning and memory. Identifying the circuit-level operations that enable the nesting of new memories in the network, while protecting previously-acquired ones from catastrophic interference, is the subject of intense investigation. Here, we present ongoing graph-theoretical work describing functional changes in hippocampal circuit properties associated with the coexistence of different neural representations. Neuronal ensembles were recorded from the dorsal CA1 of mice exploring a familiar open field environment before and after performing a conditioned place-preference (CPP) learning task. Using the spike trains of pyramidal cells monitored during active exploratory behaviour, we assessed the functional properties of weighted graphs constructed using a biophysically-inspired measure of directed firing correlations (Billeh et al., 2013). The average strength (S) of each neuron was computed as the sum of weighted functional association to all the other neurons in the network. We observed a significant increase in S during the conditioning task, independent of changes in the population average firing rate. We also noted a decrease in the mean geodesic path length and an increase in the average clustering coefficient of the network, across the task. We found these altered network structures to persist even after conditioning. However, using the Riemannian topological measures (Pennec et al., 2006), we found that the neural representation of the familiar open field environment remains stable in the re-exposure session, following the conditioning experience. Together, these findings suggest that learning dynamically alters the graph-structural properties of the dCA1 circuit to nest new mnemonic content into the network while preserving previously-acquired representations.

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Poster

161. Hippocampus: Intrinsic Hippocampal Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: FAPESP 2016/18039-9

Title: Standardized extract of Ginkgo biloba mobilizes calcium stores in hippocampal slices

Authors: C. ZAMBERLAM, J. CERUTTI, R. P. URESHINO, ***S. M. CERUTTI**;
Univ. Federal de São Paulo, Sao Paulo, Brazil

Abstract: Recent studies from our laboratory have shown that treatment with standardized extract of *Ginkgo biloba* (EGb) improves the retention of fear memory or its extinction according dose used. In addition, we found that these effects might be related to the EGb multi-target characteristic that differentially modulates GluN2B-NMDA, $GABA_A$ and $5\text{-}HT_{1A}R$ receptors in the dorsal hippocampal of rats. The NMDA receptor ion channel is highly permeable to Ca^{2+},

which activates different signal transduction pathways involved with memory formation. This study investigated the mobilization of calcium in slices of rat hippocampus following EGb treatment. For this, five adult, male *Wistar* rats were decapitated and the brains were rapidly removed and submerged in artificial cerebrospinal fluid (ACSF) and sliced on a vibratome (1000 Plus Sectioning System, Capden Instruments, IN, EUA) at 400 μm thickness. The sagittal slices containing the CA1 subfield of dorsal hippocampal formation were transferred to a holding chamber. Ca^{2+} imaging was performed using an inverted fluorescence microscope coupled to high resolution CCD digital camera 510. After baseline register the slices were treated with EGb extract 18.5 ug /mL and then, they were incubated with the Ca^{+2} Fura-2 AM (Acetoxy Methylester) fluorophore, which can be excited at wavelengths of 340 nm (Ca^{2+} bound) and 380 mM (Ca^{2+} not bound). The measurement of the cytoplasmic concentration of Ca^{2+} was performed by the ratio between Fura-2AM emissions at 340/380 nm reflecting the concentration of bound and free Ca^{2+} respectively.. The data showed that EGb 18.5 ug/mL increased intracellular Ca^{2+} in the smallest amounts applied in the slice (1.0 uL, 5.19, P=0.0076 and 3.0 uL, 8.19, P<0.0001, compared to baseline control 0.49. Conversely, treatment with the highest concentration decreased intracellular Ca^{2+} concentration: 10 uL, -3,93, P=0.0008; 30 uL,-6,35 or 50 uL, -13,21, both P<0.0001. Our preliminary findings show that EGb modulates citoplasmatic free Ca^{2+} in the hippocampal slices, adding new data on the mechanisms that explain the effects of this extract as a cognitive enhancer.

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Poster

161. Hippocampus: Intrinsic Hippocampal Circuits

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Program #/Poster #: 161.12/W23

Topic: H.01. Animal Cognition and Behavior

Title: Use of 3D printed capacitive touch objects for object recognition tasks

Authors: J. M. S. DEFILIPP, K. POTTS, K. D. STEVANOVIC, G. DRYE, *J. D. CUSHMAN;
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Abstract: Object recognition tasks are widely used learning and memory tasks that typically involve familiarizing mice with a set of objects and then presenting a novel object or displacing an object to a novel location. Learning and memory is inferred by the amount of object investigation of the novel/displaced object. These tasks are in widespread use, but unfortunately there are often large discrepancies in findings between labs. Two major contributors to these discrepancies are the lack of consistency in the method of measuring object investigation and the

lack of standardization of the objects that are used. Current video-based automated algorithms can often be unreliable, lack temporal precision and can be costly, whereas manually scoring object investigation is time consuming, tedious and more subjective. To resolve these issues, we sought to design and implement 3D printed objects, so that objects can be standardized across labs and we utilize capacitive sensing to better measure object investigation. Utilizing a 3D printer with conductive filament and low-cost off-the-shelf components we demonstrate that 3D printed capacitive touch objects are a reliable and precise way to perform object recognition tasks. Our goal is to make this an open source, community-based project so that object recognition tasks can be performed with greater standardization as well as higher precision, accuracy and throughput within and across laboratories. We envision a standardized library of objects, which have been tested and vetted for discriminability and innate preference, that can be easily printed at any institution. This will ultimately contribute to a better understanding of learning and memory mechanisms as well as improve pre-clinical models of neuropsychiatric and neurodegenerative diseases.

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Poster

161. Hippocampus: Intrinsic Hippocampal Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: Alfred P Sloan Foundation (76332-28)
PSC-CUNY TRADA-43-687

Title: Reduced Npas4 expression is associated with impaired spatial but not contextual memory retrieval in split-brain mice

Authors: *J. JORDAN[1], Y. TONG[2], C. L. PYTTE[2,1];
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Abstract: The hippocampus is critical for spatial and contextual memory and is functionally lateralized in humans and in rodents. While investigation of rodent hippocampal lateralization has been increasing, very little is known about the role of interhemispheric communication in the hippocampal system in either humans or rodents. Previously, we reported that loss of interhippocampal communication in split-brain mice impaired hippocampus-dependent spatial memory without impacting hippocampus-dependent contextual memory. Hippocampus-independent learning and anxiety were unaffected. However, it is not yet clear whether hippocampal neuronal activity is affected by loss of interhippocampal connectivity. Here we

investigated the expression of Npas4, a neuron-specific activity-dependent immediate early gene. We found that split-brain C57Bl6 mice (n=7) had reduced spatial memory-induced Npas4 expression in areas CA3 (p=0.006) and CA1 (p=0.037) compared to controls (n=7), with no differences between hemispheres. Split brain mice (n=7) did not differ from controls (n=7) in Npas4 expression following contextual fear memory retrieval in CA3 (p=0.447) and CA1 (p=0.703). Interestingly, CA3 Npas4 expression was right-lateralized across treatments following contextual fear memory retrieval (p = 0.030). Thus, split-brain mice show impaired spatial memory and bilaterally reduced spatial memory-induced Npas4 expression. There was no effect of the split-brain treatment on contextual fear memory or related Npas4 expression. These data indicate that interhemispheric communication contributes to bilateral activation required for spatial memory but is not required for the neural activation underlying contextual fear memory, providing new insight on the role of interhemispheric communication in hippocampal function and memory.

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Poster

161. Hippocampus: Intrinsic Hippocampal Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: DFG Grant YO177/4-1

Title: Hippocampal TRPC5 channels support hippocampus dependent memory and affect *in vivo* network activity

Authors: *F. M. THEISSEN[1], A. REBOREDA[2], A. ALBRECHT[3], O. STORK[3], L. LEICHERT[4], L. BIRNBAUMER[5], M. SAUVAGE[6], M. YOSHIDA[1];
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Abstract: The hippocampus is crucial for contextual- and working memory both in humans and animals. TRPC channels have been reported to support working memory and contextual memory. This suggests that TRPC channels might be important for hippocampal function. However, it has not been shown that manipulation of TRPC channels, restricted to the hippocampus, actually affects hippocampal memory. Therefore, it remains unclear whether previously reported TRPC-dependent memory deficits were caused by hippocampal dysfunction or were instead due to the absence of TRPC channels up- or down-stream of the hippocampus. In

addition, although it has been reported that synaptic plasticity is affected by TRPC knockout (KO), it remains unknown whether cellular and network activity is altered in the hippocampus due to the TRPC KO.
Here, we use a hippocampally localized KO of TRPC5 and show that this adversely affects hippocampus-dependent memory performance during a trace fear conditioning task. Specifically, KO mice showed significantly less freezing during acquisition and recall than controls. In addition, using *in vivo* electrophysiological recordings from the CA1 pyramidal cell layer, we investigated the effects of a TRPC5 KO on network and single cell activity levels during trace fear conditioning acquisition and recall, a random foraging task, and a linear track with subsequent sleep sessions. Our data indicate that TRPC5 channels in the hippocampus do indeed support hippocampus dependent memory function, as well as contributing to cell and population activity *in vivo*.

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Poster

161. Hippocampus: Intrinsic Hippocampal Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: NIH R01 AG034613

Title: Age-related modulation of functional connectivity along the long-axis of the hippocampus and the hippocampal subfields

Authors: *S. M. STARK[1], B. KIRWAN[2], C. E. STARK[3];
[1]Univ. of California, Irvine, Irvine, CA; [2]Psychology, Brigham Young Univ., Provo, UT; [3]Univ. of California Irvine, Irvine, CA

Abstract: A number of lines of evidence, from neuroanatomical through functional imaging, have supported the notion that the hippocampus demonstrates circuit-level specificity of function, both when segmented based on subfields (CA1, CA3, DG, and subiculum) and along the longitudinal axis. There is also evidence that aging may have differential effects across subfields (e.g., DG/CA3) and along the longitudinal axis. For example, there is evidence for an age-related shift in functional connectivity in the hippocampus from anterior to posterior (A-P) regions (Blum et al., 2014; Damoiseaux et al., 2016). However, the volumetric ratio of hippocampal subfields varies along the longitudinal axis of the hippocampus and may better capture these age-related alterations in connectivity. Here, we sought to evaluate the functional connectivity profile between the hippocampus and the medial temporal lobe to determine how

these segmentation models capture age-related changes in this circuit. We calculated functional connectivity during an incidental recognition memory task in 31 young (20-39 years) and 31 older (70-87 years) adults, controlling for differences in regional volume. We replicated the age-related functional connectivity shift (e.g., hippocampus to parahippocampal cortex) from the A-P hippocampus in three task-based datasets (incidental encoding of objects, incidental encoding of scenes, and a continuous recognition task). The degree of this shift also predicted mnemonic discrimination performance in older adults. Further, we observed this A-P shift for each of the hippocampal subfields, despite the fact that the subfields are disproportionally represented along the long axis of the hippocampus. To address the role of hippocampal and MTL involvement in encoding or retrieval during task-based fMRI, we explored these findings during resting-state scans. We found that task demands modulate age-related connectivity along the longitudinal axis of the hippocampus, reflecting the important role of engagement in assessing neural coordination in the aging brain.

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Poster

161. Hippocampus: Intrinsic Hippocampal Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant 1R15AG045820-01A1

Title: Dose-dependent effects of the ampakine CX614 on the development of spatial navigation in juvenile rats

Authors: *R. OGOE[1], D. G. MCHAIL[1], D. CHEN[1], N. VALIBEGI[1], S. YOON[1], T. C. DUMAS[2];
[2]Psychology, [1]George Mason Univ., Fairfax, VA

Abstract: The first three weeks of a rat's life are characterized by a period of rapid development in the rat's spatial navigation ability with postnatal day (P) 21 marking the point where adult-like spatial navigation is observed. Numerous physiological changes occurring during this time period contribute to functional maturation of the hippocampus, including alterations in the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) at glutamatergic synapses. For instance, more AMPA receptors contain GluA1subunits in pups younger than three weeks of age, but more AMPA receptors contain GluA3 subunits as animals mature beyond the end of the third postnatal week., Combined with other alterations in the AMPAR complex, an this subunit switch prolongs the synaptic response and lowers the threshold to induce long-lasting changes in synaptic strength induced by patterned activity. Our previous research has

demonstrated that the positive allosteric modulation of AMPARs (which prolongs excitatory synaptic responses) induces adult like Y-maze alternation in rats younger than three weeks of age. Because spontaneous alternation in a Y-maze does not permit separate investigation of spatial learning and memory, we recently extended this work by training juvenile rats on a Barnes maze adapted for developing rats. Preliminary results suggest that impacts of the AMPAKINE, CX614, on maze performance might differ based on age (whether younger or older than P21) and dosage (1.25 or 2.5 mg/kg). In particular, higher but not lower dosages of CX614 were associated with fewer errors during a memory probe trial in older, but not younger animals. CX614 treatment was also associated with selective spatial memory impairments that varied by age and drug dosage. Ongoing experiments with higher drug dosages (4 mg/kg) will help establish a dose-response curve for effects of CX614. These findings will help clarify the role of the maturation of hippocampal excitatory synapses in the emergence of spatial learning and memory.

Disclosures: R. Ogoe: None. **D.G. McHail:** None. **T.C. Dumas:** None.

Poster

161. Hippocampus: Intrinsic Hippocampal Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: The Crandall-Cordero Fellowship
CT Institute for the Brain and Cognitive Sciences (IBACS)

Title: Persistence of dorsal and ventral hippocampal remapping after exploring a novel environment

Authors: *S. LEE, R. TROHA, A. ANAM, M. KATZ, K. CITRIN, T. PIETRUSZEWSKI, I. H. STEVENSON, E. J. MARKUS;
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Abstract: In both humans and rats, the hippocampus is important for memory and navigation. Individual hippocampal neurons are spatially tuned, and fire in specific physical locations. Together, these “place cells” provide a representation or map of an environment. However, if the environment or behavioral context is altered, a place cell can change its firing pattern or “remap”. The hippocampus is a long structure with multiple sub-regions of diverse connections. Much is known regarding place cell activity in the dorsal region of the hippocampus, since these are close to the surface of the brain and relatively accessible. Less is known about ventral hippocampal place cell activity located much deeper in the brain. Firing characteristics were determined while recording cells simultaneously from dorsal and ventral hippocampus as rats

traversed a familiar linear track environment for food reinforcement (Day One - Session One). After the hippocampal representation of the familiar environment was determined, one of the two arms was rotated to a novel position. We measured how the spatial tuning of these neurons was modified when rats experienced a novel spatial environment (Day One - Session Two). Lastly, we re-configured the two-arm maze back to a linear track, and the rats ran in the familiar environment again (Day One - Session Three). The following day, the rats performed the same three sessions. We will compare dorsal and ventral hippocampal representations of a familiar maze configuration, the development of a new representation (in the novel location arm), and the degree to which the developed representation is stable. Taken together, these data provide insight into the regional differences of hippocampal representations of a novel environment and the persistence of their activity across sessions and days.

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Poster

162. Hippocampal Dynamics in Learning and Memory

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 162.01/W29

Topic: H.01. Animal Cognition and Behavior

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Howard Hughes Medical Institute (L.M.F.)
NIMH U01 #NS090537 (LLNL and L.M.F.)

Title: A behavioral report of spatial memory confidence and neural correlates in the rat hippocampus, orbitofrontal cortex, and nucleus accumbens

Authors: *H. R. JOO[1], H. LIANG[1], J. E. CHUNG[1], C. GEAGHAN-BREINER[1], J. FAN[1], J. A. PEBBLES[2], A. M. YORITA[2], R.-U. HAQUE[3], D. F. LIU[1], D. ROUMIS[1], L. M. FRANK[4]; [1]UCSF, San Francisco, CA; [3]Ctr. for Bioengineering, [2]Lawrence Livermore Natl. Lab., Livermore, CA; [4]Dept. of Physiol., UC San Francisco, San Francisco, CA

Abstract: There are many behavioral scenarios in which decisions must be made using degraded or corrupted information from memory. In these scenarios, *confidence* in past knowledge (*i.e.*, memory) is essential to optimize resource investment. The ability to assess the veracity of memories (memory confidence) has been demonstrated in primates performing visual memory tasks. In monkeys, functional imaging has implicated the anterior prefrontal cortex. In human subjects, a similar task has identified corresponding single unit activity in the medial temporal

lobe. However, it is not known how neural circuits compute or represent memory confidence in general, or whether organisms with a less developed cortex also have access to metacognitive variables such as memory confidence. To address these questions, we developed a novel spatial memory confidence task in the rat. In this trial-based task, a rat must visit the one of two cued locations (of six possible) that was least recently visited, then has an option to wait, for as long as it chooses, at the cued location. After the rat ends the wait, a wait length-proportional reward is received following correct choices exclusively. Here we show that behavior is consistent with an internal representation of memory confidence: rats invested more time preceding correct trials relative to error trials, and did so in a manner consistent with a memory-based model of trial difficulty. To investigate the corresponding neural activity, we have focused on three interconnected areas: the hippocampus, for its representation of spatial memories; the orbitofrontal cortex (OFC), a region of anterior frontal cortex where, in rat perceptual discrimination tasks, firing rates are correlated with decision confidence; and the nucleus accumbens (NAc), which receives inputs from both the hippocampus and OFC and is therefore well positioned to integrate those inputs to influence behavior. Using a combination of tetrodes and polymer electrode arrays (*288 total electrodes*) in behaving rats, we have begun to investigate firing patterns that may support the computation of memory confidence.

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Poster

162. Hippocampal Dynamics in Learning and Memory

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Support: Simons Collaboration on the Global Brain Postdoctoral Fellowship
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Title: Operant conditioning of hippocampal sharp wave ripples

Authors: *A. K. GILLESPIE[1], D. A. ASTUDILLO MAYA[1], D. F. LIU[1], M. E. COULTER[1], E. L. DENOVELLIS[3], S. DESSE[1], D. K. ROUMIS[1], U. EDEN[3], L. M. FRANK[4,2];
[2]Kavli Inst. for Fundamental Neurosci., [1]UCSF, San Francisco, CA; [3]Mathematics and Statistics, Boston Univ., Boston, MA; [4]Howard Hughes Med. Inst., San Francisco, CA

Abstract: Nearly every decision is influenced and informed by prior experience. This would not be possible without the hippocampus, which is critical for rapidly encoding rich, multimodal

representations of experience and coordinating the long-term storage and later retrieval of these experiences. A candidate mechanism thought to contribute to both consolidation and retrieval processes is hippocampal replay, during which the neural ensemble corresponding to an experience is reactivated in a time-compressed manner. Replay events typically coincide with distinctive, 150-250Hz oscillations in the hippocampal local field potential known as sharp wave ripples (SWRs), which can be detected as a proxy for replay. Replay during sleep generally represents recent past experience and is thought to facilitate memory consolidation. However, replay in the awake state can be predictive of upcoming movement trajectory or correct choice, and awake SWR disruption acutely impairs acquisition and performance of a spatial memory task. These findings suggest that awake replay may not simply promote consolidation, but could also underlie planning or deliberation—potentially allowing the recall of previous experience to shape upcoming decisions. To dissect the contribution of awake SWRs to memory-guided behavior, we developed an operant conditioning task that requires a rat to generate SWRs preceding the choice point in a spatial memory task. Bilateral hippocampal CA1 tetrodes were used to detect SWRs in real time and rapid auditory and reward feedback was provided when SWR detection criteria was met. Over the course of training, we found that animals learned to reliably generate SWRs at the required stage of each trial. As animals were challenged to generate higher amplitude SWRs across training days, they responded by increasing SWR prevalence approximately two-fold across events of all amplitudes. Many of these SWRs contained clear, task-relevant replay trajectories, suggesting that they could contribute to or reflect ongoing behavior. This result demonstrates the ability to promote physiologically relevant hippocampal SWRs using real-time neurofeedback. Further, the enforced period containing many SWRs preceding a choice point provides a rich opportunity to link replay content with behavior across trials of varying memory burden, reward history, goal certainty, and other task variables.

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Poster

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Title: Dorsal and ventral hippocampal sharp-wave ripples engage opposing networks in the nucleus accumbens

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Abstract: Memories of positive experiences require the brain to link places, events, and reward outcomes. Neural processing underlying the association of spatial experiences with reward is thought to depend on interactions between the hippocampus and the nucleus accumbens (NAc). Hippocampal projections to the NAc arise from both the ventral hippocampus (vH) and the dorsal hippocampus (dH), and previous studies have demonstrated that either vH or dH input to the NAc can support behaviors dependent on spatial-reward associations. It remains unclear, however, whether dH, vH, or both coordinate memory processing of spatial-reward information in the hippocampal-NAc circuit. Times of memory reactivation within and outside the hippocampus are marked by hippocampal sharp-wave ripples (SWRs), discrete events which facilitate investigation of inter-regional information processing. It is unknown whether dH and vH SWRs act in concert or separately to engage NAc neuronal networks, and whether either dH or vH SWRs are preferentially linked to spatial-reward representations. Here we show that dH and vH SWRs occur asynchronously in the awake state and that NAc spatial-reward representations are selectively activated during dH SWRs. We performed simultaneous extracellular recordings in the dH, vH, and NAc of rats learning and performing an appetitive spatial task and during sleep. We found that individual NAc neurons activated during SWRs from one subdivision of the hippocampus were typically suppressed or unmodulated during SWRs from the other. NAc neurons activated during dH versus vH SWRs showed markedly different task-related firing patterns. Only dH SWR-activated neurons were tuned to similarities across spatial paths and past reward, indicating a specialization for the dH-NAc, but not vH-NAc, network in linking reward to discrete spatial paths. These temporally and anatomically separable hippocampal-NAc interactions suggest that dH and vH coordinate opposing channels of mnemonic processing in the NAc.

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Poster

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Title: A state space model for characterizing replay dynamics

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Abstract: During sleep and immobility, hippocampal place cells fire in sequences consistent with temporally compressed versions of trajectories previously run by the animal. These replayed sequences are hypothesized to be an important mechanism for the retrieval of spatial memory in service of consolidation and decision-making. Replay events are typically evaluated based on whether they activate sequences of place cells that represent spatially continuous trajectories through the environment, but recent work has shown that these events can have more complex dynamics. For example, sequences can alternate between hovering on a particular spatial location and continuous movement (Pfeiffer and Foster 2015) or can represent continuous trajectories in other spatial environments (Karlsson et al. 2009), which may appear spatially incoherent in the context of the current environment. To quantify the structure of replay events, we develop a state space model that uses a combination of discrete and continuous latent states to decompose place cell sequences into categories based on their latent dynamics. Each discrete latent "category" is associated with a type of continuous latent dynamic—such as hovering, spatially fragmented or spatially continuous. This allows for (1) direct comparison between different categories of sequence dynamics, (2) expression of our confidence in one or more categories explaining the data, and (3) characterization of the transitions between categories. In addition, the model can function in 2D, avoiding linearization errors on more complicated environments. We demonstrate the utility of this model on simulated and real data of an animal performing a spatial memory task.

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Poster

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Topic: H.01. Animal Cognition and Behavior

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Title: Integrated statistical and machine learning approach to identify spatial receptive field structure in rat hippocampal and prefrontal cortex populations

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Abstract: Hippocampus and prefrontal cortex (PFC) play critical roles in learning spatial navigation in the rat. Much is known about the variety of signals that influence hippocampus spiking, but less is known about which behavioral variables affect spiking in PFC and whether or not they have similar effects in PFC and hippocampus. We aim to develop a complementary pair of model identification approaches, one based on classical statistical methods and another based on supervised machine learning classification algorithms, and demonstrate how these approaches can interact to enhance our understanding of coding in each of these areas. We develop the first component of this model identification framework based on point process generalized linear models (GLMs) fit using a regularized maximum likelihood procedure. This component provides well-developed methods for testing hypotheses about which signals influence the observed spiking data, for assessing model goodness-of-fit, and for iteratively refining the models. We use the resulting model structures and parameter estimates to classify neurons based on their coding properties. We will use these classifications as teaching signals for a supervised machine learning classification algorithm using a recurrent neural network architecture to explore whether the trained classifier is able to estimate the coding properties of data from newly observed neurons. Additionally, the model fit allows us to generate new simulated data across multiple classes, including those that are not well sampled in our original training dataset. We apply the statistical model identification approach to local field potential (LFP) and spiking data simultaneously recorded from hippocampus CA1 region and PFC of three male Long Evans rats performing a spatial alternation task on a W-shaped track. We analyze the degree to which neurons in PFC and CA1 code for the rats' movement trajectories including position and speed coding, theta rhythmicity, theta precession, and other forms of history dependence. We found that a substantial proportion of PFC cells are spatially selective, with position, velocity, and rhythmic firing properties. The statistical model identification identified clear differences between hippocampal and prefrontal populations, and allowed us to classify the coding properties of the population of neurons in these two regions. We will apply the machine learning algorithm to the same data to evaluate the efficiency of the classification. This model identification paradigm allows for the development of the statistically principled and computationally efficient classification of neural coding properties.

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Poster

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Support: NSF NeuroNex Award
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Title: Event-to-event variability in structural organization of population spiking during hippocampal sharp wave-ripples

Authors: *X. DENG[1], S. CHEN[2], X. WEI[1], M. SOSA[3], M. KARLSSON[4], L. M. FRANK[5]; [1]Columbia Univ., New York, NY; [2]Univ. of California, Davis, Davis, CA; [3]Neurosci. Grad. Program, Kavli Inst. for Fundamental Neurosci., [4]Univ. of California, San Francisco, San Francisco, CA; [5]Dept. of Physiol., UC San Francisco, San Francisco, CA

Abstract: The ability to flexibly remember experiences at different levels of specificity is crucial to how we learn and how we make decisions. However, the underlying mechanisms involved in flexibly storing and retrieving memories in varying degrees of detail remain elusive. Current theories suggest that memories of past experiences are stored when specific patterns of neural activity cause changes in the connections among neurons, and they are retrieved when these patterns are reinstated. For example, when an animal moves through its environment, spiking activity of hippocampal place cells is paced by an underlying "clock" with a constant rate at theta frequency. When the animal slows down or pauses, place cell population is sequentially reactivated during sharp wave-ripples (SWRs),which often represents a replay of past trajectory on a compressed time scale. Is the hippocampal clock also constant during the SWR state? Here we report that rhythmicity underlying the SWR-associated organization of population spiking exhibits high event-to-event variability on a continuous scale of 6-50 Hz. This continuous scale is observed across laps, familiarity of spatial environment, and types of replay content and is approximately lognormal. Decoding analyses using clusterless methods further suggest that during awake immobility, same spatial experience are replayed in multiple SWR events, each time with a different clock and the rate of this "clock" is randomly sampled from a lognormal distribution. We propose that such changing clock might constitute a general mechanism for flexible memory consolidation.

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Poster

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Title: Self-recognition in the mouse hippocampus

Authors: *J. YOKOSE[1], J. I. TERRANOVA[1], W. D. MARKS[1], J. YAMAMOTO[1], S. K. OGAWA[1], T. KITAMURA[1,2];
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Abstract: Episodic memory requires the concerted association of space, time, objects and individuals including the self and the other. A feature of episodic memory is autobiographical; requires own experience accompanied with representation of the self in an episode and preserves at first-person viewpoint, which allow to distinguish own episode from other's episode. Hippocampal network is thought to be crucial for the formation of episodic memory. Dorsal hippocampal CA1 neurons represent a spatial location of the self as well as that of other. Both hippocampal CA2 neurons and ventral CA1 neurons are crucial for social memory to recognize the other. However, it remains unknown whether the hippocampus is involved in the self-recognition. Here, we established a mirror self-recognition test in mouse, which was originally developed in chimpanzee. C57BL/6J mice were familiarized with a mirror for two weeks. After the familiarization, we subjected them to a mirror self-recognition test. Before the test, we marked white ink or black ink as control on their heads under anesthesia. We observed that mice with white ink more frequently groomed their heads compared to mice with black ink. The duration and frequency of head grooming was much lower when there was no mirror in the light condition or when there was a mirror in the dark condition, indicating that C57BL/6J mice recognize themselves via a mirror. Next, we chemogenetically inactivated the neural activity of dorsal or ventral hippocampal neurons during mirror self-recognition test by bilateral injection of AAV-CaMKIIα-hM4(Gi)-mCherry into hippocampus and clozapine-N-oxide (CNO) i.p. administration. We found that inactivation of either the dorsal or ventral hippocampus by the CNO injection impaired the mirror-induced head grooming compared to control PBS-hM4(Gi)-mCherry mice and CNO-mCherry mice, indicating that both the dorsal and ventral hippocampal neurons are crucial for mirror self-recognition. Currently, we are investigating how the hippocampal network represents and distinctly recognizes the self and other.

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Poster

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Title: Novel behavioral approaches for analyzing temporal-tracking, context-time integration, and time cell activity in mice

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Abstract: Episodic memory is the unique recollection of a specific event in time, requiring the processing and integration of multiple information types, including spatial, contextual, and temporal information. Previous research demonstrates that the hippocampus has the capability to integrate multiple spatially bound inputs with relevant contextual and nonspatial information into a singular representation. To better support the analysis of context-time integration and the underlying circuit mechanisms, we have designed novel behavioral tasks for use with simultaneous in-vivo calcium imaging and optogenetics to analyze time cell and place cell activity. In the first task, mice are trained to hold a 10 second nose poke in a starting box in order to receive a reward in a different location. When paired with in-vivo calcium imaging, this task allows for analysis of both time and place cells simultaneously. Importantly, it provides an alternative to motor-based assays of time cells, controlling for motor activity, head direction, and attentional processes. Time cell activity observed in this task with in-vivo calcium imaging is comparable to the readouts obtained using the running-based tasks currently in use. The context presented within the starting box or the linear track can be changed to assess the effects of context on time and place cell activity. The second task is for the assessment of temporal discrimination in mice. The apparatus, termed the Temporal I-maze, borrows from the principle of the T-maze based approaches used for temporal discrimination in rats. It consists of a linear track with a start box in the center and doors gated to a nose poke port with two arms each having a distinct contextual presentation. Mice are trained to associate different durations (2.5s or 10s) of a continuous nose poke-initiated tone presentation with a specific context/space. The animal is considered able to use temporal information to make decisions about context/space when they achieve a 70% success rate or higher on three consecutive days of testing. In both

tasks, additional inferences about temporal-contextual integration can be made through the analysis of error rate and latency to nose poke initiation when mice are presented with different or novel contexts, or during optogenetic experimentation.

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Title: A hippocampal-amygdala circuit for experience-dependent observational fear

Authors: *J. I. TERRANOVA, J. YOKOSE, W. D. MARKS, J. YAMAMOTO, S. K. OGAWA, T. KITAMURA;
Psychiatry, Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: The ability to predict dangerous situations is crucial for survival. In social fear learning, individuals can learn about harmful stimuli and environments by observing other conspecifics in aversive situations. Observational fear (OF) is a mouse model of social fear learning in which a naïve observer witnesses an unfamiliar demonstrator receive foot-shocks in a modified contextual fear apparatus. Although the observer is not shocked, it freezes in response to demonstrator foot-shock and learns to fear the context. We define this as innate OF because the observer has neither shock experience nor is familiar with the demonstrator. However, in other cases in nature, it is difficult to assess the danger of a situation solely by observing others. Therefore, in these cases, both prior experience and familiarity with the demonstrator are required for observers to exhibit OF. We define this as experience-dependent OF. While the neural mechanisms of innate OF primarily depend upon the anterior cingulate cortex (ACC) and basolateral amygdala (BLA), the neural mechanisms of experience-dependent OF are mostly unexplored. In this study, we developed a mouse model of experience-dependent OF and then investigated the underlying neural mechanisms. Surprisingly, a chemical lesion in ACC failed to block experience-dependent OF, while the same lesion results in impairment of innate OF. This is confirmed by enhanced immediate early gene expression in ACC during innate OF but not during experience-dependent OF, indicating ACC has a specific role in innate OF but in not

experience-dependent OF. Next, we investigated the role of the hippocampus in experience-dependent OF. Chemogenetic inhibition of excitatory neurons in dorsal hippocampus during own shock experience blocks experience-dependent OF. In ventral hippocampus, chemogenetic inhibition of excitatory neurons that project to the BLA during OF testing blocks experience-dependent OF. Together, our findings demonstrate that a dorsal and ventral hippocampus-amygdala network differentially contributes to experience-dependent OF. Currently, we hypothesize that dorsal hippocampus generates a fear memory engram in the BLA during own shock experience and ventral hippocampus reactivates the fear memory engram to express experience-dependent OF.

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Poster

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Title: Lateral entorhinal cortex drives strong dendritic excitation of CA1 pyramidal neurons

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Abstract: The functional interaction between the entorhinal cortex (EC) and hippocampus (HC) is critical for episodic memory formation. Direct projections from the medial and lateral entorhinal cortex (MEC and LEC) provide the hippocampus with spatial and non-spatial (contextual) sensory information, respectively. Previous studies have largely focused on MEC-HC interactions, leaving the circuit-level functional interaction between LEC and the hippocampus underexplored. Only recently have behavioral studies begun to demonstrate that LEC is involved in olfactory learning, object-related learning, and contextual learning. EC inputs synapse onto the distal dendrites of hippocampal CA1 pyramidal neurons, where they integrate with other extra- and intra-hippocampal inputs, likely influencing compartment-specific learning

rules in the hippocampus. Our study examines how LEC-CA1 inputs interact with local CA1 microcircuitry to shape dendritic integration and long-term plasticity. Using slice electrophysiology and optogenetics, we demonstrate that direct, glutamatergic LEC projections drive strong excitation and inhibition onto CA1 pyramidal neurons (PNs). Interestingly, optogenetic stimulation of LEC axons in CA1 can produce putative sodium and calcium dendritic spikes in CA1 PNs. To better understand the mechanism underlying these LEC-driven dendritic spikes, we began to probe the GABAergic microcircuit elements recruited by LEC inputs. We found that LEC inputs strongly excite vasoactive intestinal peptide (VIP)-expressing inhibitory neurons (INs) in CA1, which are known to be disinhibitory in the hippocampus and neocortex. Thus, we propose that LEC activity recruits VIP interneurons, which gate dendritic spikes in CA1 PNs through a disinhibitory mechanism. Given that *in vitro* and *in vivo* studies have previously demonstrated the role of dendritic spikes in hippocampal plasticity, LEC would be positioned to powerfully regulate plasticity changes and long-term representation in hippocampal area CA1 by promoting supralinear dendritic computation.

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Poster

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Title: Spatial selectivity in apical dendrites of area CA3 during treadmill locomotion

Authors: *J. J. MOORE, S. K. RASHID, J. BASU;
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Abstract: Hippocampal neurons known as “place cells” are tuned to specific locations in an environment, but the tuning properties of hippocampal dendrites are largely unexplored. Recent studies implicate active dendritic spikes in predicting the formation of stable place fields (Bittner et al., 2017) and shaping hippocampal activity (Basu et al., 2016). However, due to technical and analytical limitations, in vivo studies of hippocampal dendritic activity have largely been confined to basal dendrites and proximal apical dendrites. This leaves activity in the apical tuft, where direct entorhinal cortex input arrives, to be indirectly inferred from somatic activity.
To address these issues, we developed a novel experimental paradigm in which the basal dendrites, cell bodies, and apical dendrites of pyramidal neurons in area CA3 can be imaged in the same focal plane. We imaged calcium activity, reported by GCaMP6f, simultaneously in

pyramidal cell bodies and dendrites while head-fixed mice ran for random water rewards on a treadmill track. A major hurdle in processing such data is the inability to use existing automated segmentation algorithms to detect and segment fine-scale dendritic regions of interests (ROIs). Furthermore, tracing a distal dendrite to its cell body must be performed with care and precision in order to perform accurate analysis of the relationship of somatic and dendritic activity.
To overcome these difficulties, we combined functional GCaMP6f imaging with structural labeling using tdTomato expressed in excitatory CA3 neurons. Prior to imaging calcium activity, we morphologically reconstructed the imaging region using the TdTomato signal from multiple focal planes. We then developed an automatic algorithm, based on coherence clustering (Berényi et al., 2014), to automatically identify dendritic segments from the calcium signal and assign them to their putative parent cell bodies. We then initialized the CaImAn analysis software (Giovannucci et al., 2019) using these ROIs to perform spectral de-mixing to resolve branches overlapping in space.
Using this automatic algorithm, we were able to identify thousands of apical and basal dendritic branches belonging to hundreds of pyramidal soma across six mice. The algorithm ran faster than real-time on a desktop computer, taking approximately 10 minutes to process 20 minutes of video recorded at 30 frames per second. The algorithm matched manually-labeled datasets and identified many more dendritic segments at farther distances from the soma than a human expert, and allows analysis of the role of apical dendrites in computing spatial representations.

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Poster

162. Hippocampal Dynamics in Learning and Memory

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Title: Familiar and novel environments as different network states in CA3

Authors: *S. K. RASHID[1], M. A. DUFOUR[2], J. J. MOORE[2], J. BASU[3];
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Abstract: Dendrites support non-linear integration and plasticity *in vivo* in a variety of brain regions, including the hippocampus. Hippocampal neurons known as place cells are tuned to

specific locations in an environment; but the tuning properties of hippocampal dendrites and the functional impact of active dendrites on the formation, maintenance and plasticity of spatial representations is largely unexplored. We developed a novel experimental preparation in which the activity of basal dendrites, cell bodies, and apical dendrites of area CA3 were imaged in the same focal plane while mice ran head-fixed on textured treadmill tracks representing familiar and novel environments. We first explored the spatial tuning properties of CA3 pyramidal cell dendrites in both familiar and novel environments. We then correlated the dendritic and somatic activity across days in a familiar environment and examined how this relation changes with exposure to a novel spatial environment. Further, we tested how manipulation of GABAergic inhibition shapes these spatially defined somato-dendritic activity patterns. Here we provide an overview of dendritic activity in CA3 as it relates to spatial tuning, and begin to uncover mechanisms by which place cells are formed and maintained.

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162. Hippocampal Dynamics in Learning and Memory

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 162.13/W41

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant 1R01NS109362-01
Mothers Foundation Award
Klingenstein-Simon Fellowship Award in Neuroscience
Sloan Research Fellowship
Whitehall three year research Grant

Title: Hippocampus modulates cortical sensory output through parallel processing pathways

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Abstract: To make sense of our surroundings, we must extract relevant information from our environment. To do so, our brain constantly integrates and compares current information arriving from multiple sensory areas with the information derived from past experiences stored as memory representations. The comparison of relevant past and present information helps us learn and adapt our behavior to harmful or rewarding environmental conditions. The functional interaction between hippocampus (HC) and its neighboring entorhinal cortex (EC) underlies this interplay between sensory processing and memory recall. Recent studies have exclusively focused on the anatomical and functional connectivity of feed-forward projections from EC to HC. In contrast, little is known about the hippocampal projections back to the EC or their

implication on sensory processing. Novel findings from our lab suggest anatomically segregated feedback from HC to EC resulting in a true reciprocal circuit architecture for parallel information processing. While hippocampal area CA1 projects directly to superficial EC output neurons, the canonical connection from HC to deep EC neurons is mainly from the hippocampal area Subiculum. My proposed novel circuit model prompts a revision of the existing EC-HC circuit model. My work aims to elucidate the circuit principles and plasticity rules by which hippocampus modulates cortical sensory information processing, thus allowing for quick behavioral adaptation to changing environmental demands.

Disclosures: **T. Butola:** None. **L. Peng:** None. **J. Basu:** None.

Poster

162. Hippocampal Dynamics in Learning and Memory

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 162.14/W42

Topic: H.01. Animal Cognition and Behavior

Support: Grant-in-Aid for JSPS Research Fellow

Title: Calcium imaging of hippocampal CA1 neurons during contextual fear memory encoding, retrieval, and extinction

Authors: *K. KOBAYASHI[1], R. TAKAKURA[2], J.-N. TERAMA[2], N. MATSUO[1];
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Abstract: Animals acquire a fear memory upon encounter a dangerous situation, and they make full use of the memory upon re-encounter the same situation to survive. Contextual fear conditioning is a powerful behavioral paradigm to study the neural mechanism of learning and memory. In this paradigm, a mouse is placed in a neutral context, which is characterized by a variety of environmental cues, such as floor textures, lighting intensity, background noise, odors of the chamber. Several minutes later, electrical foot shock is administered and the mouse is removed from the context. When the mouse is returned to the same context, it exhibits freezing behavior as a result of fear memory retrieval. Subsequent repeated re-exposure to the context without foot shocks eventually results in an attenuation of freezing behavior. Thus, contextual fear conditioning is useful for the analysis of memory encoding, retrieval, and extinction. Although a number of studies have demonstrated that the hippocampus is indispensable for contextual learning, a number of mysteries still remain unexplained: (1) what kind of cell would become a memory engram cell, (2) how hippocampal neurons encode contextual information, and (3) how hippocampal context representations are affected by fear memory extinction. To tackle these issues, we monitored calcium dynamics of 2,359 hippocampal CA1 pyramidal cells from 11 mice using a head-mounted microendoscope at single-cell resolution during contextual

fear learning. We thereby analyzed the activity of each neuron at different time points (before, during, or after foot shocks), different contexts (fear-conditioned context, distinct context, or home cage), and different behavioral states (freezing or non-freezing). In this meeting, we will present our recent findings about the neuronal activity patterns observed in the CA1 and discuss the association between the neural activity and behavior.

Disclosures: K. Kobayashi: None. **R. Takakura:** None. **J. Terama:** None. **N. Matsuo:** None.

Poster

162. Hippocampal Dynamics in Learning and Memory

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Program #/Poster #: 162.15/W43

Topic: H.01. Animal Cognition and Behavior

Support: Biotechnology and Biological Sciences Research Council UK award BB/N0059TX/1
Medical Research Council UK award MC_UU_12024/3

Title: Plastic neuronal allocation to hippocampal signatures of behavioural contingencies supports flexible learning

Authors: *M. EL-GABY[1], H. REEVE[1], V. LOPES DOS SANTOS[1], I. LUKÁCS[2], P. PERESTENKO[1], N. CAMPO-URRIZA[1], O. PAULSEN[3], D. DUPRET[1];
[1]MRC Brain Network Dynamics Unit, [2]Pharmacol., Univ. of Oxford, Oxford, United Kingdom; [3]Physiol. Develop. and Neurosci., Univ. of Cambridge, Cambridge, United Kingdom

Abstract: The ability to flexibly express distinct reward-predicting behaviours provides a key survival advantage. Hippocampal neurons are thought to collectively form cognitive maps that uniquely represent distinct environments. However, it is unclear whether distinct, task-defined contingencies within the same spatial environment are encoded by such neurons. To address this, we designed a conditional discrimination task in which mice learned to associate two sets of LED displays, each defining a distinct ‘contingency’, with different locations of rewarding and aversive stimuli within the same enclosure. Mice learned to make such a conditional discrimination within one day and remembered the association in a subsequent memory probe test. We find that hippocampal assembly patterns represent spatial and contingency-related aspects of an environment. While neurons with similar place fields were rapidly allocated to coactivity patterns representing space during pre-learning exploration, spatially incongruent neurons were gradually allocated to contingency-representing patterns as animals learned. The activation of contingency-representing patterns, but not of their individual member neurons, predicted subsequent performance in the probe test. Crucially, optogenetically silencing a subset of plastic intrahippocampal synapses selectively impaired allocation of neurons to contingency

patterns. At the behavioural level, such a manipulation severely impaired flexible behaviour in the probe test. We therefore identify a mechanism by which hippocampal neurons are plastically allocated to contingency-representing coactivity patterns to support flexible memory-guided behaviour.

Disclosures: **M. El-Gaby:** None. **H. Reeve:** None. **V. Lopes dos Santos:** None. **I. Lukács:** None. **P. Perestenko:** None. **N. Campo-Urriza:** None. **O. Paulsen:** None. **D. Dupret:** None.

Poster

162. Hippocampal Dynamics in Learning and Memory

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Program #/Poster #: 162.16/W44

Topic: H.01. Animal Cognition and Behavior

Support: Whitehall Foundation Grant 2017-05-35 to A.M.M.
Georgia State University Second Century Initiative Neurogenomics Fellowship to M.A.G.

Title: Neural coding in the dorsal CA1 hippocampus during behavioral flexibility

Authors: *M. A. GHANE[1], K. M. DALEY[2], Z. D. ALLEN[1], I. BELYKH[2], A. M. MABB[1]; [1]Neurosci. Inst., [2]Mathematics and Statistics, Georgia State Univ., Atlanta, GA

Abstract: Common among a vast number of neuropsychiatric and neurodegenerative illnesses is a diminished ability to re-evaluate and update task strategies in the face of changing allo- and ego-centric contingencies, a set of behaviors grouped under the category *behavioral flexibility*. Despite the fact that behavioral inflexibility severely diminishes patient quality of life, its circuit-level mechanisms are still not well understood. To approach this question, we are performing *in-vivo* calcium imaging in freely behaving mice during attentional set-shifting and Barnes maze tasks, allowing us to examine the contribution of the dorsal CA1 hippocampus (dCA1) to shifts in task dimensionality and spatial strategy, respectively. We find activity changes in the dCA1 during specific phases of learning with the execution of different strategies. dCA1 activity also appears to be linked to task accuracy in some phases of behavior. Finally, neural ensembles appear to encode different task demands in both behaviors. Our findings contribute to a more thorough understanding of neural coding of flexible behaviors in dCA1, and will inform interrogations of the cellular, molecular, and functional underpinnings of behavioral flexibility in health and disease.

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Poster

162. Hippocampal Dynamics in Learning and Memory

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Program #/Poster #: 162.17/X1

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant MH100349
NIH Grant NS086947

Title: Hippocampal unit spiking becomes selective for ripples of specific duration over extended spatial memory retention periods

Authors: *S. AHMADI[1], C. K. LUONG[1], S. LEUTGEB[1,2], J. K. LEUTGEB[1];
[1]Neurobio. Section and Ctr. for Neural Circuits and Behavior, [2]Kavli Inst. for Brain and Mind, Univ. of California San Diego, La Jolla, CA

Abstract: Hippocampal sharp-wave ripples are critical for learning and memory, and extended replay in long ripple events has been observed in behaving rats (Davidson *et al*., 2009). How ripple duration affects neuronal recruitment, coding reorganization, and memory performance, especially over long memory retention intervals, has not been elucidated. To address this question, we trained 6 Long-Evans rats on a spatial memory task in which the rats had to explore an 8-arm radial maze to find and remember an arm location that was novel on each day (*i.e.*, target arm) and where they received a large reward (CUE) over 5 trials. The rats were then tested for their memory of the target arm in a single trial (TEST) after a retention period of 6 hours (REST) during which they were placed in a box while their brain activity and movements were recorded. To control for factors other than the memory of the target arm, we pseudorandomly switched the target arm from trial to trial in a separate group of rats (control group). We initially asked whether ripples that occurred in the intervening REST would be prolonged by the memory demand of the task. We observed that, under various selection criteria, the distribution of ripple lengths did not differ between the memory and control groups. We then examined whether hippocampal neurons were differentially recruited as a function of ripple duration. This analysis revealed two distinct patterns across the memory group and the controls. First, in the memory group, cells became more selective for a narrow range of ripple durations in which they participated, whereas in the control group, cells were more broadly tuned to participate in ripples of various lengths. Second, in the memory compared to the control group, a larger proportion of cells was recruited during longer ripples. Next, we asked whether neurons that were more frequently recruited in long ripples had a more stable spatial representation across CUE and TEST. A linear regression analysis indicated no significant ($p = 0.11$) correlation between a neuron's preferred ripple length and its spatial coding stability as assessed by spatial correlation. In future analyses, we will test the functional implications of the differential recruitment of cells

by ripple duration. Specifically, we will test whether the memory of the target arm (as assessed by the rank of entry into the target arm at TEST) can be predicted by the level of unit recruitment and network reorganization in the hippocampus. Taken together, these experiments will contribute to revealing the role of sharp-wave ripples for long-term memory retention.

Disclosures: S. Ahmadi: None. **C.K. Luong:** None. **S. Leutgeb:** None. **J.K. Leutgeb:** None.

Poster

162. Hippocampal Dynamics in Learning and Memory

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Program #/Poster #: 162.18/X2

Topic: H.01. Animal Cognition and Behavior

Support: NIH grant MH100349
NRC grant 230413

Title: The ventral hippocampus is necessary for acquisition of innate fear memory

Authors: *L. RAGAZZI[1,2], M. SABARIEGO[2], I. ZUTSHI[2], S. LEUTGEB[2,3], J. K. LEUTGEB[2], V. H. BRUN[1,4], K. B. KJELSTRUP[1,4];
[1]Institutt for Klinisk Medisin (IKM), UiT The Arctic Univ. of Norway, Tromso, Norway; [2]Div. of Biol. Sciences, Neurobio. Section, [3]Kavli Inst. for Brain and Mind, UCSD, La Jolla, CA; [4]Univ. Hosp. of North Norway, Tromso, Norway

Abstract: The expression of fear in appropriate situations is critical for survival, and the ventral hippocampus (VH) has been identified as necessary for context-dependent fear responses (Jin & Maren, 2015; Kjelstrup et al., 2002; Vetere et al., 2017; Zhu et al., 2014). However, the specific contribution of this region is still unclear. In order to evaluate whether VH contributes to the expression of innate fear or to remembering where innate fear was experienced, we either silenced or lesioned the VH in Long-Evans rats while rats were exposed to a predator odor (coyote urine) and a snake-like robot in one side of a two chambered environment. Subsequent preference for the safe compartment compared to the compartment with prior odor/snake exposure was then tested in a series of tests (at 24 h, 48 h, and 72 h after exposure). Fear-related behaviors such as freezing and avoidance of the conditioned chamber were assessed at each time point in 4 experimental groups: 1) AAV-hm4D virus targeted bilaterally to VH with CNO inactivation only on the day of exposure to the odor/snake, 2) CNO-sham, 3) bilateral VH ibotenic acid lesions, and 4) VH lesion shams. Control animals showed a marked preference for the compartment without predator urine both during exposure and during testing after 24 h, 48 h, 72 h (sham group, p-value < 0.001 over all time points; CNO-sham group p-value < 0.001 during exposure, > 0.05 24 h, < 0.01 48 h, < 0.01 72 h) indicating the existence of innate fear as well as of contextual fear memory. In contrast, the lesion and CNO inactivation groups did not show

preference for either compartment except at 72 hours after urine/snake exposure (lesion group p-value > 0.05 during exposure, > 0.05 24 h, > 0.05 48 h, < 0.02 72 h; CNO-hm4D group p-value > 0.05 during exposure, > 0.05 24 h, > 0.05 48 h, < 0.02 72 h). Compared to their respective controls, VH lesions and inactivation therefore prevented or reduced the development of place preference and suppressed freezing responses (sham vs lesion group p-value < 0.02; CNO-sham vs CNO-hm4D group p-value < 0.04). Our data suggest that activity of neurons in the VH is necessary for the expression of innate fear as well as for the acquisition of a contextual fear memory, evident from both the removal of the VH as well as its temporary inactivation. The finding that inhibition of VH activity during exposure blocks the later contextual fear response suggests a role of ventral hippocampus in not only the expression of innate fear memory but also in the encoding of fear memory.

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Poster

162. Hippocampal Dynamics in Learning and Memory

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Program #/Poster #: 162.19/X3

Topic: H.01. Animal Cognition and Behavior

Support: Northern Norwegian Health Authority (Helse Nord) grant number SFP1165-14

Title: Hippocampal growth hormone modulates relational memory by changing the dendritic spine density in CA1

Authors: *K. HAUGLAND[1], A. OLBERG[1], F. STETTE[1], A. LANDE[1], K. KJELSTRUP[1,2], V. BRUN[2];
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Abstract: The hippocampus is a crucial site for memory and learning processes which is greatly influenced by neuromodulators. Although functions of some neuromodulators have been enlightened the effects of neuromodulators like growth hormone (GH) remain elusive. GH is commonly known as a peptide hormone that mainly targets the liver, however GH is also involved in cognitive functions. GH deficiency is associated with reduced capacity for memory and learnnig, and may occur in endocrine disorders but also during normal ageing. To determine how intrahippocampal GH affects hippocampal memory, we injected recombinant adeno-associated virus (rAAV) in male rats to express green fluorescent protein (GFP) combined with either GH, antagonizing GH (aGH), or no hormone in the dorsal CA1. We found that aGH disrupted memory in the Morris water maze task, and that these animals needed one more day of

training to re-learn a novel goal location. In a one-trial spontaneous location recognition test, the GH treated rats had better memory performance for object-locations than the two other groups. Histological examinations revealed that GH increased the dendritic spine density on apical dendrites of CA1, while aGH reduced the spine density. Furthermore, we used phosphorylated signal transducer and activator of transcription 5 (P-Stat5) as an indicator for activavted GH receptors. We found increased levels of P-Stat5 in the GH group compared to controls, and reduced P-stat5 in the aGH group. These findings imply that GH is a neuromodulator with strong influence over hippocampal plasticity and relational memory.

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Poster

162. Hippocampal Dynamics in Learning and Memory

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Topic: H.01. Animal Cognition and Behavior

Support: Office of Naval Research Grant N00014182114
NIH UL1 TR001414
NIMH MH082042
NIH T32 AG00096-34

Title: Characterization of a reverberating network in hippocampal field CA3 required for the acquisition of temporal order

Authors: *B. G. GUNN, B. M. COX, C. D. COX, C. M. GALL, G. LYNCH;
Anat. and Neurobio., Univ. of California Irvine, Irvine, CA

Abstract: Episodic memory, an essential element of orderly thinking, requires the organization of serial events into narratives about the identity of cues along with their locations and temporal order (‘what’, ‘where’, and ‘when’). The hippocampus plays a central role in the acquisition and retrieval of episodes with two of its subsystems being separately linked to ‘what’ and ‘where’. The substrates for the third temporal element are the most computationally challenging and, in the above context, remain poorly understood. The dense CA3 commissural-associational (C/A) system and its presumed recurrent connectivity has led to the suggestion that this region mediates aspects of memory processing including rapid short term storage and pattern completion. Here we propose that the C/A network, via prolonged reverberating activity, provides a mechanism capable of generating episodic time. Specifically, we demonstrate that hippocampal field CA3 maintains self-sustained activity for remarkable periods (minutes long) following a brief input and the ability of such input to initiate this prolonged activity is frequency dependent. Using

pharmacological and chemogenic tools, in conjunction with computational modelling, we explore features of this network that are required for the initiation, maintenance and termination of prolonged reverberating activity. We found that initiation was state-dependent, and as anticipated with any complex system, this network was highly sensitive to perturbations and prone to catastrophic collapse. Consistent with these electrophysiological findings, we recently demonstrated, using novel behavioral tests which like human episodic learning do not involve training or explicit rewards, that partial silencing of the network in mice blocks acquisition of temporal order, but not the identity or location, of cues.

Disclosures: B.G. Gunn: None. **B.M. Cox:** None. **C.D. Cox:** None. **C.M. Gall:** None. **G. Lynch:** None.

Poster

162. Hippocampal Dynamics in Learning and Memory

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Support: Office of Naval Research Grant N00014182114
NIMH MH082042
NIH T32 AG00096-34
NIH UL1 TR001414

Title: Hippocampal field CA3 is necessary for encoding temporal order: A proposed ‘when’ component of episodic memory

Authors: *C. M. GALL[1], B. M. COX[1], A. A. LE[1], B. G. GUNN[1], J. QUINTANILLA[1], C. D. COX[1], G. LYNCH[2];
[1]Anat. and Neurobio., [2]Psychiatry and Human Behavior, Univ. of California, Irvine, CA

Abstract: Episodic memory, a key element in information processing, involves the formation of associations between elements in a sequence into a narrative about what happened, where particular features occurred, and the order in which they appeared ('what', 'where', and 'when'). The hippocampus plays a central role in episodic memory formation. While extensive human and animal research showed that 'what' and ‘where’ information is associated with the lateral and medial perforant path projections from the entorhinal cortex, respectively, the ‘when’ element is poorly understood. As episodic memories in humans are routinely acquired as a part of everyday life without practice or explicit rewards, we developed one-trial, non-rewarded behavioral protocols to assess the three basic elements of an episode. We show that partial silencing of the field CA3 network in mice blocks acquisition of temporal order (‘when’), but not the identity or location (‘what’ and ‘where') of cues. We also examine the roles played by the diverse

mechanisms that govern self-sustained activity in the network in producing different features of episodic time (e.g., temporal elasticity). These results suggest a solution to the question of how hippocampus adds time to episodic memories.

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Poster

162. Hippocampal Dynamics in Learning and Memory

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Program #/Poster #: 162.22/X6

Topic: H.01. Animal Cognition and Behavior

Support: ONR Grant N00014182114

Title: Temporary inactivation of field CA3 of hippocampus in different forms of learning in enriched rats

Authors: *L. C. PALMER[1], B. M. COX[3], C. D. COX[2], C. PETREE[4], B. HENRIQUEZ[1], C. M. GALL[2], G. LYNCH[1];
[2]Anat. and Neurobio., [1]Univ. of California, Irvine, CA; [3]Anat. and Neurobio., Univ. California, Irvine, CA; [4]Irvine, Irvine, CA

Abstract: In humans, episodic memory includes the ability to recall the sequential order in which events occurred. Prior work from our group discovered that the dense bilateral system of recurrent connections that characterize hippocampal field CA3 generates self-sustained firing activity lasting for remarkable periods (minutes) following brief stimulation, and that transient unilateral silencing of a subpopulation of CA3 neurons with DREADD virus blocked this effect. The same manipulation applied unilaterally to the hippocampus eliminated the ability of mice to retain temporal order for a sequence of odors, leaving recognition of cue identity and location intact. Here we examined the effects of this treatment in rats tested on episodic encoding and their interactions with a complex environment. Our previous studies [Cox et al 2017] found that rats without prior experience with complexity show poor within- and between-trial learning in this environment, indicating that as with humans they transfer earlier experience to a novel situation. For the present study, rats given six 5-hour sessions in a large four-level chamber containing diverse objects had subpopulations of neurons in the unilateral field CA3 transfected with Gi-DREADD virus. After surgical recovery, they were injected with DREADD agonist (CNO) i.p. 30 minutes before being placed in a test arena having multiple compartments with various objects and a darkened refuge. There were no evident gross effects of activating the DREADD receptors: animals explored willingly, did not spend more time in the refuge, and within-session habituation rates were not negatively affected. However, the patterning of

foraying behaviors was disrupted compared to controls. The animals were then tested in episodic 'when' and 'what' assays using odor cues; as with mice, CNO injections completely blocked recognition of the temporal order without blocking detection of novelty. We hypothesize that the bilateral CA3 system used to acquire a series of presented cues across time, in a relatively simple test situation with little choice, may also be involved in processing the self-directed generation of perceptual sequences as the animal explores a complex situation. This question relates to a key cognitive constraint first recognized by the German philosopher Kant, who emphasized the need for the perceptual system to determine if an incoming sensory sequence comprises successively sampled percepts of an enduring complex (object or place), or successive perceptions of an objectively real sequence in the world (an event or episode).

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Poster

162. Hippocampal Dynamics in Learning and Memory

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Program #/Poster #: 162.23/X7

Topic: H.01. Animal Cognition and Behavior

Title: Distance and position coding in CA1 place cells during septal inactivation

Authors: *M. NORDLUND, D. LALAINA, G. MARTI, R. BOURBOULOU, F.-X. MICHON, J. EPSZTEIN, J. KOENIG GAMBINI;
Inst. of Neurobio. of the Mediterranean Sea (INMED), INSERM UMRS1249, Marseille, France

Abstract: Hippocampus and medial entorhinal cortex (MEC) are critically involved in the ability to navigate using an allocentric reference frame necessary to locate points of interest (nest, food sources, ⋯) from any starting points in the environment. These brain regions also contain a constellation of spatially modulated cells such as place cells in the hippocampus and grid cells in the MEC that exhibit a striking allocentric position code during random foraging in 2D environment. In 1D environment such as in linear tracks however, and at least for place cells, these cells can exhibit different spatial coding regime such as position coding (firing fields at the same position in both direction) or distance coding (firing fields at the same distance from both starting points) in bidirectional cells as well as directional modulation (unidirectional cells). The presence of these types of spatial coding depends on the richness of local cues and the experience of the animal in the environment and likely reflect differences in the integration of landmarks versus self-motion cues. Our goal in this work was to interrogate how distance, position and directional modulation react to an inactivation of the medial septum, a manipulation known to affect hexagonal firing patterns of grid cells. This inactivation was tested on CA1 pyramidal cells recorded in several behavioral tasks relying or not on the use of an allocentric strategy. Indeed, it

is still not clear how grid cells contribute to allocentric coding in the hippocampus (Koenig et al, 2011, Wang et al, 2014). To investigate this question, we tested first the effects of an inactivation of the medial septum on hippocampal spatial coding in mice running back and forth in a virtual linear track deprived or enriched with proximal virtual 3D-objects (Bourboulou, Marti, et al, 2019). In this task, the paucity of visual landmarks favored distance coding while the presence of 3D-objects favored position coding. In a second experiment, we tried to manipulate the presence of position/distance coding among CA1 place cells depending on the behavioral strategy the animal is using in a spatial memory task in the virtual linear track. We trained animals in two different behavioral protocols in which the animal can use only an allocentric strategy (to locate a hidden goal from any point in the maze) or in which it could rely on a path integration strategy (to locate a hidden goal from two fixed points in the track). Mice were able to learn both tasks. Dorsal CA1 neurons were recorded in these tasks before and during inactivation of the medial septum.

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Poster

162. Hippocampal Dynamics in Learning and Memory

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Program #/Poster #: 162.24/X8

Topic: H.01. Animal Cognition and Behavior

Support: Human Frontiers Science Program LT000835/2018

Title: Characterizing the activity of neural assemblies in the hippocampus across the full sleep-wake cycle

Authors: *R. BOYCE, R. COSSART;
Inst. De Neurobiologie De La Méditerranée, Marseille, France

Abstract: Sequences of hippocampal place cells encode spatial trajectories as a subject navigates a given environment. During periods of restful wakefulness and non-REM sleep, condensed sequences corresponding to prior navigation recur during sharp-wave-ripple (SWR) events in the hippocampus, the disruption of which disturbs memory. Enabled by the use of large-scale neural imaging, recent work has revealed a more structured organization behind these sequences as they appear to be composed of multiple discrete assemblies connected together. Understanding this activity during various behavioral conditions and across different vigilance states is critical as assemblies may represent the basic unit upon which memories are encoded. However, the functional organization of the microcircuits, including assembly activity, recruited during REM sleep (REMs) remains unknown despite the recently confirmed role for REMs in the formation

of spatial memory. To address this issue, we have employed large-scale 2-photon imaging in fully habituated head-fixed mice, enabling the simultaneous recording of hundreds of CA1 pyramidal neurons across multiple sleep-wake cycles, and under several tightly controlled experimental conditions (baseline in a cued / un-cued environment and following spatial learning in a cued environment). Cumulatively, these ongoing experiments provide a novel characterization of the recruitment and mechanistic role of neural assemblies in the hippocampus across the full sleep-wake cycle and under different environmental conditions.

Disclosures: R. Boyce: None. **R. Cossart:** None.

Poster

162. Hippocampal Dynamics in Learning and Memory

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Topic: H.01. Animal Cognition and Behavior

Support: Ministerial Grant
ERC Grant NeuroPionniers

Title: How direct thalamic inputs modulate hippocampal dynamics during development?

Authors: *E. LEPRINCE, M. BOCCHIO, R. COSSART, A. BAUDE;
INMED UMR 1249, Marseille, France

Abstract: In adult mice, the activity of hippocampal CA1 neurons is controlled by many converging inputs, including inputs from the hippocampus as well as other cortical or subcortical structures. The CA1 region is therefore a highly integrative brain area. Among those various pathways, the ventral midline thalamus (VMT), composed by the nuclei Reuniens (Re) and Rhomboïd (Rh), directly projects to the CA1 *stratum lacunosum moleculare* (slm). The VMT modulates CA1 dynamics by acting on the excitation/inhibition balance (Dolleman-van der Weel et al., 1997). Through its interconnections with both the medial prefrontal cortex and the hippocampus (Vertes et al., 2007), Re is involved in many cognitive processes in adult mice. Recently, the VMT has been shown to be an anatomical and functional relay between the hippocampus and the medial prefrontal cortex as early as P8 in rats (Hartung et al., 2016). It therefore suggests that the VMT may shape early hippocampal dynamics and contribute to the establishment of functional circuits. However, the impact of VMT inputs on developing CA1 dynamics at cellular level remains unknown. By using *in vitro* electrophysiological recordings and calcium imaging coupled with optogenetic stimulations, we have probed the effect of photostimulating thalamic afferents on the activity of hippocampal neurons. We report that the stimulation of thalamic afferents as early as P5 induces direct monosynaptic responses onto CA1 GABAergic neurons located in the slm. In addition, we observe in pyramidal cells that

photostimulation trains evoke polysynaptic responses (GDPs) which are blocked with a $GABA_A$ antagonist. These preliminary results suggest that thalamic afferents may contribute in shaping early hippocampal dynamics as early as the first postnatal week through a direct drive of GABAergic neurons.

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Poster

162. Hippocampal Dynamics in Learning and Memory

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 162.26/X10

Topic: H.01. Animal Cognition and Behavior

Support: FRM Grant FDM20170638339

Title: A deep-learning-based toolbox for inferring *in vivo* large scale neuronal activity from calcium imaging movie in the region CA1 of the developing hippocampus

Authors: *J. DENIS, R. DARD, M. PICARDO, R. COSSART;
INMED Mediterranean Inst. of Neurobio., Marseille, France

Abstract: Two-photon calcium imaging is now widely used to indirectly infer multineuronal dynamics from changes in fluorescence of a reporter protein or dye. However, analyzing this kind of data can still represent a computational challenge. Part of the challenge is to offer an analytical tool that would be scalable to the wide variety of calcium imaging data while providing reliable analysis.State of the art computational tools are still not optimized for the analysis of highly active neurons in densely packed regions such as the CA1 pyramidal layer of the hippocampus during early postnatal stages of development. Indeed, the accurate segmentation of the activity from overlapping neurons is not achieved by the latest analytic tools such as CaImAn. To meet this challenge, we have developed a method based on deep-learning.First, we have developed a graphical user interface (GUI) allowing for a precise manual detection of all transients (from onset to peak) from detected neurons, based on the activity from the calcium imaging movie. We collected and combined a corpus of manual annotations from three human experts' analyses on 6 mouse pups from 7 to 13 days old. Part of the labeled data was used to train our model, while the rest was kept to benchmark the performance. Then, we processed our data using a convolutional neural network and a bidirectional long-short term memory network. The proposed method is capable of learning long term sequences and can process long videos. We find that our method achieves better performance than CaImAn to identify neural activity in the developing CA1 without any user intervention.

Disclosures: J. Denis: None. **R. Dard:** None. **M. Picardo:** None. **R. Cossart:** None.

Poster

162. Hippocampal Dynamics in Learning and Memory

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 162.27/X11

Topic: H.01. Animal Cognition and Behavior

Title: Emergence of cell assemblies in the developing hippocampus

Authors: *R. F. DARD, J. DENIS, R. COSSART, M. A. PICARDO;
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Abstract: The classic view of hippocampal function is that of a structure providing a cognitive map of space, involved in navigation, learning, and episodic memory. However, within the last decade, the understanding of hippocampal function started to move away from this classical vision and a more computational and less representational version of its role started to emerge. In that framework, the hippocampus is best described as a “sequence generator”, i.e. a circuit with the ability to produce sequences of transient neuronal activation segmenting a multisensory context onto a continuous variable space. These sequences arise from the interaction between external sensory inputs and internally-generated self-organized activity. We have been exploring the internal self-organization of activity in the adult mouse CA1 hippocampus and uncovered the building-blocks of this internal scaffold, in the form of functionally orthogonal assemblies. During rest, these assemblies reactivate discrete temporal segments of neuronal sequences observed during run. These stable internal modules may therefore represent the default building blocks of hippocampal organization that can be combined to encode or retrieve spatio-temporal information. It is therefore essential to determine when and how these modules emerge in the course of development. To do so, we perform in vivo two-photon calcium imaging in the hippocampus of un-anesthetized pups from early postnatal stages to adulthood. We use a custom designed algorithm to infer neuronal activity from calcium transients recorded in hundreds of neurons. As animals age, we observe a desynchronization of network dynamics. Additionally, preliminary results revealed the emergence of cell assemblies around the end of the first postnatal week.

Disclosures: **R.F. Dard:** None. **J. Denis:** None. **R. Cossart:** None. **M.A. Picardo:** None.

Poster

163. Learning and Memory: Physiology I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 163.01/X12

Topic: H.01. Animal Cognition and Behavior

Support: Simons Foundation (542955, E.A.B)
McKnight Endowment Fund for Neuroscience (E.A.B)
National Science Foundation Graduate Research Fellowship (Y.B.)

Title: Monkey hippocampal neurons respond to structured task events in virtual reality

Authors: *Y. BROWNING[1], J. W. RUECKEMANN[2], A. L. FAIRHALL[2,3], E. A. BUFFALO[2,4];
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Abstract: It is not clear what aspects of experience drive hippocampal neurons to fire and how this activity contributes to the information processing that supports hippocampal function. Previous electrophysiological studies in rodents have demonstrated that task structure has a strong influence on hippocampal activity. Recent work has shown that hippocampal neurons reliably respond as an animal progresses through a predictable task, suggesting that salient task events are sufficient to structure hippocampal activity. This raises the compelling possibility that the ongoing activity in the hippocampus functions to link salient events into a continuous, unitary episode.
To characterize task-relevant activity in the primate hippocampus, we analyzed data from three rhesus macaque monkeys while they performed a spatial delayed alternation task in virtual reality. Each monkey received a chronically implanted hyperdrive targeting the medial temporal lobe with 124 independently-movable single wire electrodes. Recorded neurons were localized using a fiducial-registered MRI, and the sampled population spanned all major hippocampal subfields along the longitudinal axis. We used a generalized linear modeling framework to rigorously characterize the activity of more than 650 hippocampal neurons across 55 behavioral sessions. Approximately 25% of neurons active in the maze showed activity that was significantly reliable and informative about position in virtual space. Spatial fields for the majority of these neurons clustered around the key events in the maze, including the start zone, the choice point, and the goal locations. A smaller neuronal population sparsely represented the intervening areas of the maze, bridging the gaps between salient regions with continuous activity. By analyzing activity relative to the timing of salient events, additional neurons were identified that had significant responses to reward (15%) and also responses that tiled the inter-trial delay (8%). While some of these task-related neurons “remapped” when the monkey

performed the same task in a visually distinct context, other neurons maintained their task-specific responses irrespective of the visual cues defining the virtual environment. The stability of activity across mazes provides support for the idea that the hippocampus may abstract task structure across individual exemplars. Taken together, these findings emphasize the central role that task structure plays in shaping hippocampal activity. Further, these data are congruent with the theory that activity in the hippocampus tracks consistent task features and links them as a sequence of events within a cohesive episode.

Disclosures: Y. Browning: None. **J.W. Rueckemann:** None. **A.L. Fairhall:** None. **E.A. Buffalo:** None.

Poster

163. Learning and Memory: Physiology I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 163.02/X13

Topic: H.01. Animal Cognition and Behavior

Support: Simons Foundation (542955, E.A.B)
McKnight Endowment Fund for Neuroscience (E.A.B)
NIMH (R01MH117777, E.A.B)
NIH (P51OD010425)
NSF GRFP (18-573)
Washington Research Foundation Innovation Graduate Fellowship
NIH (T32NS099578)

Title: Spectral phenotypes of the monkey hippocampus

Authors: *A. D. GARCIA[1,2,3], J. W. RUECKEMANN[1], B. W. BRUNTON[2], E. A. BUFFALO[1]; [1]Physiol. and Biophysics, [2]Biol., [3]Grad. Program in Neurosci., Univ. of Washington, Seattle, WA

Abstract: Each subfield of the hippocampus reflects a distinct cytoarchitecture composed of different microcircuits, each with unique dynamic physiological processes. Recruitment of these neuronal circuits into coordinated patterns of temporally-structured activity is reflected in oscillations of the local field potential (LFP). It is hypothesized that microcircuits resonate within a range of characteristic frequencies when they become active, and that the local circuitry responsible for particular dynamics can be identified through the oscillatory patterns observed in the LFP. Additionally, rhythmic inputs to the hippocampus contribute to the oscillatory dynamics of the LFP, revealing the topographic organization of afferent terminations. A characterization of the spectral motifs of hippocampal physiology would provide an framework for demarcating subregions within this structure. Identification of these phenotypes is critical for elucidating the nature of interactions between hippocampal subfields, as well as illuminating how the local

circuitry of the hippocampus operates on its inputs in primates. Here, we examined oscillatory profiles unique to distinct subregions along the longitudinal axis of the monkey hippocampus. To accomplish this, we recorded LFPs across the longitudinal extent of the hippocampus from three rhesus monkeys, using chronically implanted hyperdrives while the animals performed tasks in virtual reality. Each drive contained 124 independently-moveable electrodes whose exact position could be tracked using MRI. Through employment of time-frequency analysis, spectral averaging, time-domain feature extraction, and machine learning algorithms, we characterized recorded LFPs and identified features that were unique to distinct portions of the hippocampus. Preliminary findings suggest that recording locations can be clustered by their LFP content in a topographic fashion, with key spectral features such as beta- (10-20Hz) and gamma-band (25-100Hz) dynamics varying across the structure.
These data reveal oscillatory profiles unique to distinct portions of hippocampus. Because the dynamics of the LFP over time reflect changes in the engagement of hippocampal circuitry during distinct behavioral states, these spectral phenotypes can be used to characterize active processing states within the hippocampus and to disentangle the nature of interactions between the primate hippocampus and downstream structures in the service of learning and memory.

Disclosures: **A.D. Garcia:** None. **J.W. Rueckemann:** None. **B.W. Brunton:** None. **E.A. Buffalo:** None.

Poster

163. Learning and Memory: Physiology I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 163.03/X14

Topic: H.01. Animal Cognition and Behavior

Support: Simons Foundation-542955
NIMH-R01MH117777
McKnight Endowment Fund for Neuroscience
NIH-P51OD010425
NIMH-R01MH080007

Title: Traveling sharp-wave ripples in the monkey hippocampus

Authors: *J. W. RUECKEMANN[1], A. DEDE[2], Y. BROWNING[3], E. A. BUFFALO[2,4];
[2]Physiol. and Biophysics, [3]Grad. Program in Neurosci., [1]Univ. of Washington, Seattle, WA;
[4]Washington Natl. Primate Ctr., Seattle, WA

Abstract: Hippocampal sharp-wave ripples reflect some of the most synchronous neural events in the brain. This coherent activity is thought to facilitate learning by coordinating spiking within a viable window for spike-timing dependent plasticity. Sharp-wave ripple events not only recruit

neurons through the intrinsic connectivity of the hippocampus, but they also coordinate activity through extrinsic projections across the cortical mantle and subcortical targets. The efferents from the hippocampus are topographically organized along the longitudinal axis, so characterizing the propagation of sharp-wave ripples through the hippocampus is key to understanding how the hippocampus interacts with reader structures across the brain.
We have analyzed the spread of sharp-wave ripple activity in the right hippocampus of four rhesus monkeys with chronically implanted hyperdrives. Each drive contains 124 independently-movable electrodes that span the full extent of the hippocampus. We have recorded over 10,000 events during between-task rest intervals. Similar to previous research in monkeys, we find that sharp-wave ripples occur somewhat less often (0.13/sec) and have a lower ripple frequency (115-130Hz) than has been observed in rodents. By analyzing the temporal offsets of each event across electrodes, we find that sharp-wave ripples vary in their point of origin. In addition, the progression of temporal offsets across anatomically arranged electrodes demonstrates that the sharp-wave ripples travel as a longitudinal wave. Interestingly, we identified several distinct anatomical trajectories traversed by the different ripple events, indicating that there is no fixed path for the travel of sharp-wave ripples through the hippocampus. Preliminary analyses demonstrate that sharp-waves spread farther along the longitudinal axis than the rat, and we have not observed any discontinuity between the posterior (dorsal in rat) and anterior (ventral in rat) hippocampus. Although there are many similarities between monkey and rodent hippocampus, the differences in longitudinal connectivity within CA3 may support a traveling wave than can more freely traverse the longitudinal axis - giving rise to coordinated activity that is more distributed throughout the hippocampus and its downstream targets.

Disclosures: **J.W. Rueckemann:** None. **A. Dede:** None. **Y. Browning:** None. **E.A. Buffalo:** None.

Poster

163. Learning and Memory: Physiology I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 163.04/X15

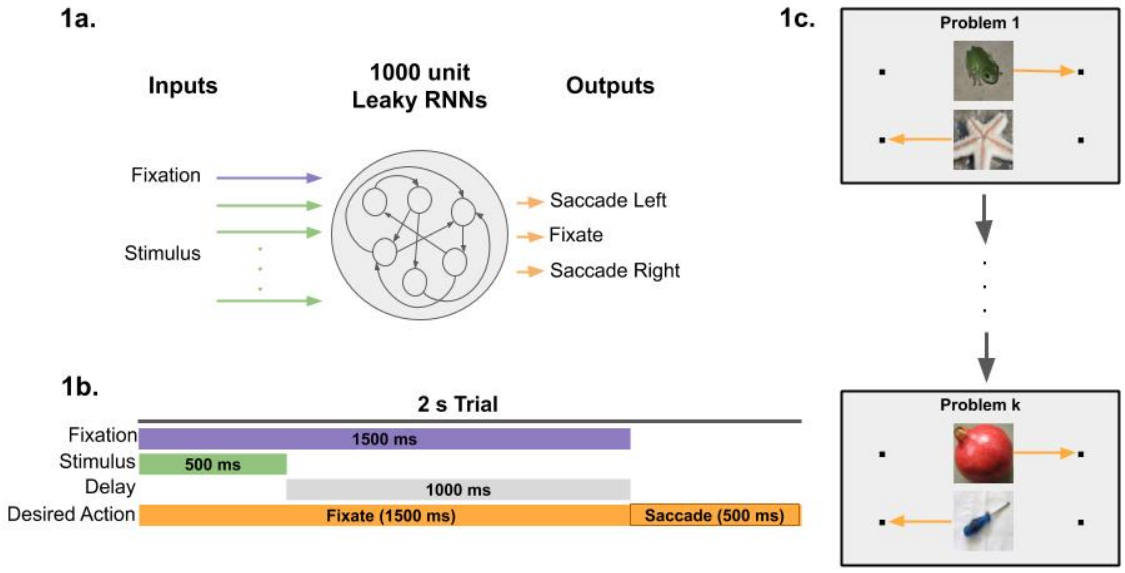
Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant U19NS107609
ONR Grant N00014-13-1-0297

Title: Elucidating the neural mechanisms of learning-to-learn

Authors: *V. GOUDAR[1], B. PEYSAKHOVICH[2], E. A. BUFFALO[4], D. J. FREEDMAN[3], X.-J. WANG[1];
[1]New York Univ., New York, NY; [3]Neurobio. and Computat. Neurosci., [2]Univ. of Chicago, Chicago, IL; [4]Physiol. and Biophysics, Univ. of Washington, Seattle, WA

Abstract: Our capacity to rapidly adapt to novel environments stems from our ability to learn how to efficiently solve frequently encountered problems. Behavioral studies have shown that as primates learn a series of discrimination problems, later problems are learned in fewer trials. This implies that the animals are not just learning rewarded stimulus-action associations, but also how to efficiently learn novel associations. While monkey lesion studies strongly implicate the prefrontal cortex in this ability, its circuit-level mechanisms remain unknown. To elucidate these mechanisms, we trained a recurrent neural network (RNN) model on a series of delayed sensorimotor association problems (Fig. 1a-b). In each problem, a pair of stimuli had to be uniquely mapped to a pair of actions (Fig. 1c). The stimuli were represented as random vectors in a high-dimensional feature space and changed from problem to problem, while the actions stayed fixed. We show that RNNs trained on a series of such problems demonstrate learning-to-learn: early problems were learned in hundreds of trials, while later problems were learned in fewer than ten trials on average. Interestingly, this ability only weakly depends on the stimulus dimensionality. Dimensionality reduction on the population activity showed that the learned representations exhibited a shared low-dimensional structure across problems, despite the plasticity-driven changes in these representations that ensued from learning new problems. Also, the number of trials to learn a problem correlated strongly with the magnitude of the change in synaptic weights, while the small changes in weights on rapidly learned problems resulted from a reuse of the dynamics learned from earlier ones. Collectively, these results indicate that the network extracts the latent task structure shared by the problems - and reuses it to learn new problems more efficiently. This study offers a mechanistic explanation, and testable experimental predictions, for learning-to-learn by linking it to changes in neural network representations and structure.



Disclosures: **V. Goudar:** None. **B. Peysakhovich:** None. **E.A. Buffalo:** None. **D.J. Freedman:** None. **X. Wang:** None.

Poster

163. Learning and Memory: Physiology I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 163.05/X16

Topic: H.01. Animal Cognition and Behavior

Support: U19NS107609, E.A.B
NIH P51OD010425

Title: Modeling of cognitive flexibility in Rhesus monkey

Authors: *A. J. O. DEDE[1,3], V. GOUDAR[4], S. AHMAD[1,3], S. A. SCHLEUFER[1,3], C. I. O'LEARY[1,3], N. GERMANOS[1], E. A. BUFFALO[2];
[2]Physiol. and Biophysics, [1]Univ. of Washington, Seattle, WA; [3]Washington Natl. Primate Ctr., Seattle, WA; [4]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Rules facilitate adaptive behavior with abstract mappings between stimuli and responses, but it is inefficient to learn mappings for every situation. By applying previously learned rules to new situations, we facilitate new learning. In effect, we learn how to learn. Two aspects of this ability include choosing which rule to apply when a situation changes and representing rules generally so that they may be applied to many stimuli. Here, we used a version of the Wisconsin Card Sorting Task to examine how monkeys flexibly switch between well-learned rules with familiar stimuli. On each trial, monkeys were presented with an array of four stimuli on a computer monitor. Each stimulus was comprised of three stimulus features: color, shape, and texture, e.g., a red striped star, and was chosen from a pool of 64 unique stimuli. Within a block of trials, one stimulus feature was designated as the target, resulting in 12 possible rules. The monkey received food reward for saccading to and fixating the stimulus that contained the target feature. Following 8 consecutive correct responses, the target feature changed without any cue to the monkey. Recursive Bayesian updating models were applied to the behavioral data (Wilson and Niv, 2012). An ideal Bayesian inference model capable of simultaneously considering all possible rules fit the data less well than a selective attention model that serially tests alternative hypotheses. These data replicated findings previously reported for humans. The goodness of the fit was similar to that observed in human data (mean model-predicted choice probability: .41; session range: .37-.43; chance: .25). However, the monkey deviated from model predictions early in the learning of new rules. These trials formed a low peak in the histogram of model-predicted choice probability. By contrast, human data had a unimodal distribution. The monkey's eye movements exhibited a spatial bias in these early trials that was not present in later trials, suggesting a shift of strategy as the monkey learned. An

advantage of this modeling approach is that it permits trials to be grouped based on the monkey's modeled mental state: guessing (high spatial bias); early learning (low spatial bias and low model-predicted choice probability); late learning (low spatial bias and high model-predicted choice probability). These distinct trial classifications will be correlated with neural data obtained from the hippocampus and prefrontal cortex in order to identify the neural mechanisms that underlie the application of abstract rules during new learning.

Disclosures: **A.J.O. Dede:** None. **V. Goudar:** None. **S. Ahmad:** None. **S.A. Schleufer:** None. **C.I. O'Leary:** None. **N. Germanos:** None. **E.A. Buffalo:** None.

Poster

163. Learning and Memory: Physiology I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 163.06/X17

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant NS094368
NIH Grant DC014686

Title: Heterogeneous coding of spatial behavioral variables across the macaque medial temporal lobe

Authors: *D. MAO[1], E. AVILA[2], G. C. DEANGELIS[3], J. DICKMAN[1], D. E. ANGELAKI[2]; [1]Dept. of Neurosci., Baylor Col. of Med., Houston, TX; [2]Ctr. for Neural Sci., New York Univ., New York, NY; [3]Brain Cognitive Sci., Univ. of Rochester, Rochester, NY

Abstract: Extensive studies have identified and characterized various functional cell types in the hippocampal formation and its connected regions in rodents, notably place cells, grid cells, and head direction cells. Whether the primate hippocampal system encodes spatial information in a similar manner remains to be determined. We recorded neuronal activity with chronically implanted tetrodes or single electrodes in several areas within the medial temporal lobe (MTL), including the hippocampus, (pre/para) subiculum, entorhinal cortex, and parahippocampal cortex, while monkeys (rhesus macaques) foraged for randomly scattered food pellets or fruits on the floor in a circular arena (3.3 m in diameter), a typical behavioral paradigm used to probe hippocampal spatial activity in rodents. We used a marker-based approach to accurately track the monkeys' position and 3D head orientation. By using an unbiased model-based method, we found that a large fraction of neurons across the MTL encode spatial behavioral variables in a heterogeneous way. Despite a low prevalence of sharp, isolated rodent-like place fields, position was prominently encoded in all areas by broadly modulating background firing, although neuronal activity was as sparse as that in rodents. Consistent with rodent studies, (pre/para) subiculum and entorhinal cortex carried significant head direction information. The firing of

many neurons throughout the MTL was also influenced by locomotion speed, head elevation, and pitch, but in diverse ways. For example, some neurons showed monotonically increasing or decreasing firing as a function of elevation whereas others showed U-shaped tuning curves. The activity of many neurons was best modelled by a combination of two or more variables. Distinct from rodents, local field potential activity exhibited peak power in the low beta band (~12-15 Hz) associated with slow motion or immobility. Taken together, these results suggest that the primate MTL system may encode spatial behavioral variables with neural correlates that are different in a variety of ways from those observed in rodents.

Disclosures: D. Mao: None. **E. Avila:** None. **G.C. DeAngelis:** None. **J. Dickman:** None. **D.E. Angelaki:** None.

Poster

163. Learning and Memory: Physiology I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 163.07/X18

Topic: H.01. Animal Cognition and Behavior

Support: IRP, NIMH, NIH, dHHS

Title: Monkeys with temporal-prefrontal disconnections are slow to learn a recognition memory task

Authors: *J. E. PEARL, M. A. G. ELDRIDGE, R. C. SAUNDERS, E. A. MURRAY, B. J. RICHMOND;
LN, Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Learning of visual recognition tasks relies on both temporal and prefrontal areas. We studied the role of the rhinal-orbitofrontal functional connection (Rh x OFC) in the learning of a visual recognition memory task. In the task, monkeys learned to discriminate between first and second presentations of never-before-seen images, of animals in natural scenes, to earn liquid rewards. In each trial, monkeys indicated whether the current image was a first or second presentation by releasing a touch bar in one of two intervals signaled by the change in color of a central target. Monkeys performed 400 trials per day (200 images). To assess learning, the set of possible intervals between first and second presentations expanded when monkeys reached criterion (75% correct, averaged across intervals) on the current set. The sets were: {0}, {1}, {1,2}, {0,1,2,4}, {0,1,2,4,8,16}, and {0,1,2,4,8,16,32,64,128}. The treatment groups were as follows: Rh: bilateral aspiration lesions of rhinal cortex. Hippocampus (HPC): bilateral excitotoxic lesions of hippocampus. Ventromedial prefrontal cortex (vmPFC): bilateral excitotoxic lesions of Walker's area 14. Rh x OFC: contralateral aspiration lesions of Rh and OFC (Walker's areas 11, 13, and 14).The Rh x OFC group learned the task more slowly than

controls (i.e. required more trials to reach criterion), and their average performance on the first 10 sessions of the final interval set was significantly worse than that of controls. The performance of the bilateral Rh removal group was also significantly worse than that of controls on the first 10 sessions of the final interval set. The two vmPFC monkeys learned more slowly than controls across interval sets, but were not consistently impaired in the final interval set. The HPC monkeys' learning and performance was indistinguishable from that of controls.
We conclude that 1) functional connections between Rh and OFC are necessary for the learning and performance of this serial recognition task, and 2) vmPFC contributes to learning but may not be essential for performing the task once learning has taken place.

Disclosures: **J.E. Pearl:** None. **R.C. Saunders:** None. **M.A.G. Eldridge:** None. **B.J. Richmond:** None. **E.A. Murray:** None.

Poster

163. Learning and Memory: Physiology I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 163.08/X19

Topic: H.01. Animal Cognition and Behavior

Support: IRP, NIMH, NIH, DHHS

Title: Object recognition and categorization in monkeys with aspiration removals of inferior temporal areas TEO and/or TE

Authors: *M. A. ELDRIDGE[1], T. SETOGAWA[2], J. E. PEARL[1], G. P. FOMANI[1], R. C. SAUNDERS[1], B. J. RICHMOND[1];
[1]NIMH, Bethesda, MD; [2]Univ. of Tsukuba, Tsukuba, Japan

Abstract: Introduction: Visual functions that require the processing of whole objects are known to depend on inferior temporal cortex (IT). We sought to determine the relative contributions of IT subdivisions, TEO and TE, on two forms of visual object processing.
Methods: To test recognition, monkeys were presented with a series of new images of animals in natural scenes every day. A single image was presented on each trial. The images were repeated once, after a delay of between 0 and 128 intervening image presentations. On each trial, the monkey had to indicate whether the current image was a first or second presentation by releasing a touch bar in one of two intervals.
To test visual categorization, the same monkeys were trained on a task that required them to make perceptual generalizations. Monkeys learned to classify the images as ‘cats’ or ‘dogs’. Initially, monkeys were presented with a set of 20 dogs and 20 cats. A single image was presented on each trial, and the monkey was rewarded for releasing a lever in the correct interval to indicate category membership. After the monkeys had learned to respond accurately to the

initial set of cat and dog images, a new set of 240 cats and 240 dogs was introduced to avoid the possibility of solving the task via rote learning...
Results: Recognition memory task performance of monkeys with TEO lesions was indistinguishable from that of controls. Monkeys with aspiration removals of area TE performed significantly less well than controls. Monkeys with combined TE and TEO removals performed as badly as, but no worse than, the monkeys with TE-only removals.
Categorization performance was moderately impaired after area TE removals. Removals of TEO caused only small transient impairments in categorization. Combined removals of areas TEO and TE produced marked, near-total, impairments in categorization.
Conclusions: The results show that processing in the ventral visual stream is not a strictly serial feed-forward circuit; in both experiments the effect of bilateral removal of TEO (earlier) was less severe than removal of TE (later). Therefore, visual afferents must transmit information in parallel, some of which bypass area TEO and some of which pass through TEO, to reach late stages of visual object processing.

Disclosures: **M.A. Eldridge:** None. **J.E. Pearl:** None. **G.P. Fomani:** None. **R.C. Saunders:** None. **B.J. Richmond:** None. **T. Setogawa:** None.

Poster

163. Learning and Memory: Physiology I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 163.09/X20

Topic: H.01. Animal Cognition and Behavior

Support: IRP
NIMH
NIH
dHHS

Title: Silencing the ChATter: Coexpressing an shRNA suppressing choline acetyltransferase activity and a chemogenetic DREADD receptor in monkey striatum

Authors: *S. MUMUNEY, W. LERCHNER, A. ADIL, R. FALCONE, J. N. TURCHI, B. J. RICHMOND;
Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Tonically active interneurons are cholinergic interneurons found throughout the striatum. Despite their sparse distribution (1-2% of striatal neurons) they are presumed to play important functional roles in reward seeking behavior. We wish to manipulate their function during behavioral and physiological studies using modern genetic techniques in old-world monkeys. Existing technologies, such as chemogenetics are powerful for modulating whole

regions but cell-type specific activity modulation remains difficult. Vector mediated RNA interference (RNAi) can in principle be used to target cell-specific pathways. Here we show a lentivirus with a human Synapsin promoter that co-expresses a microRNA scaffold against the Choline-Acetyl Transferase (ChAT) mRNA with either mCherry or a hM4Di-CFP fusion protein. To create shRNAs specific to ChAT mRNA, we used the DSIR (Designer of Small Interfering RNA) webtool to rank potential candidates; we then further selected them for targeting all ChAT isoforms and against targeting of other monkey genes. Three candidates were selected, and each was cloned into a microRNA MirE scaffold in the 3' UTRs of the hM4Di-CFP open reading frame (ORF). The best ranked candidate was also cloned into a MirE scaffold of the 3' UTR of an mCherry reporter. In addition, we created a polycistronic array of four different shRNAs in the 3' UTR of either ORF. Two monkeys were injected into multiple sites for each construct. Following a minimum of six weeks, to ensure adequate expression, brain sections were stained with fluorescent antibodies and confocally visualized for ChAT protein and reporter expression. All ChAT positive cells were counted in the injected striatum region, without visualizing the reporter, and categorized as either strongly, medium or weakly expressing. Then regions with cellular reporter expression were outlined with MBF Neurolucida, and ChAT cells in the injection region were additionally categorized for no, weak, medium, and strong reporter expression. All mirE shRNA constructs showed reporter correlated shifts of varying magnitudes from strong to weak ChAT expressing cells, with one construct also showing a 40% overall reduction in ChAT cells. The polycistronic constructs, on the other hand, had no discernible effects.

Disclosures: S. Mumuney: None. **W. Lerchner:** None. **A. Adil:** None. **R. Falcone:** None. **J.N. Turchi:** None. **B.J. Richmond:** None.

Poster

163. Learning and Memory: Physiology I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 163.10/X21

Topic: H.01. Animal Cognition and Behavior

Support: NIH IRP

Title: Retrograde and anterograde lentivirus transduction in old-world monkey amygdala for examining connections to and from frontal cortex, striatum, and temporal lobe

Authors: *W. LERCHNER, K. DASH, T. SETOGAWA, J. TURCHI, M. G. A. ELDRIDGE, V. DER MINASSIAN, B. J. RICHMOND;
NIH, Bethesda, MD

Abstract: We use chemogenetic tools to study the integration of sensory and reward value processing in old-world monkeys. Previous experiments have identified a circuit that involves frontal cortex, striatum and temporal lobe structures, including the rhinal cortex and the amygdala, but low efficiency of neuronal virus transduction has slowed progress in the past. Here we report that we can target a chemogenetic DREADD receptor (hM4Di-CFP) to all nuclei of the amygdala with a consistently high (50-100%) neuronal transduction efficiency (penetrance) in regions of virus expression. We injected 15 x 20ul (300ul total) of lentivirus at a titer of 2*10E9 infectious units per ml into both hemispheres of the amygdala of a single monkey. The overall regions with high penetrance DREADD coverage varied between the two hemispheres, with 32% of the left amygdala and 44% of the right amygdala covered by DREAD expressing neurons. Coverage percentages of individual nuclei also differed between the two sides, with lateral basal nucleus (>62% of volume) on the left side and the accessory basal nucleus (>66% of volume) on the right side having the most region coverage.
In addition, we were able to express genetic material at high penetrance in the lateral amygdala and cortical regions by injecting a retrogradely transported FuGE-lentivirus with a GFP and mCherry reporter into ventromedial striatum and orbitofrontal cortex (OFC) respectively. Histological analysis revealed that many neurons in the basolateral nucleus of the amygdala project to either OFC or ventromedial striatum. There is also a subpopulation of individual neurons in the basolateral nucleus projecting to both OFC and striatum. Overlap was strongest in amygdala regions that also showed the highest dopaminergic innervation as visualized by tyrosine hydroxylase. These results show that a combined approach of retrograde and anterograde lentivirus provides a means to use genetic tools to study circuit connectivity in old-world monkey.

Disclosures: W. Lerchner: None. **K. Dash:** None. **T. Setogawa:** None. **J. Turchi:** None. **M.G.A. Eldridge:** None. **V. Der Minassian:** None. **B.J. Richmond:** None.

Poster

164. Cortical Hippocampal Circuits: Time and Memory

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

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ONR DURIP N00014-17-1-2304
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NIMH R01MH052090
NIMH R01MH112169

Title: Consistent sequences on the scale of tens of minutes in mice hippocampus

Authors: *Y. LIU[1], S. J. LEVY[2], W. MAU[2], M. W. HOWARD[2,1];
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Abstract: Longstanding theories of memory from cognitive psychology postulate that the brain maintains a scale-invariant temporal context which serves as a substrate for episodic memory. Previous studies have shown 'time cells' in the hippocampus that fire sequentially over the scale of seconds (Pastalkova, et al., 2008; MacDonald, et al., 2011). In parallel, other studies have shown that hippocampal populations drift slowly over much longer time scales from tens of minutes (Manns, et al., 2007) to hours (Mankin, et al., 2012; Hyman, et al., 2012) to days (Rubin, et al., 2015; Mau et al., 2018). While the existence of these two phenomena is consistent with the hypothesis that memory representations have the same properties over different scales, there is an important distinction between time cells on the scale of seconds and drift over longer time scales. While time cells follow a consistent sequence during different delay intervals, it is not known whether population drift also follows consistent sequences. Here we analyze two *in vivo* calcium imaging datasets recorded from hippocampal region CA1 with mice performing different tasks. We show that the population dynamics for each session follow similar trajectories. This confirms that the slowly changing population code follows a reliable sequence over the course of a session rather than merely randomly drifting over time. This means that the population can be used to decode time since the beginning of the experimental session, on the scale of tens of minutes.

Disclosures: Y. Liu: None. **S.J. Levy:** None. **W. Mau:** None. **M.W. Howard:** None.

Poster

164. Cortical Hippocampal Circuits: Time and Memory

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Title: Identifying the contribution of medial septum cell types in generating the entorhinal grid cell code

Authors: *J. C. ROBINSON[1], M. P. BRANDON[2,3], M. E. HASSELMO[1];
[1]Psychological and Brain Sci., Boston Univ., Boston, MA; [2]Psychiatry, McGill Univ., Montreal, QC, Canada; [3]Douglas Hosp. Res. Ctr., Montreal, QC, Canada

Abstract: The medial entorhinal cortex (MEC) is essential for spatial navigation and contains a myriad of spatially tuned neurons including grid cells, which fire in multiple locations that form a repeating hexagonal pattern. Grid cells are known to require input from the medial septum (MS), a structure which is has been shown to be critical for the generation of theta oscillations in the MEC. However, while it is known that the complete inactivation of the MS results in the disruption of grid cell spatial firing, we have yet to resolve which of the three cell populations in the MS (GABAergic, glutamatergic, and cholinergic) are critical for grid cell function. To date, we have evaluated the role of MS GABAergic and glutamatergic neurons in grid cell generation. To do this, we specifically targeted either GABAergic or glutamatergic neurons in the MS using optogenetics to reversibly and selectively silence each cell type while recording grid cells in the MEC. A cre-dependent viral vector (AAV-Flex-ArchT-GFP) was injected into the MS of two transgenic mouse lines (VGAT-Cre and VGLUT2-Cre) to drive expression of ArchT, an optogenetic silencer, in either GABAergic or glutamatergic neurons. For both groups, a fiber optic was placed just above the MS for light delivery, and a four-tetrode microdrive was implanted into the MEC for grid cell recordings. Our results indicate that while silencing of septal glutamatergic neurons does not significantly reduce MEC theta power, this manipulation induced an enlargement of a subset of grid fields and a distortion of the overall grid pattern. These results suggest that the glutamatergic MS to MEC projection plays a subtle role in modulating the grid cell firing pattern. In contrast, we found that optogenetic silencing of septal GABAergic neurons caused a 50-80% reduction in MEC theta power and a complete disruption in grid cell spatial firing. These data highlight the critical importance of the long-range GABAergic projection from the MS to MEC in generating the spatial firing pattern of grid cells. Given the role of the MS GABAergic population in setting the baseline theta frequency of the MEC-hippocampal circuit, these data further link grid cell spatial periodicity to network rhythmicity.

Disclosures: J.C. Robinson: None. **M.P. Brandon:** None. **M.E. Hasselmo:** None.

Poster

164. Cortical Hippocampal Circuits: Time and Memory

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Title: Trisynaptic input mediates remapping of the CA1 rate code by entryway

Authors: A. T. KEINATH[1], A. NIETO-POSADAS[1], J. C. ROBINSON[2], M. P. BRANDON[1,3]; [1]Psychiatry, McGill Univ., Montreal, QC, Canada; [2]Boston Univ., Boston, MA; [3]Douglas Hosp. Res. Ctr., Montreal, QC, Canada

Abstract: Extensive research has shown that areas CA1 and CA3 of the hippocampus represent spatial location through the coordinated activity of place cells: sparsely active cells tuned to different preferred locations that tile the entire navigable space. In addition to spatial location, the hippocampus also represents a diverse range of contextual cues through reorganization of place cell firing rates, a phenomenon termed 'rate remapping'. Though much is known about the cues which induce rate remapping, less is known about the circuit mechanisms that underlie this coding scheme. Previous computational and theoretical work has implicated the trisynaptic circuit - from EC to DG to CA3 to CA1 - as a potential mediator of rate remapping due to pattern separation and/or attractor dynamics implemented in DG and CA3 respectively. Here, we introduce a novel rate remapping paradigm and subsequently test whether trisynaptic input is necessary for the observation of remapping in downstream CA1. To this end, we first recorded the activity of large populations of place cells in CA1 via calcium imaging as mice freely explored an environment with multiple entryways. Firing rates were inferred from the deconvolved calcium traces via a second-order autoregressive model. We observed that the population activity of CA1 place cells reliably remapped according to the most recent entryway. This remapping was driven by reliable differences in the firing rates, but not the preferred locations, of place cells - the hallmark of rate remapping. Next, to determine whether remapping in CA1 could feasibly be driven by trisynaptic input, we recorded from CA3 place cells where we also observed reliable entryway rate remapping. Finally, to causally test whether trisynaptic input is necessary for the observation of rate remapping, we again recorded from CA1 during chemogenetic inactivation of trisynaptic input on a subset of trials in a separate cohort of mice. We found that suppression of trisynaptic input fully eliminated remapping of the CA1 rate code by entryway while sparing other representational characteristics such as place field stability. Together, these results indicate that the hippocampus represents the point of entry, a purely latent variable, via rate remapping. Moreover, these results causally demonstrate that trisynaptic input mediates remapping of the CA1 rate code in this paradigm.

Disclosures: A.T. Keinath: None. **A. Nieto-Posadas:** None. **J.C. Robinson:** None. **M.P. Brandon:** None.

Poster

164. Cortical Hippocampal Circuits: Time and Memory

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ONR MURI N00014-16-1-2832
DURIP N00014-17-1-2304

Title: The role of medial septum and visual inputs for theta rhythmicity and coding of location and running speed in medial entorhinal cortex

Authors: *H. DANNENBERG, M. E. HASSELMO;
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Abstract: The medial septal input is critical for the generation of theta oscillations in the hippocampus and entorhinal cortex. Theta frequency and amplitude positively correlate with the running speed of an animal providing a running speed signal to the hippocampus and entorhinal cortex. Medial septum inactivation disrupts the spatial periodicity of grid cell firing, which has been utilized in models of path integration. Both the running speed signal by theta frequency as well as the positional coding by grid cells may be updated by visual cues to prevent the accumulation of error in models of path integration. However, little is known so far about the impact of visual inputs on theta oscillations and how the sudden absence of visual inputs affects theta oscillations and grid cell firing. We therefore recorded theta oscillations and grid cells in the medial entorhinal cortex in mice exploring a familiar open field environment during alternating epochs of light and darkness. This allowed us to study the effect of darkness on the theta frequency vs. running speed relationship and to test the hypothesis that visual inputs stabilize grid cell firing fields. We found that the onset of complete darkness reduced the slope of the theta frequency vs. running speed relationship in a bimodal way with a fast initial decrease of the slope followed by a slower further decrease in slope over minutes. No change in the y-intercept of the theta frequency vs. running speed relationship was observed.
In rats, grid cells have been shown to retain their periodic firing pattern during darkness in the absence of visual inputs. However, introducing mice to an open field environment in complete darkness results in significant disruption of the spatial periodicity of grid cell firing. This points to a possible role of visual input in maintaining a stable grid field by preventing an accumulation of error inherent to path integration mechanisms.
When the animals enter an environment that is initially lighted, we find that grid cells largely maintain their hexagonal firing pattern even during longer epochs of exploration in complete

darkness consistent with their proposed role in path integration, but that grid fields become wider and spatial information decreases in complete darkness, consistent with a role of visual cues for updating the spatial code by grid cell firing.
These results are relevant for models of path integration and for our understanding of how visual inputs modulate the coding of location and running speed in the entorhinal cortex.

Disclosures: H. Dannenberg: None. **M.E. Hasselmo:** None.

Poster

164. Cortical Hippocampal Circuits: Time and Memory

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ONR DURIP N00014-17-1-2304

Title: A cortical-hippocampal network supporting the temporal organization of memory

Authors: *W. NING[1], J. H. BLADON[1], J. CHEN[1], S. STEINWENTER[2], A. HOYLAND[1], M. E. HASSELMO[1];
[1]Psychological and Brain Sci., Boston Univ., Boston, MA; [2]Neurolog. Sci., Univ. of Vermont, Burlington, VT

Abstract: Episodic memory binds the temporal and spatial relationships between the elements of experience so that one can reconstruct the past. The dorsal hippocampus (dHPC) has been well studied in its role in temporal coding and previous research has shown that hippocampal CA1 time cells fire sequentially at specific time points in a temporally organized experience (Pastalkova et al., 2008; Kraus et al. 2013). Upstream regions of the hippocampus, such as the lateral entorhinal cortex (LEC) exhibit clear ramping activity and may provide temporal information to the HPC (Tsao et al., 2018). However, the temporal coding properties of the medial prefrontal cortex (mPFC), a region thought to participate in memory retrieval and consolidation, have yet to be explored. In order to better understand the neural network that supports the organization of memory across time, we utilized simultaneous in vivo tetrode recordings in the mPFC, HPC and LEC in Long-Evans rats during a mnemonic delay. mPFC, dHPC, and LEC units exhibited temporally modulated firing, but the mechanism by which individual units did so varied across regions. We found hippocampal time cells and object

responsive cells that showed theta phase precession. While we found that some temporally modulated cells in the mPFC also exhibited theta phase precession, or showed slow shifts in phase across the delay period, we failed to observe precession of LEC time-modulated units. These data suggest that the theta rhythm may support the organization of firing in the mPFC and dHPC to represent the passage of time. The LEC may use a different, complementary mechanism to organize experiences across time.

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Poster

164. Cortical Hippocampal Circuits: Time and Memory

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CIHR Master's Training Grant

Title: Impaired path integration correlates with selective disruption of grid cells in an amyloid beta mouse model of preclinical Alzheimer's disease

Authors: *J. YING[1], A. T. KEINATH[2], R. LAVOIE[4], S. KIM[5], M. P. BRANDON[3]; [1]McGill Univ., Montreal, QC, Canada; [2]Psychiatry, McGill Univ., Verdun, QC, Canada; [3]Psychiatry, McGill Univ., Montreal, QC, Canada; [4]Neurosciences, [5]Douglas Mental Hlth. Univ. Inst., Verdun, QC, Canada

Abstract: Despite being considered a hallmark of Alzheimer's disease (AD), the presence of amyloid beta (Aβ) plaques in the brain weakly correlates with cognition in patients. In fact, plaques are present in cognitively normal adults, suggesting that the pathophysiology of AD takes place during the early stages of Aβ pathology. Indeed, general soluble Aβ deposition in the brain occurs decades prior to the onset of clinical AD and is linked to poorer cognitive performance. This highlights the importance of establishing reliable biomarkers of early Aβ pathology, and by extension, preclinical AD, to identify healthy individuals at risk. In line with this goal, it's been shown that healthy individuals at genetic risk for AD have path integration (PI) deficits (Kunz et al., 2015). Concurrently, functional magnetic resonance imaging (fMRI) reveals that a grid-like signal is impaired in these individuals, suggesting that the PI deficits

result from a disrupted grid cell network in the medial entorhinal cortex (MEC). Notably, the onset of these impairments match time points during which soluble Aβ deposition increases in the brain (Lesné et al., 2013). However, it remains unclear if PI and grid cell fidelity are truly specific to early Aβ processes or a different phenomenon altogether. To establish a direct link between grid cells and PI deficits in the early stages of Aβ deposition, we carried out PI behavioral testing and *in-vivo* electrophysiological recordings in the MEC of the transgenic J20 mouse model of Aβ pathology. We demonstrate an aged-related deficit in the spatial tuning of grid cells. Conversely, the firing representations of all other spatially-tuned cells in the MEC, along with place cells in region CA1 of the hippocampus, were undisrupted. On a behavioral level, J20 mice exhibited worsened PI across age in a food-foraging task in darkness, but their allocentric navigation remained intact. These results are similar to existing PI data in individuals at genetic risk for AD, indicating homogeny between rodent and human spatial navigation. Taken together, our data show that PI performance and grid cell fidelity have predictive utility in determining those at risk of AD and pinpoint the MEC as a target to direct preclinical treatments.

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Poster

164. Cortical Hippocampal Circuits: Time and Memory

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Title: Neural responses in retrosplenial cortex associated with environmental objects

Authors: *L. C. CARSTENSEN[1], A. S. ALEXANDER[2], G. W. CHAPMAN, IV[2], M. E. HASSELMO[2];
[1]Grad. Program for Neurosci., [2]Psychological and Brain Sci., Boston Univ., Boston, MA

Abstract: Most complex behavior requires flexible and efficient spatial navigation through the external world. The retrosplenial cortex (RSC) is an association area that is interconnected with regions of the brain that are known to display spatial correlates, including the hippocampal formation (HPC), rhinal cortices, anterothalamic nuclei (ATN), and the anterior cingulate cortex (ACC). Accordingly, neurons in these regions have been shown to encode the past or present location of objects, proximity to objects or boundaries, and position in the observable

environment. Similarly, studies in humans and rodents have shown that RSC is important for spatial memory, such as tracking distance from a location, orientation in an environment, and knowledge of distances between objects. For these reasons and the connectivity of RSC in the spatial circuit, it is important to determine precisely how individual neurons in RSC represent available cues such as objects or boundaries and their relationship to the local environment. In the current work, we performed in vivo electrophysiological recordings in RSC while rats explored arenas containing objects that had a stable location, moved locations, or changed in size between sessions. In some manipulations, the configuration of the arena (local environment) was changed between sessions to be in conflict with distal cues present in the room while the objects were kept in the same allocentric location. Building upon previous work, we report that RSC neurons display spatial correlates that are reliable across sessions and can anchor to objects or environmental cues. These may be objects in the arena, boundaries, or other local or distal cues. Some RSC neurons exhibited differences in firing rate when a new object was inserted into the environment. In addition, a portion of neurons exhibited other egocentric related firing, such as boundary and speed coding. These results distinguish RSC as a region important in orientation and mapping features of spatial environments, together with the interconnected regions of the spatial circuit.

Disclosures: L.C. Carstensen: None. **A.S. Alexander:** None. **G.W. Chapman:** None. **M.E. Hasselmo:** None.

Poster

164. Cortical Hippocampal Circuits: Time and Memory

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ONR DURIP N00014-17-1-2304

Title: Egocentric boundary vector tuning of the retrosplenial cortex

Authors: *A. S. ALEXANDER, L. C. CARSTENSEN, W. G. CHAPMAN, F. RAUDIES, J. R. HINMAN, M. E. HASSELMO;
Dept. of Psychological and Brain Sciences, Ctr. for Systems Neurosci., Boston Univ., Boston, MA

Abstract: Substantial evidence suggests that the retrosplenial cortex (RSC) is critical for spatial cognition. However, spatial sensitivity of RSC neurons has primarily been characterized by recordings during behavior in linear environments whereas other regions within the broader neural spatial circuitry, such as the hippocampus (HPC) or medial entorhinal cortex (MEC), have been primarily studied during behavior in two-dimensional (2D) free foraging tasks. Given that RSC is anatomically positioned to route spatial information between sensory or motor cortices and HPC or MEC, it is important to analyze RSC spatial responses in the 2D environment with respect to place cells, grid cells, and head-direction cells. In the current work, we sought to further characterize RSC spatial representations during free exploration by performing in vivo electrophysiological recordings while rats explored familiar arenas. We report the existence of a subset of RSC neurons whose spatial receptive field defines an egocentrically-referenced vector to environmental boundaries. These neurons were primarily localized to the dysgranular sub-region of RSC and were observed across the entirety of the anterior-posterior axis of RSC. Arena rotations, expansions, and the removal of environment walls confirmed that both distance and orientation components of the egocentric vector were anchored to arena boundaries. Further, RSC neurons with egocentric boundary tuning were insensitive to manipulations of environment geometry. An analysis of the influence of self-motion on egocentric boundary vector tuned cells demonstrated that neither turning movements nor speed could explain the phenomenon. Finally, a subset of RSC neurons exhibiting this property were theta modulated, thus providing a potential temporal window by which egocentric boundary vector cells could synchronize with HPC or MEC ensembles.

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Poster

164. Cortical Hippocampal Circuits: Time and Memory

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Title: Input-output organization of VIP interneurons in the medial entorhinal cortex

Authors: *S. BADRINARAYANAN[1], F. MANSEAU[3], A. KEINATH[2], S. WILLIAMS[3], M. P. BRANDON[2,3];

[1]Integrated Program in Neurosci., [2]Dept. of Psychiatry, McGill Univ., Montreal, QC, Canada; [3]Douglas Hosp. Res. Ctr., Montreal, QC, Canada

Abstract: The medial entorhinal cortex (MEC) is a region involved in episodic memory and spatial navigation. Through a careful balance of excitation and inhibition, the MEC microcircuit generates a myriad of spatially modulated cells to form a map of the animal's environment. Of the various spatially tuned cells, grid cells present in the MEC are one of the most prominent cell type. These cells tend to fire in distinctive triangular patterns that map the entire environment the animal traverses. They also compute for the animal's displacement, trajectory and angular motion. Interestingly, computational models and experimental data from stellate cells (putative grid cells) cells in the MEC predict that in this network, where grid cells are constantly under inhibition, an increase in the cell's firing rate as the animal traverses a grid field could be a result of a disinhibitory mechanism. While the role of excitatory and certain inhibitory cell populations has been studied extensively, the interplay required to generate and maintain the excitation-inhibition balance remains elusive. By employing viral-mediated circuit tracing, optogenetics and *in vitro* electrophysiology, we aim to uncover the role of vasoactive intestinal peptide (VIP)-expressing interneurons in the MEC. As VIP cells are known to inhibit other interneuron populations in various cortical structures, our first set of experiments were designed with the aim to characterize the electrophysiological and morphological profile of VIP cells, as well as their input-output organization within the MEC. Using *in vitro* electrophysiological recordings in brain slices, we find that these cells in the deep layers of the MEC have a higher input resistance and firing threshold when compared to cells in the superficial layers. VIP cells in the deep layers have distinct dendritic and axonal morphologies in comparison to the cells in the superficial layers. To identify the post-synaptic targets of the VIP cells, we expressed the excitatory opsin Channelrhodopsin in a Cre dependent manner and will patch various cell types in the MEC. To investigate the areas and cell types that can activate these cells, we visualized brain-wide inputs to VIP neurons in the MEC using retrograde rabies virus tracing. Our findings from these experiments are preliminary and in the process of quantification. Together this study will provide insight into how VIP cells maintain the excitation-inhibitory balance that governs the functioning of the MEC.

Disclosures: S. Badrinarayanan: None. **F. Manseau:** None. **A. Keinath:** None. **S. Williams:** None. **M.P. Brandon:** None.

Poster

164. Cortical Hippocampal Circuits: Time and Memory

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Title: Examination of non-spatially tuned hippocampal neurons in a context fear discrimination task

Authors: *R. R. ROZESKE, L. RUNTZ, A. T. KEINATH, A. SOSSIN, M. P. BRANDON; Psychiatry, McGill Univ., Montreal, QC, Canada

Abstract: Appropriate defensive behavior is critical for an organism's survival and depends heavily upon whether a context is identified as safe or threatening. The hippocampus is essential for associative memories, but the neuronal ensembles that encode non-spatial information to assign valence to context during fear conditioning remain relatively unexplored. To examine hippocampal activity in safe and dangerous contexts we used one photon micro-endoscope (miniscope) calcium imaging of large neuronal populations in both the dorsal and ventral hippocampus in a novel context fear discrimination task. Mice underwent context fear conditioning in a cylindrical arena constructed of LED wall panels that spanned 360 degrees, which allowed instantaneous alteration of visual and auditory features. Miniscope recordings were performed throughout the fear discrimination paradigm. Context A was associated with footshock while context B remained neutral, resulting in clear context discrimination as measured by freezing behavior. We then characterized the activity of hippocampal neurons and found that a subset of hippocampal neurons was directly activated by footshock and/or freezing regardless of the animal's spatial location. To examine how these cell populations contribute to the discrimination between safe and dangerous contexts, the following day mice were rapidly "teleported" between contexts A and B. Analyses of dorsal and ventral hippocampal cell populations during transitions between safe and dangerous contexts will be presented. Moreover, mice were exposed to the elevated plus maze to contrast hippocampal activity among innate and learned threatening contexts. Together, these experiments contribute to our understanding of how the hippocampus encodes non-spatial features to support dynamic switching of fear behavior in contexts of differing threat.

Disclosures: R.R. Rozeske: None. **L. Runtz:** None. **A.T. Keinath:** None. **A. Sossin:** None. **M.P. Brandon:** None.

Poster

164. Cortical Hippocampal Circuits: Time and Memory

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Title: Linking hippocampal remapping to memory retrieval in a context fear teleportation task

Authors: *L. RUNTZ, R. R. ROZESKE, A. T. KEINATH, A. SOSSIN, M. P. BRANDON; Dept. of Psychiatry, McGill Univ., Montreal, QC, Canada

Abstract: The encoding of episodic memories depends upon the brain's capacity to rapidly generate and retrieve representations of spatial context. Spatial memory encoding depends on the hippocampus to generate a cognitive map via large ensembles of place cells that each fire reliably at a position in a given context. When exploring other contexts, the firing positions of hippocampal cells are altered to form orthogonal spatial maps, a phenomenon known as remapping. As animals enter a familiar context, a process of pattern separation and pattern completion is performed to retrieve the spatial map that was previously formed in this environment. To study the neural dynamics of this retrieval process, one prior study (Jezek, et al. 2011) teleported animals between contexts by manipulating visual cues. Here, we extend on this approach to link retrieval of spatial maps to memory in a similar teleportation task after the animal has been conditioned in one of the contexts. Our large cylindrical apparatus contains interior walls composed of a continuous LED panel that allows for control of visual scenes, as well as speakers to control auditory, to be able to display and immediately transition between contexts. The floor of the apparatus is a shock grid (90 cm diameter). For two days prior to teleportation experiments, animals were introduced to contexts A and B, receiving 12 foot shocks in context A. For teleportation experiments, animals were teleported between the contexts every 2 minutes. As expected presentation of context A and context B produced respectively high and low levels of freezing behavior. Next, to link freezing behavior to the retrieval of spatial maps in the hippocampus, we performed calcium imaging 'miniscope' in both the dorsal and ventral hippocampus during the conditioning and teleportation phases of the task. Preliminary results demonstrate that the hippocampus does form orthogonal maps for contexts A and B, and that conditioning induced significant changes to the spatial map in context A. We are currently working to characterize the retrieval dynamics when animals are teleported between the two contexts to find a link between map retrieval and memory expression (freezing behavior).

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Poster

164. Cortical Hippocampal Circuits: Time and Memory

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Title: Heterogeneous stability in hippocampal spatial memory representations

Authors: *S. J. LEVY, N. KINSKY, D. SULLIVAN, M. HASSELMO;
Boston Univ., Boston, MA

Abstract: Recent work suggests that a major role of the hippocampus in memory and navigation is to disambiguate memories by forming unique neural representations when experiences involve overlapping elements. Previous studies have shown that hippocampal neurons modulate their activity according to context-dependent cognitive demands, even while behavior is stable and spatial cues are identical (splitter cells). We recorded cells in dorsal CA1 of the hippocampus in mice performing a delayed non-match to place task on a continuous T-Maze over several sessions. In tracking the same populations of cells, we found that there was heterogeneity in the stability of task related representations. Many single cells exhibited modulation in their firing activity according to cognitive task variables while the animal was in the same spatial location. The distribution of these responses over the course of recordings was stable for cells active on the return arm, but this was not the case on the central stem. We observed that the proportion of active cells that showed context-dependent responses to task phase (sample versus test) was stable, but the proportion showing selective responses for turn direction (left versus right) increased over days, and the proportion that coded both task phase and turn direction decreased. We found this change was not attributable to variable stability of these different selective responses or by maze locations, but instead a change in in the allocation of newly active cells among different response phenotypes. In spite of these changes, the ensemble-level discriminations of task dimensions were highly stable. Current experiments are examining whether modulation of firing activity by these same cognitive demands is reliable across distinct spatial contexts. These experiments contribute to an account of hippocampal function which includes both episodic memory and spatial navigation.

Disclosures: S.J. Levy: None. **N. Kinsky:** None. **D. Sullivan:** None. **M. Hasselmo:** None.

Poster

164. Cortical Hippocampal Circuits: Time and Memory

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 164.13/X34

Topic: H.01. Animal Cognition and Behavior

Support: ONR MURI N00014-16-1-2832
NIMH R01MH095297
NIMH R01MH112169
NIBIB R01EB022864

Title: Compressed representations of time and space in the hippocampus

Authors: *D. W. SULLIVAN, J. CHEN, M. W. HOWARD;
Dept. of Psychological and Brain Sci., Boston Univ., Boston, MA

Abstract: In many experimental paradigms, hippocampal neurons fire as if they have receptive fields that identify contiguous subsets of position within an enclosure, time since a relevant event, and other variables as well. These receptive fields vary across neurons such that the receptive fields of the population collectively span the entire range of places or times that are experienced. However, it is not necessary that the spacing and density of receptive fields in the neural population is constant across the continuous dimension. Indeed, previous reports have shown that the receptive fields of time cells become broader going from the beginning to the end of the sequence. This suggests that the neural representation of time in the hippocampus is compressed analogous to the Weber-Fechner law. It is unknown whether the hippocampal representation of position is also compressed, nor has the form of compression for time been systematically compared to that of space. In order to better understand the nature of hippocampal neuronal sequences and the processes governing them, we used calcium imaging and miniaturized fluorescence microscopy to monitor hippocampus CA1 neuronal activity in freely moving mice performing two separate behaviors that generate spatial and temporal sequences: movement along a variable-length linear track, and an alternating fixed/variable interval cued water delivery paradigm. Along the linear track we observe "place" cells that fire with significant reliability some distance from a movable landmark. In the cued water delivery paradigm we observe "time" cells that fire with significant reliability some time after a cue. By comparing activity in hundreds of simultaneously recorded neurons across these spatial- and non-spatial-dependent behaviors, we compare the compression of neural representations of space and time in the hippocampus.

Disclosures: D.W. Sullivan: None. **J. Chen:** None. **M.W. Howard:** None.

Poster

164. Cortical Hippocampal Circuits: Time and Memory

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 164.14/X35

Topic: H.01. Animal Cognition and Behavior

Support: CIHR Project Grant # 367017
CIHR Project Grant # 377074
NSERC Discovery Grant # 74105
Canada Research Chairs Program

Title: Optogenetic inactivation of medial septum GABAergic neurons induces partial remapping of delay encoding neurons

Authors: *H. YONG[1], M. P. BRANDON[2];
[1]Integrated Program in Neurosci., [2]Psychiatry, McGill Univ., Montreal, QC, Canada

Abstract: Recent work has shown that neurons in the hippocampus are sequentially activated during the delay period of a delayed spatial alternation task. Experimental data and a model proposed in Wang et al. (2015) suggest that the readout of this sequential activity required hippocampal theta oscillations. Further, the readout of this sequence has been proposed to be necessary for successful performance in the delayed spatial alternation task. However, this prior study used a long-acting GABA receptor agonist to inhibit the activity of all medial septum neurons for the entire testing session, raising the possibilities that 1) septal contributions other than theta generation may support delay encoding, and 2) that memory deficits could be the result of septal inactivation outside of the delay period. To assess these possibilities, we applied an optogenetic strategy to selectively silence the theta-generating GABAergic population in the medial septum (MS) specifically during the delay period as mice performed the delayed spatial alternation task. Mice were trained to run on a treadmill for 10 seconds between each alternation lap on the T-maze, and were deemed to have learned the task after reaching 80% correct or better for two consecutive days. Once they reached the criteria, septal GABAergic neurons were pseudo-randomly inhibited in 50% of the trials, for the entire 10 second duration of the delay period. We show that inhibition of these neurons significantly reduced the amplitude of hippocampal theta oscillation (mean theta reduction: 56%), and induced remapping in many of hippocampal neurons that encoded the delay period. Thus, we observed a new delay sequence only on trials when the septal GABAergic input to the hippocampus was suppressed. This phenomenon was not observed in laser-on GFP-only expressing control experiments. This result suggests the possibility that region CA1 of the hippocampus receives competing delay sequences from CA3 and the medial entorhinal cortex (MEC), both of which are known to contain delay cells. We suggest the possibility that MS inactivation disrupts the MEC delay sequence (similar

to the effect of MS inactivation on MEC grid cells), which then gives the CA3 input more influence over CA1 sequences. Finally, we observed that remapping of the delay sequence had no impact on behavioral performance on the T-maze delayed alternation task, suggesting the possibilities that either 1) the identity of the sequence is not critical for performance, and/or 2) that the MS GABAergic neurons are critical to performance outside of the delay period of the task. We are exploring these possibilities now.

Disclosures: H. Yong: None. **M.P. Brandon:** None.

Poster

164. Cortical Hippocampal Circuits: Time and Memory

Location: Hall A

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Program #/Poster #: 164.15/X36

Topic: H.01. Animal Cognition and Behavior

Support: CIHR Project Grant # 367017
CIHR Project Grant # 377074
NSERC Discovery Grant # 74105
Canada Research Chairs Program

Title: Network dynamics of the thalamic head direction system during reorientation

Authors: *Z. AJABI[1], A. NIETO-POSADAS[1], M. P. BRANDON[2];
[2]Psychiatry, [1]McGill Univ., Montreal, QC, Canada

Abstract: Computational models of the head direction (HD) system propose a 'continuous ring attractor', whereby an 'activity bump' along a ring-shaped manifold is maintained by recurrent excitation and lateral inhibition. Movement around the manifold is proportional to angular movement of the head. Several key experimental findings offer support for this model, most notably, the coherence between head direction cells is maintained across contexts, during sleep, and after the removal of upstream angular velocity inputs. These data show that the HD network is 'hard-wired', but have yet to reveal the intrinsic geometrical structure of the neural dynamics during behavior. For example, imagine that a familiar visual landmark becomes available and 'resets' the animal's sense of direction. Does activity rotate around the ring-manifold, or does the visual landmark induce immediate resetting through a process similar to competition between discrete attractors? To answer this, we recorded in the anterior dorsal thalamic nucleus (ADN) in mice using the UCLA miniscope, yielding between 100-250 HD cells simultaneously. To induce many resetting events, we designed an open field surrounded by a 360 degree LED screen to display a given cue at different locations with a predefined phase shift. We alternated between two-minute periods of complete darkness and the display of the cue. Using a deep neural network, we reveal that the manifold of the thalamic HD system may be described by a cone-like

geometry. Complete darkness induced drifts along this manifold. Appearance of the shifted cue induced 'resetting' whereby activity rotated around the cone-shaped manifold at varying velocities that appear to be a function of the distance to the center of the said structure. Changes in this distance highly correlate with changes in firing rates of active neurons. Although in some cases, resetting was almost immediate to occur, often times it took the system surprisingly prolonged periods to reset (up to one minute), indicating a slow correction mechanism. We offer the possibility that visual information does not simply reset the HD system, but may work in concert with vestibular inputs to directly induce rotations along the HD manifold during navigation.

Disclosures: Z. Ajabi: None. **M.P. Brandon:** None. **A. Nieto-Posadas:** None.

Poster

164. Cortical Hippocampal Circuits: Time and Memory

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 164.16/X37

Topic: H.01. Animal Cognition and Behavior

Support: NIMH Grant MH094263

Title: Mixed selectivity across task dimensions

Authors: *C. MIKKELSEN[1], M. W. HOWARD[2];
[2]Dept. of Psychological and Brain Sci., [1]Boston Univ., Boston, MA

Abstract: Mixed selectivity in a population of neurons provides a computational advantage over a singularly selective encoding scheme (Rigotti et al., 2013). It allows for a group of neurons to encode properties of multiple task dimensions, which becomes critical as the number of task dimensions increases. Mixed selectivity has been extensively reported in working memory tasks in frontal regions in macaques. Here we report mixed selectivity in a contextual association task believed to be dependent on hippocampal function. We report mixed selectivity from six data sets using the same task while recordings were taken from the orbitofrontal cortex, medial prefrontal cortex, hippocampus, lateral entorhinal cortex, and medial entorhinal cortex of rodents. Although there were some subtle differences in the coding of task dimensions in the various regions, the most dramatic finding is that there was mixed selectivity in all of these regions during performance of this task. This suggests that large segments of the brain use mixed selectivity to represent task variables conjunctively to enable behaviorally-appropriate responses.

Disclosures: C. Mikkelsen: None. **M.W. Howard:** None.

Poster

164. Cortical Hippocampal Circuits: Time and Memory

Location: Hall A

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ONR MURI N00014-16-1-2832
ONR DURIP N00014-17-1-2304
NIBIB R01EB022864

Title: Applying hierarchical Bayesian models to time cells

Authors: *R. CAO[1], S. CHARCZYNSKI[1], Z. TIGANJ[2], M. W. HOWARD[3];
[2]Ctr. for Memory and Brain, Dept. of Psychological and Brain Sci., [3]Dept. of Psychological and Brain Sci., [1]Boston Univ., Boston, MA

Abstract: The sequential firing of "time cells" after the presentation of a salient event can be used to decode the passage of time (Eichenbaum, 2014). Many studies have shown time cells across a variety of brain regions and species. Because the neural sequence does not proceed at a constant rate there must be some form of compression of the neural code for time. The goal of this study is to quantitatively describe the form of this compression by studying populations of time cells using datasets of extracellular recording of single units in different brain regions from multiple species. Here we applied hierarchical Bayesian modeling techniques to characterize the distribution of time field centers and the relationship between time field width and time field centers. We compare several theoretical distributions, with special attention to the power-law distribution.

Disclosures: R. Cao: None. **S. Charczynski:** None. **Z. Tiganj:** None. **M.W. Howard:** None.

Poster

164. Cortical Hippocampal Circuits: Time and Memory

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Office of Naval Research Grant MURI N00014-16-1-2832

Title: A temporal record of the past with a spectrum of time constants in the monkey entorhinal cortex

Authors: *I. M. BRIGHT[1], M. L. MEISTER[2], N. A. CRUZADO[1], Z. TIGANJ[1], M. W. HOWARD[1], E. A. BUFFALO[2];
[1]Dept. of Psychological and Brain Sci., Boston Univ., Boston, MA; [2]Physiol. and Biophysics, Univ. of Washington, Seattle, WA

Abstract: Episodic memory is believed to be intimately related to our experience of the passage of time. Indeed, neurons in the hippocampus and other brain regions critical to episodic memory code for the passage of time at a range of time scales. The origin of this temporal signal, however, remains unclear. Here, we examined temporal responses in the entorhinal cortex of macaque monkeys as they viewed complex visual images. Many neurons in the entorhinal cortex were responsive to image onset, showing large deviations from baseline firing shortly after image onset but relaxing back to baseline at different rates. This range of relaxation rates allowed for the time since image onset to be decoded on the scale of seconds. Further, the ensemble carried information about image content suggesting that neurons in the entorhinal cortex carry information not only when an event took place but also the identity of that event. Taken together these findings suggest that the primate entorhinal cortex uses a spectrum of time constants to construct a temporal record of the past in support of episodic memory.

Disclosures: I.M. Bright: None. **M.L. Meister:** None. **N.A. Cruzado:** None. **Z. Tiganj:** None. **M.W. Howard:** None. **E.A. Buffalo:** None.

Poster

164. Cortical Hippocampal Circuits: Time and Memory

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 164.19/X40

Topic: H.01. Animal Cognition and Behavior

Support: NSF IIS 1631460
NIBIB R01EB022864

Title: A physical model for pattern completion of temporally correlated patterns for episodic memory

Authors: *Z. G. ESFAHANI, M. W. HOWARD;
Dept. of Psychological and Brain Sci., Boston Univ., Boston, MA

Abstract: It is widely believed that the hippocampus uses pattern completion in order to recover episodic memories. However, it is believed that episodic memory is associated with retrieval of a slowly changing temporal context and it is known that representations throughout the subregions of the hippocampus change slowly over time. This means that a pattern completion mechanism must somehow overcome the similarity induced by this temporal autocorrelation. We approach this problem from two perspectives and consider both behavioral and neurophysiological constraints.
First, we consider the properties of content-addressable memory dynamics that can recover temporally correlated patterns. We consider the problem of attractor dynamics between stored patterns learned from a population of sequentially activated time cells. Each of the time cells is triggered by a presented stimulus at a particular time scale. The primary result is that synaptic plasticity must be localized to cells with similar time scales in order to enable appropriate dynamics. We study the effect of recency on content-addressable memory retrieval by enabling the current state of time cells to participate in the dynamics. We consider both symmetric and asymmetric plasticity among time scales and discuss this in the context of phase precession and spike-timing dependent plasticity.
Second, we consider mechanisms that could be used to implement “address-addressable” memory. In many experiments, such as free recall, human subjects can direct their memory search to particular events without an explicit content cue. In this case, time itself functions much like a pointer to retrieve the content of a memory. We consider two possible physical mechanisms to implement address-addressable memory search.

Disclosures: Z.G. Esfahani: None. **M.W. Howard:** None.

Poster

164. Cortical Hippocampal Circuits: Time and Memory

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 164.20/X41

Topic: H.01. Animal Cognition and Behavior

Support: SFB 874

Title: Imaging and optogenetically manipulating memories over half a life time reveals a time-limited and preferential role of CA3 for the retrieval of precise memories when compared to CA1

Authors: *E. ATUCHA, S. KU, M. LIPPERT, F. OHL, M. SAUVAGE;
Leibniz Inst. For Neurobio., Magdeburg, Germany

Abstract: If the hippocampus contributes to the retrieval of precise memories and if it participates to the retrieval of remote/very remote memories is still controversial. Also, the specific contribution of the hippocampal subfields CA1 and CA3 within this frame is either unclear or not known. We recently showed that the hippocampal subregion CA1 is recruited for the retrieval of up to one-year-old contextual fear memories in mice (comparable to 40 years in humans, based on life expectancy), unlike CA3 that is no longer engaged for retrieving very remote memories (6 months and 1 year-old; Lux *et al*, Elife, 2016). The extent to which CA1 and CA3 are necessary for memory retrieval and the specific nature of their contribution over time remain however unclear. To address these questions, we used an ArchT optogenetic approach to inhibit cell firing in CA1 or CA3 at retrieval (i.e upon exposure to the conditioning context and subsequently to a similar context 1 day, 6 months and 1 year after conditioning). In parallel, we imaged medial temporal lobe activity patterns by detecting the RNA of the immediate-early gene *Arc*. Results show that controls discriminated between both contexts when memories were recent (1 day-old) while optogenetic inhibition of CA1 led to memory retrieval impairments. Conversely, the same manipulation for CA3 led to contextual generalization. For very remote memories, inhibiting CA1 still led to impairments, while controls showed this time contextual generalization, and inhibition of CA3 did not affect freezing levels for either context. These findings suggest that CA1 is necessary for the retrieval of memory traces independently of the age of the memory trace. In contrast, CA3 contributes especially to the retrieval of most recent memories and is involved in retrieving precise memory representations to a larger extent than CA1. (See also Atucha E, Fuerst C, Sauvage M's poster for imaging of object-in-place remote memories.)

Disclosures: E. Atucha: None. **S. Ku:** None. **M. Lippert:** None. **F. Ohl:** None. **M. Sauvage:** None.

Poster

164. Cortical Hippocampal Circuits: Time and Memory

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 164.21/X42

Topic: H.01. Animal Cognition and Behavior

Support: SFB 874

Title: Imaging object-in-place memories over half a life time

Authors: E. ATUCHA, C. FUERST, ***M. SAUVAGE**;
Functional Architecture of Memory, Leibniz Inst. for Neurobio., Magdeburg, Germany

Abstract: The role of the hippocampus in retrieving memories over time has mainly been investigated in fear conditioned memories in rodents as these memories last longer than memories without fear content. Thus, virtually nothing is known about the contribution of the hippocampus to the retrieval of remote/very remote memories devoid of fear content, nor whether the contribution of the CA1 and CA3 subfields differ within this frame, despite accumulating evidence for a functional segregation between these areas for the retrieval of recent memories. We recently showed that CA1 is recruited during the retrieval of up to 1-year-old contextual fear memories in mice (comparable to 40 years in humans, based on life expectancy), unlike CA3 that is no longer recruited for the retrieval of very remote memories (6 months and 1-year-old; Lux *et al*, Elife, 2016). To assess whether the disengagement of CA3 for the retrieval of very remote memories reflects a general mechanism subserving memory consolidation or whether it is selectively bound to the fearful nature of fear conditioned memories, we adapted a standard murine object-in-place task to the detection of very remote memories traces by extending the duration of the study phase. In addition, we imaged CA1 and CA3 patterns of activity 1 day, 1 week, 1 month, 6 months and 1 year after mice had been exposed to an arena containing two identical objects by exposing them again to the same arena containing duplicates of the objects, one at the same location, the other displaced. CA1 and CA3 activity was imaged by detecting the RNA of the immediate-early gene *Arc*. These results will enable a better general understanding of the mechanism underlying memory consolidation at the system level. (See also Atucha E, Ku SP, Lippert MT, Ohl FW, Sauvage M's poster for optogenetic manipulations and imaging of precise memories)

Disclosures: E. Atucha: None. **C. Fuerst:** None. **M. Sauvage:** None.

Poster

165. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 165.01/X43

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant AG049090 (AAW)
NSF Grant 1631465 (BLM)
NSERC Grant RGPIN-2017-03857 (BLM)

Title: Functional and pathological changes in the parietal-hippocampal network underlie impaired spatial reorientation in AD mice

Authors: *A. C. STIMMELL[1], J. DIXON[1], S. MOSELEY[1], V. LAPOINTE[2], R. COOK[1], L. SANTOS-MOLINA[1], L. JENNINGS[1], R. BERLIN[1], B. L. MCNAUGHTON[2,3], A. A. WILBER[1];

[1]Florida State Univ., Tallahassee, FL; [2]Dept. of Neurosci., The Univ. of Lethbridge, Lethbridge, AB, Canada; [3]Univ. of California, Irvine, Irvine, CA

Abstract: Getting lost is one of the first impairments to emerge in pre-clinical Alzheimer's disease (AD). Individuals with pre-clinical AD have hyperactivation in the hippocampus and parietal cortex (PC; Machulda et al., 2003; Oh et al., 2015; Putcha et al., 2011), particularly in the PC-hippocampal network (O'Neil et al., 2014; Buckner et al., 2005). We set out to look for a similar pattern of brain and behavior changes in mouse models of AD. We demonstrated that impaired spatial navigation may result from a failure to use distal cues to get oriented in space (Stimmell et al., 2019). The impaired use of distal cues for spatial reorientation in mice emerges early in disease progression before the onset of plaques and tangles, suggesting the impairments we observed in mice may be similar to those observed in pre-clinical AD in humans (Henderson et al., 1989; Weintraub & Salmon, 2012; Allison et al., 2016). Male mice were not impaired either early or late (post-plaque formation) in disease progression. We set out to explore the relationship between these early changes in female mice and impaired use of distal cues for spatial orientation. We used a semi-automated whole brain approach to quantify the proportion of neurons positive for pathology markers (6e10, pTau, M22, M78). Female mice had significantly more intracellular amyloid pathology in dorsal hippocampus and ventral subiculum than male mice. In female mice the proportion of Aβ positive cells was robustly correlated with impaired spatial reorientation in ventral subiculum (SUB), dorsal retrosplenial cortex (RSC), dorsal CA1, and PC (but not dorsal SUB, agranular RSC, ventral RSC, or ventral CA1), the same brain network proposed to underlie use of landmarks for spatial orientation (Wilber et al., 2018). In order to assess functional changes that could underlie the relationship we observed between intracellular pathology and impaired spatial orientation, we assessed the proportion of active cells during the spatial reorientation task using cFos immunohistochemistry. We found that larger proportions of active cells were apparent in every brain region assessed, with more pronounced increases in the PC-hippocampal network (e.g., subiculum). Thus, getting lost in pre-clinical AD may be a consequence of impaired use of landmarks and this impairment may be related to early pathological changes that contribute to hyperactivation.

Disclosures: A.C. Stimmell: None. **J. Dixon:** None. **S. Moseley:** None. **V. Lapointe:** None. **R. Cook:** None. **L. Santos-Molina:** None. **L. Jennings:** None. **R. Berlin:** None. **B.L. McNaughton:** None. **A.A. Wilber:** None.

Poster

165. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 165.02/X44

Topic: H.01. Animal Cognition and Behavior

Support: NIA Grant AG049090

Title: Impaired memory reactivation in a mouse model of Alzheimer's disease

Authors: *S. D. BENTHEM[1], I. SKELIN[2], S. MOSELEY[1], J. R. DIXON[1], A. S. MELILLI[1], A. C. STIMMELL[1], A. A. WILBER[1];
[1]Dept. of Psychology, Florida State Univ., Tallahassee, FL; [2]Neurol., Univ. of California Irvine, Irvine, CA

Abstract: In preclinical Alzheimer's disease (AD), spatial learning and memory is impaired (Allison et al., 2016). We reported similar navigational impairments in 6-month 3xTg-AD female mice on a task that requires learning and memory for landmark use to get oriented in space (*spatial reorientation task* from Rosenzweig, 2003; Stimmell et al., 2018). Pathology in the parietal cortex (PC)-hippocampal network (but not other regions) was robustly correlated with impairments which emerged early in disease progression before the onset of plaques and tangles, suggesting the impairments in mice may be similar to those in human pre-clinical AD (Henderson et al., 1989; Weintraub & Salmon, 2012; Allison et al., 2016). Hippocampal-PC coordination likely underlies performance on the *spatial reorientation task* (Wilber et al, 2018), memory replay during sleep is critical for learning related plasticity (Ego-Stengel & Wilson, 2009; Jadhav et al, 2012; Maingret et al, 2016), and hippocampal-cortical dysfunction is a potential mechanism for memory impairments in individuals with AD (Gennaro et al, 2017; Khan et al, 2014). Thus, the spatial reorientation deficit in AD mice may be a consequence of AD related changes to the PC-hippocampal network that produce memory impairments. Thus, we assessed modular (multi-unit activity) memory replay in 6-month female 3xTg-AD mice that were learning the *spatial reorientation task*. Mice were implanted with a 16 tetrode recording arrays targeting right PC and hippocampus and underwent daily recording sessions of rest-task-rest as they learned to locate the unmarked reward zone. Multi-unit activity template matching compared activity patterns observed during slow wave sleep (SWS) to patterns from the approach to the reward zone (Wilber et al., 2017). We assessed sleep quality metrics, multi-unit activity patterns and delta waves (DW) in PC and markers of memory replay in the hippocampus (SWRs) during SWS. AD mice had significantly longer sleep bouts and SWS, and SWR density was reduced. However, the longer duration of SWS and sleep bouts appeared to compensate for reduced SWR density so that the total number of SWRs was not reduced. There was a non-significant reduction in proportion of template matches in PC in AD mice. In control mice hippocampal SWRs slightly preceded DW in PC and this correlation was strengthened in post-task sleep. However, in AD mice SWR-DW cross-correlations were significantly reduced and were significantly correlated with impaired performance on the *spatial reorientation task*. Thus, AD may cause PC-hippocampal network changes which impair spatial orientation because of impaired learning related plasticity during SWS.

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Poster

165. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

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Topic: H.01. Animal Cognition and Behavior

Support: NIH/NIAAA Grant AA024983
Alzheimer's Association Grant AARG-17-531572
NIA Grant AG049090

Title: Role of the anterior thalamic nuclei-parietal cortex network in an action-orientation task

Authors: *C. SIMMONS[1], M. BAIAMONTE[1], B. J. CLARK[2], A. A. WILBER[1];
[1]Florida State Univ., Tallahassee, FL; [2]Psychology Dept., Univ. of New Mexico, Albuquerque, NM

Abstract: The anterior thalamus (ATN) is known to contain head direction cells that are responsible for signaling the directional orientation of an animal within an environment. These cells have direct and indirect connections with the parietal cortex (PC), an area hypothesized to play a functional role in coordinating viewer-dependent and viewer-independent spatial reference frames. This coordination between reference frames would allow an individual to translate movements toward a desired location from memory. Functional connectivity between the ATN and PC would thus be critical for orienting and maneuvering toward remembered locations. This hypothesis was tested by running rats through an action-orientation task that required the rats to associate an appropriate action (left or right turn) with a particular directional heading. In this task, there are four arms of equal length positioned around a central starting point. Each arm is offset from the next by 90 degrees and has a unique allocentric direction (north, south, east, or west) in the room. A trial begins with the rat in the central starting point. A pseudorandom selection determined which of the four arms the rat would leave from. After exiting an arm, the rat had to turn and then displace the correct object covering one of two feeding stations located to the left and the right in order to receive a reward. For a pair of arms facing opposite directions (i.e., east and west), the reward was located on the left, and for the other pair, the reward was located on the right. Thus, each of the four reward locations were associated with a different combination of allocentric heading direction and egocentric action. Complete removal of an object was scored as correct or incorrect. Trials in which the rat did not respond were scored as 'no response' trials. After an object was removed, the rat returned to the center starting position and the maze was reset for the next trial. To investigate the role of the PC, ATN, and the ATN-PC network (Fresno et al., 2019), muscimol (GABAa agonist) infusions were targeted to inactivate bilateral PC, bilateral ATN, or ATN-PC network (Left ATN-Right PC or Right ATN-Left PC). Muscimol sessions were counterbalanced and compared to saline sessions within the

same animal. Inactivations resulted in decreased performance and increased response latency. Impairments were largest following bilateral ATN inactivation, with bilateral PC and network inactivations producing similar disruptions. Together, these results suggest that the ATN-PC network is critical for linking the appropriate action to a spatial orientation for navigation.

Disclosures: C. Simmons: None. **M. Baiamonte:** None. **B.J. Clark:** None. **A.A. Wilber:** None.

Poster

165. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation II

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Searle

Title: Neural mechanisms of social foraging in flying bats

Authors: *N. M. DOTSON, K. SOMAN, M. M. YARTSEV;
Bioengineering, Hellen Wills Neurosci. Inst., Univ. of California Berkeley, Berkeley, CA

Abstract: Sociality is one of the key forces that enables us to thrive as a species. To further our understanding of how social information is utilized by individuals engaged in cooperative behavior and the neural computations that support it, we utilize Egyptian Fruit Bats, a highly social species that can commonly be found in large groups at foraging sites. Using a controlled yet ethologically realistic behavioral paradigm, computational behavioral modeling, and wireless neural recordings in foraging bats, we address the neural underpinnings of social foraging. Specifically, we aim to understand how personal and social foraging information is acquired, integrated, and transformed into a decision. In two sets of experiments - solo and social - we trained individual bats and pairs of bats to perform an n-armed bandit task using a fully automated flight room experimental setup. There, the animals had to choose (on each trial) between one of four navigational goals (feeders), each with a different underlying reward probability that dynamically changed throughout each session. The robustness of the behavior and controlled conditions allowed us to leverage reinforcement learning models to extract value estimates corresponding to the navigational choices on a trial-by-trial basis. To model the behavior of the paired bats, we developed a novel reinforcement learning model that includes the choices of the other bat, allowing us to quantify the fractions of personal and social information

used to drive the individual bats decisions. Neural recordings in posterior parietal cortex and area CA1 of the hippocampus have been carried out in bats performing the task solo, and pairs of bats performing the task in tandem (both bats implanted). In the case of the solo experiments, we find that many of the neurons in posterior parietal cortex exhibit highly reproducible and spatially restricted firing fields in freely flying bats performing the task. Strikingly, a significant fraction of neurons exhibited value-modulated neural activity ("value fields") that reliably tracked the value of navigational choices on a moment-by-moment basis in a spatially restricted region of the environment. The social experiment is ongoing, however, preliminary behavioral findings indicate that social information is indeed utilized by the bats to make choices. Further analyses are aimed at unraveling the intriguing relationship between spatial and value coding, and to determine how social information is integrated with personal information to guide behavior.

Disclosures: N.M. Dotson: None. **K. Soman:** None. **M.M. Yartsev:** None.

Poster

165. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 165.05/Y1

Topic: H.01. Animal Cognition and Behavior

Support: AFOSR Grant FA-9550-17-1-0412
New York Stem Cell Foundation
Searle Scholars Program
Packard fellowship

Title: Planar representation in the hippocampus of freely flying bats

Authors: *K. SOMAN[1], N. M. DOTSON[1], A. GOLDSHTEIN[2], Y. YOVEL[2], M. M. YARTSEV[1];
[1]Bioengineering, Univ. of California Berkeley, Berkeley, CA; [2]Tel-Aviv Univ., Tel-Aviv, Israel

Abstract: Navigation in three dimensional (3D) environments likely requires dedicated spatial codes that extend beyond those described in 2D environments. Here, we took a multi-level approach, which combined studies of bat navigation in the wild, computational modeling of 3D navigation and wireless electrophysiological recordings in flying bats. In doing so, we reveal a novel hippocampal code for 3D spatial representation – that of ethologically relevant 2D planes embedded in 3D space. For the behavioral analysis in the wild, we tracked the flight paths of ten Egyptian fruit bats across multiple nights. We observed that each bat showed two distinct modes of navigation: (i) stereotypical large-scale commute and (ii) local foraging. Subsequently, we brought bats from the wild to the lab where they performed a foraging flight task and their flight behavior was measured at high temporal and spatial resolutions. Analysis of the bat foraging in

flight within the lab environment revealed similarities to those exhibited in the wild. Next, the empirically recorded 3D movements were used as input for a neural network model of 3D navigation (Soman et al., 2018) which predicted (i) previously described fine grain 3D place cells (ii) and a previously undescribed coarser, plane like representation of 3D space. To test for these predictions, we utilized wireless electrophysiological methods to record neural activity from the hippocampal dorsal CA1 region of bats foraging inside the flight room. We found that many of the recorded neurons were 3D place cells and those showed localized isotropic spatial activity, in agreement with previous findings (Yartsev & Ulanovsky, 2013). However, and in agreement with the theoretical model, we identified an additional class of neurons that activated along 2D planes cutting through the 3D volume. The represented planes were mostly horizontal and non-directional. To assess the robustness of this novel spatial code to environmental changes, we manipulated the environment by elevating the floor which induced global remapping of the 3D place cells. Plane cells also underwent global remapping by randomly re-orientating the represented planes in the major Euler angles (azimuth and pitch). Importantly, even after the significant environmental manipulation, plane cells maintained their functional identity. Lastly, using a Bayesian decoder we could show that the combination of both place and plane cells allowed accurate reconstruction of the bat's 3D position, pointing at the complementary aspects of both spatial codes. Taken together, these results reveal a novel hippocampal spatial code for ethologically relevant 3D navigation.

Disclosures: K. Soman: None. **N.M. Dotson:** None. **A. Goldshtein:** None. **Y. Yovel:** None. **M.M. Yartsev:** None.

Poster

165. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation II

Location: Hall A

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Program #/Poster #: 165.06/Y2

Topic: H.01. Animal Cognition and Behavior

Support: New York Stem Cell Foundation
Searle Scholars Program
AFOSR (grant #FA-9550-17-1-0412)

Title: A new experimental framework for studying spatial navigation in flying bats: The Automated Flight Room and its utilization to investigate multiplexed coding in the retrosplenial cortex

Authors: D. GENZEL[1], ***M. M. YARTSEV**[2];
[1]Helen Wills Inst. of Neurosci. and Dept. of Bioengineering, Univ. of California, Berkeley, CA;
[2]Univ. of California Berkeley, Berkeley, CA

Abstract: Bats are the only mammals that can fly and thus offer important insight into the neural mechanisms that contribute to three-dimensional (3D) navigation. To obtain a detailed and quantitative understanding about bat navigation behavior while studying its relevant neural circuits, we developed a fully automated experimental framework for the study of bat navigation. In this new experimental setup, flight behavior, echo-acoustic attention and neural activity can all be recorded simultaneously and importantly, controlled in a highly efficient manner while allowing bats to navigate freely without the presence or involvement of a human experimenter. Here, we describe this setup as well as its initial implementation for neurophysiological studies of cue-guided navigation in freely flying bats. While bats are renowned for their ability to extract information about the environment using echolocation, they in fact also rely on visual, passive auditory and even olfactory signals for navigation as well (Genzel et al., 2018). The Egyptian fruit bat for example, is a highly visual species that most likely relies predominantly on visual cues for long-range navigation. We therefore initially, studied the navigation towards a visual cue in a key candidate structure for this form of navigation, the retrosplenial cortex (RSC), which receives direct inputs from visual cortices and is believed to contribute to spatial navigation. Here, Egyptian fruit bats were trained to approach in flight one of four targets to obtain a food reward, where a correct target was marked by a visual cue. By varying the intensity of the light cue, we could further modulate the reliability of the sensory cue in a controlled fashion and ask how this might affect the bat's performance and ongoing neural activity in the RSC. We find that the RSC contains a robust and multiplexed representation of multiple task variables, including the bat's spatial position and reward and intensity of the visual cues. The study of navigating bats coupled to cellular-resolution measurements of brain activity during free flight and under ethological yet controlled conditions, will provide important insight into how the mammalian brain supports complex forms of three-dimensional navigation.
Genzel, D., Yovel, Y. and Yartsev, M. M. (2018). Neuroethology of bat navigation. *Curr. Biol.* **28**, R997–R1004.

Disclosures: D. Genzel: None. **M.M. Yartsev:** None.

Poster

165. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 165.07/Y3

Topic: H.01. Animal Cognition and Behavior

Title: Representation of large-scale spaces in the hippocampus of flying bats

Authors: *T. ELIAV, S. R. MAIMON, L. LAS, N. ULANOVSKY;
Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Most mammals navigate daily over distances spanning from hundreds of meters to many kilometers. However, nothing is known about hippocampal neural codes for such large spatial scales: over the last fifty years, hippocampal research focused on spatial representations in small laboratory environments. Here we addressed this question for the first time, by developing a unique setup - including a very large environment with an ethologically-relevant spatial scale. We studied Egyptian fruit bats - flying mammals that are excellent navigators over large scales, and which have rodent-like hippocampal spatial representations in small laboratory environments. We developed an on-board wireless neural-logging system, which allows recording single-units over unlimited distances. We built a 200-m long tunnel where bats can fly freely; bat's position was tracked using an RF localization device that measures distances to a ground-based antenna array - yielding high accuracy of ~10-cm, much better than GPS. Bats flew back-and-forth along the tunnel, more than 100 laps per-session (>20-km total distance). Recordings from dorsal hippocampal area CA1 of 5 bats showed some firing properties that were similar to findings in small-scale laboratory environments, such as directionality. However, most properties were very different: (i) Individual cells exhibited multiple fields per neuron. (ii) field size could reach up to 20-30 meters. (iii) A given neuron could exhibit multiscale spatial coding - with different place-fields of the same neuron having very different sizes, ranging from sub-meter to 20-30 m. These large variations in scale were unrelated to local landmarks, and could not be explained by the animal's flight speed, which was extremely stable along the tunnel - suggesting that CA1 neurons have a genuine multi-scale representation. Taken together, the firing properties of CA1 neurons in this large-scale environment suggest a representation that is very different from findings reported in the laboratory.

Disclosures: **T. Eliav:** None. **S.R. Maimon:** None. **L. Las:** None. **N. Ulanovsky:** None.

Poster

165. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 165.08/Y4

Topic: H.01. Animal Cognition and Behavior

Support: ERC
ISF

Title: Episodic cells for self and other in the bat hippocampus

Authors: *D. OMER[1,2], L. LAS[2], N. ULANOVSKY[2];
[1]Edmond and Lily Safra Ctr. for Brain Sci., The Hebrew Univ. of Jerusalem, Jerusalem, Israel;
[2]Neurobio., Weizmann Inst. of Sci., Rehovot, Israel

Abstract: The hippocampal formation is essential for forming episodic memories of where-and-when. Extensive research has revealed place cells and grid cells that encode spatial position (where), and time cells that encode elapsed time (when). However, very little is known about how the brain encodes both space and time simultaneously. Furthermore, it is unknown whether and how the brain encodes elapsed time for other individuals, in a social context. Here we show, for the first time, that CA1 neurons in the bat hippocampus encode simultaneously elapsed time × space, and also encode elapsed time for another bat - in a social task. We trained an observer bat to watch, remember, and imitate the flights of a demonstrator bat to different positions in the room. We found time-cells in dorsal CA1 of the observer bat - neurons which fired transiently at specific times after the observer bat has landed and was hanging motionlessly. Different time-cells had different preferred times at which they fired - and together, ensembles of these time-cells formed internally-generated firing sequences that encoded elapsed-time, and spanned the entire waiting-time of the observer bat. Importantly, these cells generated different temporal sequences at different locations in the room, thus encoding simultaneously space × time (spacetime): hence we termed them 'episodic cells'. A distinct subgroup of neurons exhibited the same preferred-time irrespective of position - thus purely encoding elapsed time. Surprisingly, we also found episodic-cells that encoded elapsed time from the landing-moment of the other bat. Together, our results demonstrate neuronal coding of spacetime for self and other in the hippocampus - which may support both perception of interval timing and episodic memories for self and other.

Disclosures: D. Omer: None. **L. Las:** None. **N. Ulanovsky:** None.

Poster

165. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 165.09/Y5

Topic: H.01. Animal Cognition and Behavior

Support: ERC

Title: Representation of 3D space in the entorhinal cortex of flying bats

Authors: *G. GINOSAR[1], J. ALJADEFF[2], Y. BURAK[3], H. SOMPOLINSKY[3], L. LAS[1], N. ULANOVSKY[1];
[1]Weizmann Inst. of Sci., Rehovot, Israel; [2]Dept. of Bioengineering, Imperial Col. London, London, United Kingdom; [3]The Edmond and Lily Safra Ctr. for Brain Sciences, and Racah Inst. of Physics, Jerusalem, Israel

Abstract: Grid cells are neurons in medial entorhinal cortex (MEC) that are activated when the animal passes through multiple locations ('firing-fields') on the 2D surface that the animal is

exploring. These firing-fields are arranged in a hexagonal 2D lattice that spans the entire 2D surface. Although many animals navigate in 3D space, the volumetric 3D firing-pattern of grid cells remains unknown. Here we recorded MEC neurons in freely-flying bats, and found a variety of spatial cells - including 3D border-cells, 3D head-direction cells, and neurons with multiple 3D firing-fields. The multi-field neurons displayed an increased inter-field-spacing along the dorso-ventral axis of MEC - as in rodent grid-cells. Many of the multi-field neurons were 3D grid cells - exhibiting a local order in their field arrangement, with fields separated by a characteristic distance; however, they were not organized in a global lattice. We modeled grid-cells as emerging from pairwise-interactions between fields, which yielded a hexagonal lattice in 2D, but only local order in 3D - thus explaining both 2D and 3D grid-cells within one model.

Disclosures: G. Ginosar: None. **J. Aljadeff:** None. **Y. Burak:** None. **H. Sompolinsky:** None. **L. Las:** None. **N. Ulanovsky:** None.

Poster

165. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 165.10/Y6

Topic: H.01. Animal Cognition and Behavior

Title: Effects of early experience on spatial representation of large-scale environments in the bat hippocampus

Authors: *S. R. MAIMON, T. ELIAV, L. LAS, N. ULANOVSKY;
Weizmann Inst. of Sci., Rehovot, Israel

Abstract: The proper formation and function of many brain circuits require normal experience during development. While long-term effects of altered sensory experience on the brain have been extensively studied, little is known about the long-term neurophysiological effects of abstract, cognitive experiences. The hippocampus offers an excellent substrate for addressing this question, because this high-level brain region is very far removed from the sensory periphery, and it contains abstract representations of space. A number of studies have examined the development of spatial representations in the hippocampal formation of rat pups, during normal ontogeny; however, it is unknown how alterations in early experience affect spatial representation in the adult hippocampus. We are addressing this question by investigating how a very large environment (a 200-meter long tunnel) is represented in the hippocampus of laboratory-born bats, which were never exposed to large-scale environments (larger than a 6×6 meter room). We are using a miniature wireless electrophysiology system to record place cells in hippocampal area CA1 of laboratory-born bats that are flying in this very long tunnel from the very first exposure. We compare the spatial maps of these place-cells and their temporal dynamics to those of place-cells recorded from wild-caught bats performing the same task in the

same tunnel. The main difference between the ontogeny of lab-born and wild-born bats is in a very abstract parameter: the spatial scale of the environment they have experienced during ontogeny. Here we will present preliminary neural recordings that aim to elucidate how hippocampal representations are shaped by abstract features of early experience.

Disclosures: **S.R. Maimon:** None. **T. Eliav:** None. **L. Las:** None. **N. Ulanovsky:** None.

Poster

165. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 165.11/Y7

Topic: H.01. Animal Cognition and Behavior

Title: Dynamics of hippocampal spatial representation upon brief attentional switches during navigation

Authors: *A. SAREL, D. BLUM, L. LAS, N. ULANOVSKY;
Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Navigation is a rich and dynamic behavior that unfolds in complex environments. It requires the animal to know its own location within the environment, but also be attentive to brief and sudden events such as obstacles along the way, or other conspecifics or predators that may appear suddenly and which the animal should avoid. Most studies about the neural basis of navigation focus on the representation of the current position of the animal in small, empty, and static setups that do not imitate well the complexity and rich dynamism of real-world navigation. Here we set out to test how brief attentional switches to 'things out-there' affect the representation of space in the hippocampus. To this end, we designed a task in which two bats flew together in a 120-meter flight tunnel, and had to be attentive both to the environment and to the position of the other bat, in order to avoid collisions during events of 'cross-overs' between the bats. We recorded the neural activity in hippocampal area CA1 and tracked the position of the bats in the tunnel using wireless-electrophysiology and custom tracking devices. Preliminary results showed that during cross-overs, many place-cells in CA1 transiently switched their activity from representing the position of the bat in the tunnel to representing the distance from the other bat (inter-bat distance). This neuronal representation of the other bat was very brief, lasting for only a few hundred milliseconds, and then it switched back to the normal place-tuning of the tunnel. Thus, hippocampal neurons dynamically and rapidly switched between an allocentric representation of the tunnel (place tuning) and an egocentric representation of the distance from the other bat. We also found neurons that exhibited pure egocentric distance-tuning but no allocentric place-tuning. Interestingly, most cells were tuned to egocentric inter-bat distances within a range of ±10 m, which roughly matched the distance at which bats attended to each other, as indexed by their echolocation signals. Together, these preliminary results suggest

that attentional switches during navigation - which in bats can be measured directly based on their echolocation signals - elicit rapid dynamics of hippocampal spatial coding.

Disclosures: **A. Sarel:** None. **D. Blum:** None. **L. Las:** None. **N. Ulanovsky:** None.

Poster

165. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation II

Location: Hall A

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Program #/Poster #: 165.12/Y8

Topic: H.01. Animal Cognition and Behavior

Support: NSERC Discovery Grant #40352
NSERC Discovery Grant #03857
CIHR Project Grant

Title: Mutual-information based method for detecting coordinated neural activity in two-photon calcium imaging data

Authors: *H. CHANG[1], M. TATSUNO[1], M. H. MOHAJERANI[1], B. L. MCNAUGHTON[1,2]; [1]Dept. of Neurosci., Univ. of Lethbridge, Lethbridge, AB, Canada; [2]Dept. of Neurobio. and Behavior, Univ. of California Irvine, Irvine, CA

Abstract: Coordinated activation of neurons is believed to underlie complex cognitive functions. Recently *in vivo* 2-photon calcium (Ca^{2+}) imaging has enabled new opportunities to conduct large-scale recordings of single neurons. Whether traditional methods used in detecting neuronal ensembles in electrophysiology such as Principal Component (PC)-based approaches can be applied to Ca^{2+} data still needs investigation. These methods must account for specific constraints of Ca^{2+} data including spatiotemporal noise, low sampling rates and nonlinear signal dynamics caused by saturation levels. We show that PC-based methods do suffer from these constraints. In response, we developed a novel Mutual Information (MI)-based method specifically for Ca^{2+} data. First, we evaluated three representative PC-based approaches (Lopes-dos-Santos et al. 2011, Carrillo-Reid et al. 2016 and Malvache et al. 2016) with real and simulated data. Real data consisted of 10-minute imaging sessions of CA1 neurons from Thy1-GCaMP6s mice running on a treadmill. Simulated data consisted of inhomogeneous Poisson spikes with time-dependent firing rate estimated from deconvolved CA^{2+} time series. Performance on real data was evaluated based on the fraction of detected cells that expressed recurrent activity patterns regarding the animals' location. For simulated data, we quantified accuracy in detecting ensembles embedded in time series with varying degrees of noise. We found that detection accuracy deteriorated rapidly with small amounts of jitter, increased background noise, low ensemble activity and reduced linear correlation between ensemble cell-pairs. These results demonstrated limitation of PC-based approaches on Ca^{2+} data. Next, we

developed a novel method using MI as the measure of similarity between cell-pairs. We chose MI as it is capable of detecting nonlinear relationships that may arise from saturation or inherent dynamics of the Ca^{2+} indicator. MI was estimated by a modified Kraskov-Stoegbauer-Grassberger algorithm. Agglomerative clustering was applied to the resulting similarity matrix to assign neurons membership to distinct ensembles, with additional steps to account for overlaps between ensembles. We showed that detection accuracy for our method decreased at a significantly slower rate with increasing jitter, background noise, and decreasing ensemble activities. Accuracy was unaffected by reduced linear correlation between cell-pairs. A larger fraction of place cells were also recovered in real data. In summary, we showed that PC-based methods were limited by the constraints of Ca^{2+} data and proposed a MI-based method more robust to those constraints.

Disclosures: H. Chang: None. **M. Tatsuno:** None. **M.H. Mohajerani:** None. **B.L. McNaughton:** None.

Poster

165. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation II

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Program #/Poster #: 165.13/Y9

Topic: H.01. Animal Cognition and Behavior

Support: DARPA Lifelong Learning Machines (L2M) Program
National Science Foundation
National Eye Institute Award Number F30EY029589

Title: Rapid global remapping in retrosplenial cortex

Authors: *Z. NAVRATILOVA, D. BANERJEE, F. MUQOLLI, S. LONDONO-MUNOZ, S. P. GANDHI, B. L. MCNAUGHTON;
Neurobio. and Behavior, Univ. of California Irvine, Irvine, CA

Abstract: A prevalent theory of the role of the hippocampus in memory retrieval and consolidation is that it creates a unique ‘index’ for each experience and stores it in neocortex in association with the contents or attributes of the experience. Autoassociative retrieval of an index in the hippocampus then triggers retrieval of the attribute representations in cortex. Episodic memories are thought to be consolidated by replay of the index, which then activates event-related cortical neurons, and thus permits strengthening of synaptic connections among them. Dorsal hippocampus can produce novel, sparse codes for spatial location within minutes when an animal is exposed to a new environment (Wilson & McNaughton, 1993). Moreover, the ‘index’ patterns are replayed during sleep or quiet rest after the experience (e.g. Wilson & McNaughton, 1994). A further requirement of the index hypothesis is that the cortex receives and encodes this

spatial signal from the hippocampus. Recently, Mao et al. (2017, 2018) have shown spatial coding among many cells in superficial layers of retrosplenial cortex (RSC) that has a sparseness similar to dorsal hippocampus (in mice running on a virtual track), and that appearance of this sparse sequential pattern is impaired when hippocampus is lesioned bilaterally. Finally, hippocampus-guided memory replay must occur after a single experience, and so we looked for a spatial signal in RSC during novelty. We exposed mice to a highly familiar virtual track, while imaging activity in single neurons in the RSC using 2-photon calcium imaging, and then 'teleported' them to a novel VR environment to determine if spatial activity can form in RSC within a single session. We compared the spatial neural activity in the familiar and novel environments, and observed a complete rearrangement of the activity of RSC cells, indicating that the novel environment was coded essentially orthogonally to the familiar environment. Complete remapping occurred during the first 5 laps in the novel environment. The peak firing rates of superficial RSC cells were reduced during the first 5-10 laps in the novel environment, but then rebounded to the same levels as in the familiar environment. Correlations between spatial maps during intervals of 5 laps in the novel environment indicated that spatial activity was not fully developed during the first 10 laps, but after that the activity became stable. This stability was comparable to that in the familiar environment. Thus, spatial activity in the retrosplenial cortex can emerge and stabilize on the same timescale as in the hippocampus (~10 min), suggesting that patterns produced in the hippocampus can guide memory formation in cortex.

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Poster

165. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation II

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CIHR Grant 390930 (to M.H.M)
NSERC DG Grant 40352 (to M.H.M)

Title: Probing the contribution of self-motion versus sensory cues in generating responses of hippocampal CA1 place cells in mice

Authors: *S. INAYAT[1], J. SUN[1], B. L. MCNAUGHTON[3,4], M. H. MOHAJERANI[2]; [1]Neurosci., [2]Dept. of Neurosci., Univ. of Lethbridge, Lethbridge, AB, Canada; [3]Dept. of Neurosci., The Univ. of Lethbridge, Lethbridge, AB, Canada; [4]Univ. of California, Irvine, CA

Abstract: There remains some controversy over the relative primacy of a self-motion-based distance calculation or external cues (landmarks) in the determination of where hippocampal 'place cells' fire, and these signals may not be mutually exclusive. We investigated this issue using a novel behavioral paradigm and calcium imaging of dorsal CA1 pyramidal cells. Head-restrained mice were trained to run in the dark on a linear treadmill in response to a continuous mild air puff (AP) as an aversive stimulus. After the AP onset, mice had to run one lap of the belt (142 cm) before the AP was turned off. The intertrial interval was 15 s and mice were free to move the belt between trials. The trial onset was therefore variable with respect to belt coordinates. Since the treadmill was initially devoid of any sensory cues, place cells (PCs) were observed encoding distance from the AP onset (nominally the only available 'landmark'). Most place field centers were in the first half of the trajectory. An increase in average place field widths was observed with increasing distances from the AP onset suggesting distance-calculation based on self-motion as the primary input for PC responses (increasing accumulation error with distance from AP onset). Four identical visuo-tactile cues were then placed on the treadmill belt at approximately equidistant locations. As the animal was free to move within the intertrial period the cue locations were variable with respect to AP onset. The number of PCs did not increase markedly but there was some remapping of PCs as well as emergence of new PCs and loss of previous ones. Most of the place field centers were still in the first half of the belt suggesting that distance calculation from the AP onset was still the primary determinant of PC responses. When the belt was subsequently locked during the inter-trial interval, the cues became fixed relative to the AP onset. Although there were only four sensory cues, PCs were observed almost uniformly distributed over the length of the belt, indicating that sensory cues recruited more PCs driven by distance calculation from self-motion. When finally, sensory cues were removed, an increase in place fields widths was observed for many cells suggesting that sensory cues reset the distance calculation reducing accumulation error and increasing place encoding precision by decreasing place field widths. Our data therefore support the long-standing idea (McNaughton et al., 1996) that path-integration is the primary determinant of place-field location, while sensory cues become secondarily bound to place fields and act as anchoring points to reset distance calculations for the reduction of cumulative error.

Disclosures: S. Inayat: None. **J. Sun:** None. **B.L. McNaughton:** None. **M.H. Mohajerani:** None.

Poster

165. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation II

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Program #/Poster #: 165.15/Y11

Topic: H.01. Animal Cognition and Behavior

Support: American Epilepsy Society Junior Investigator Award (OJA)
MICDE Catalyst Grant (OJA)

Title: Retrosplenial high frequency oscillations demarcate high activity REM sleep frames

Authors: *M. GHOSH, F. C. YANG, A. GONZALEZ, V. HETRICK, O. AHMED;
Psychology, Univ. of Michigan,Ann Arbor, Ann Arbor, MI

Abstract: The Retrosplenial Cortex (RSC) is heavily interconnected to an array of brain regions, serving as a major hub in the episodic memory system. Both the CA1 and subiculum regions of the hippocampal formation project to the RSC. In addition, it has reciprocal connections to multiple cortical and subcortical regions, including the posterior parietal cortex (PPC), visual cortex and the anterior thalamus. These anatomical connections and experimental evidence suggest that the RSC might be specifically involved in spatial cognition and memory retrieval. We have studied the RSC using large-scale simultaneous tetrode recordings from the visual cortex, CA1, PPC and RSC in male Long-Evans rats. Here, we report the dynamics of a unique and precisely theta-coupled high frequency oscillation (HFO) that is localized to the RSC. We present a comparative analysis of the HFO across REM and awake states, its properties across brain regions and variations within the RSC. We also present our results of in-vivo single cell activity with respect to these RSC HFOs. Our results show that RSC HFOs are unique markers of high activity states across the episodic memory system during REM sleep.

Disclosures: M. Ghosh: None. **F.C. Yang:** None. **A. Gonzalez:** None. **V. Hetrick:** None. **O. Ahmed:** None.

Poster

165. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation II

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MICDE Catalyst Grant (OJA)

Title: Computational modeling reveals novel biophysical mechanisms underlying profound theta phase-amplitude coupling in the retrosplenial cortex

Authors: *S. SUDHAKAR[1], E. K. W. BRENNAN[2], O. J. AHMED[1];
[1]Dept. of Psychology, [2]Univ. of Michigan, Ann Arbor, MI

Abstract: The retrosplenial cortex (RSC) plays a crucial role in learning, memory, cognition and successful navigation. The RSC displays unique oscillatory signatures during active behaviors, as well as rapid eye movement (REM) sleep in rodents. Fast brain oscillations such as gamma (40-70 Hz) and high frequency oscillations (120-160 Hz) have both been reported in the retrosplenial cortex during these states. These fast oscillations often exhibit remarkably strong and precise phase amplitude coupling with the slower theta rhythm (4-12 Hz). The biophysical mechanisms responsible for these unique oscillatory patterns in the retrosplenial cortex remain poorly understood. In this study, using computational modeling, we unravel the cellular and network mechanisms of retrosplenial gamma and high frequency oscillations, as well as their unique phase amplitude coupling with theta rhythms. Our computational modeling reveals that the retrosplenial cortical network is endowed with unique neurophysiological mechanisms that can intrinsically generate theta oscillations. We highlight the distinct biophysical mechanisms that can lead to gamma versus high frequency oscillations in the RSC. We also model the effect of medial septal inputs on the robustness of retrosplenial oscillatory patterns. The findings of our computational modeling provide novel insights into the computational schemes used by the retrosplenial cortex to carry out its spatial navigation and memory functions.

Disclosures: S. Sudhakar: None. **E.K.W. Brennan:** None. **O.J. Ahmed:** None.

Poster

165. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 165.17/Y13

Topic: H.01. Animal Cognition and Behavior

Support: NIH T32
Rackham Merit Fellowship, University of Michigan

Title: Comparison of retrosplenial oscillatory dynamics during head-fixed vs unrestrained behaviors

Authors: *S. P. RICE[1], M. GHOSH[2], D. SIU[3], V. HETRICK[2], O. J. AHMED[4];
[1]Neurosci. Grad. Program, Bioinformatics Grad. Program, Kresge Hearing Res. Inst., [2]Psychology, [3]LS&A Psychology, [4]Dept. of Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: The retrosplenial cortex is an important structure in spatial navigation and contains head direction cells. The retrosplenial cortex receives dense projections from multiple regions including the subiculum and anterior thalamus, two regions that themselves contain neurons that encode head direction. Here, we investigate how the retrosplenial cortex responds in unrestrained conditions where vestibular inputs change through active control by the mouse versus head-fixed conditions where vestibular inputs are controlled by the experimenter. In particular, we focus on

network oscillations recorded in the local field potential. Our results reveal key differences between head-fixed vs unrestrained behaviors in multiple rhythmic frequency bands, including theta. This approach highlights the care that must be used when interpreting the results of head-restrained studies of the retrosplenial cortex.

Disclosures: S.P. Rice: None. **M. Ghosh:** None. **V. Hetrick:** None. **O.J. Ahmed:** None.

Poster

165. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 165.18/Y14

Topic: H.01. Animal Cognition and Behavior

Support: NSF Graduate Research Fellowship (TJ)
American Epilepsy Society Junior Investigator Award (OJA)
MICDE Catalyst Grant (OJA)

Title: Stability and flexibility in the hippocampal theta sequence coding circuit

Authors: *T. JOHN[1], O. J. AHMED[2];
[1]Neurosci. Grad. Program, [2]Dept. of Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: Brain circuits must represent information in a way that is robust against noise and yet receptive to updates based on changing external inputs, i.e. in a way that is both stable and flexible. The requirements for stability and flexibility are particularly prominent in the circuits responsible for representing space since the code is stable over time yet highly receptive to environmental cues. The processes determining the timing of spikes relative to the theta rhythm and relative to other cells coding for space are not completely understood, but they are likely required to exhibit both stability and flexibility in order to constitute an effective sequence coding scheme.
Here, we explore intrinsic and synaptic contributions to balancing stability and flexibility within sequence coding schemes used by spatial navigation circuits. This is done using conductance-based computational modeling in order to adequately capture processes that influence spike timing in ways that a rate model could not. For this we adapt the single CA1 cell model that L. Stan Leung (2011) developed to explain subthreshold properties of place cells in behaving animals.
We examine the consequences of intrinsic heterogeneity in the conductances thought to be responsible for these oscillations combined with plausible synaptic network structures in the hippocampus. We measure stability as the extent of speed and sensory input values for which the position-phase correlation is sufficiently high when varying these inputs at both fast or slow timescales. We measure flexibility as the extent of output values, i.e. phase values and

differences, that can be produced by the circuit. Internal parameters such as the dynamics and heterogeneity of subthreshold oscillating conductances and the strength of recurrent synapses were varied to evaluate their influence on stability and flexibility of the temporal code as defined in this way. Results suggest that the combination of network-level and intrinsic properties contribute to affording both stability and flexibility in models of the hippocampal theta sequence coding circuit.

Disclosures: T. John: None. **O.J. Ahmed:** None.

Poster

165. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 165.19/Y15

Topic: H.01. Animal Cognition and Behavior

Support: American Epilepsy Society Junior Investigator Award
MICDE Catalyst Grant
NIDA T32DA007281

Title: Anatomical and behavioral correlates of retrosplenial oscillations

Authors: *A. P. LORENZO GONZALEZ, M. GHOSH, V. HETRICK, O. J. AHMED; Dept. of Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: The retrosplenial cortex (RSC) is a midline structure deeply interconnected with a number of cortical and subcortical areas in the brain, serving as a hub for the integration of sensory, motor and memory-related information. Damage to this structure results in memory deficits, as well as impaired spatial navigation. While the importance of the RSC in learning and memory has been firmly established, relatively few studies have examined the precise circuit mechanism via which the RSC can support these functions. Our group has characterized a unique high frequency oscillation HFO specifically localized to the RSC (rscHFOs). These rscHFOs are prominent theta-coupled 120-160 Hz rhythms, are only generated during high-activity states, and they show increased power during REM sleep. Here, we carefully use multi-electrode microdrives, and silicon probes to localize the precise laminar correlates of these HFOs. The implanted animals were recorded during specific behavioral tasks that require instrumental, contextual, or spatial learning, and careful analysis of single neuron activity and brain rhythms was conducted. Our results shed light on the precise behavioral correlates of these oscillations in the context of spatial navigation and memory.

Disclosures: A.P. Lorenzo Gonzalez: None. **M. Ghosh:** None. **V. Hetrick:** None. **O.J. Ahmed:** None.

Poster

165. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 165.20/Y16

Topic: H.01. Animal Cognition and Behavior

Support: American Epilepsy Society Junior Investigator Award (OJA)
MICDE Catalyst Grant (OJA)
NSF GRFP

Title: Connections of hyperexcitable pyramidal neurons in the retrosplenial cortex

Authors: *E. K. BRENNAN, S. SUDHAKAR, I. JEDRASIAK-CAPE, S. RICE;
Univ. of Michigan, Ann Arbor, MI

Abstract: The retrosplenial cortex plays a critical role in learning, memory, and spatial navigation and shares extensive connections with other brain regions involved in these functions. However, the nature of these precise connections and the neuronal subtypes involved remain largely unknown. Our study aims to identify the precise neuronal subtypes within the superficial layers of the retrosplenial cortex as well as local and extrinsic connectivity. We show that unique and distinct subtype of pyramidal neuron is highly intrinsically excitable within these layers. However, it rarely synapses with its local neighbors and instead sends its axons into deeper layers and the corpus callosum. Conversely, local FS cells provide a strong source of inhibition onto the novel pyramidal neurons in these layers, creating an inhibition dominated network. We present implications of these circuit properties in the context of our in vivo observations.

Disclosures: E.K. Brennan: None. **S. Sudhakar:** None. **I. Jedrasiak-Cape:** None. **S. Rice:** None.

Poster

166. Human Perception and Imagery I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 166.01/Y17

Topic: H.02. Human Cognition and Behavior

Title: Taking (virtual) aim: A study of symptoms related to training in VR

Authors: *A. TORRES LOPEZ[1], C. Y. DELGADO[2], A. X. KRAUSE[2], D. LEVATON[2], B. GABAY[1], J. HINKEL-LIPSKER[1], S. A. DREW[1];
[1]California State Univ. Northridge, Northridge, CA; [2]California State Univ. Northridge, Tarzana, CA

Abstract: Due to the increasing popularity of virtual reality (VR) and the linked increase of asthenopia, also known as visual discomfort, it is critical to better understand how VR usage relates to visual discomfort associated with the accommodative and vergence systems. Previous studies have shown that there has been an increase in visual discomfort symptoms associated to computer usage and virtual reality devices (Ames et al., 2005). Utilizing an acute symptom survey, we investigated ocular symptoms reported by VR-trained participants compared to real-world (RW) trained participants in a darts throwing task. Pre- and post-acute measurements were made and preliminary data shows marked differences between the two groups, with VR-trained participants showing an increase in symptoms of visual discomfort as compared to RW-trained participants. Implications of these findings will be discussed.

Disclosures: A. Torres Lopez: None. **C.Y. Delgado:** None. **A.X. Krause:** None. **D. Levaton:** None. **B. Gabay:** None. **J. Hinkel-Lipsker:** None. **S.A. Drew:** None.

Poster

166. Human Perception and Imagery I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 166.02/Y18

Topic: H.02. Human Cognition and Behavior

Title: Comparing human and network similarity judgments on adversarial image scenes and captions

Authors: *R. M. FLEMMING[1], J. SCHWARTZ[2], P. R. SCHRATER[2];
[1]Psychology, Univ. of Minnesota, Twin Cities, Minneapolis, MN; [2]Univ. of Minnesota, Minneapolis, MN

Abstract: Neural network architectures have made huge strides in object recognition over the last decade, and have even expanded into image captioning, where a network learns to assign labels to parts of an image or to the image as a whole based on the parts' relation to each other. While such captioning networks perform on some images, they mislabel other images—especially those with strange or uncommon scenes—in surprisingly incorrect ways, indicating differences from humans in the networks' learned semantic scene representations and/or the process by which those representations are translated into natural language. To understand network and human caption generation, confidence, and exchangeability, we design an adversarial image set aimed at altering human captioning of images. These images exhibit

human-tweaked minimal distortions which aim to alter the captions generated by people, or the semantic interpretation of the scene. Humans are shown these images and asked to make two types of judgments: 1) a caption for the image and confidence rating for their caption, and 2) to rate the similarity of images by the exchangeability of their captions. By computing these human and network image-similarity matrices, we can compare the semantic scene representations of people and image captioning networks. Understanding how these representations differ could be a critical step in determining how these networks fail in non-human ways and what augmentations may be necessary to exhibit more human-like labeling.

Disclosures: R.M. Flemming: None. **J. Schwartz:** None. **P.R. Schrater:** None.

Poster

166. Human Perception and Imagery I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 166.03/Y19

Topic: H.02. Human Cognition and Behavior

Support: NEI-EY025872

Title: Environmental priors and stimulus history shape early visual processing

Authors: *T. C. SHEEHAN[1], C. CHUNHARAS[2], J. SERENCES[3];
[1]UC San Diego, La Jolla, CA; [2]Neurology, department of Intrnl. Med., King Chulalongkorn memorial hospital, Chulalongkor, Bangkok, Thailand; [3]Psychology, UCSD, La Jolla, CA

Abstract: It is well established that memory for low level stimuli are systematically biased and that these biases are shaped by both our prior expectations (Wei & Stocker, 2015) and short term assumptions of stimulus contiguity (Fischer & Whitney 2014). These biases have been identified in orientation reporting tasks as cardinal and serial biases respectively. Both types of biases can be understood in the context of an efficient coding scheme which uses Bayesian inference from noisy sensory encoding to minimize the variance of reported orientations at the cost of introducing bias (Wei & Stocker, 2017). While both types of bias have been explored theoretically, little work has looked at the neural underpinnings of these biases or how they interact. We developed a novel task paradigm whereby sequences of oriented stimuli were constructed to maximize cardinal and serial biases. Behavioral results in a change detection task demonstrate robust increases in perceptual sensitivity when cardinal or serial biases shifted perception away from probe stimuli, with helpful cardinal bias increasing performance from 65 to 85% (t(19)=5.76, p<1e-7) and serial bias from 65% to 81% (t(19)=3.10, p<.005). We then used fMRI and multivariate encoding/decoding models to examine the neural underpinnings of these behavioral biases in early areas of human visual cortex. We found that cardinal and serial biases shift orientation reconstructions across the visual hierarchy and that these biases

emerge at the time of perception. We further observed that serial bias is strongly predicted by poor reconstruction fidelity on the current trial and strong fidelity on the previous one. These results suggest that both cardinal and serial behavioral biases are influenced by corresponding biases in representations of orientation in early visual cortex.

Disclosures: T.C. Sheehan: None. **J. Serences:** None. **C. Chunharas:** None.

Poster

166. Human Perception and Imagery I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 166.04/Y20

Topic: H.02. Human Cognition and Behavior

Support: N

Title: Estimating behavioral and neural stimulus-response functions in auditory processing

Authors: *M. ALIZADEH SHALCHY[1], K. C. YAGHOUBI[2], X. CHEN[3], J. LANGLEY[3], I. J. BENNETT[1], X. P. HU[4], M. A. K. PETERS[5], A. R. SEITZ[1];
[1]Psychology, [2]Psychology, Bioengineering, [3]Ctr. for Advanced Neuroimaging, [4]Bioengineering, [5]Bioengineering,Psychology,Interdepartmental Grad. Program in Neurosci., UCR, Riverside, CA

Abstract: In the current project, we sought to characterize the relationship between psychophysical and neurometric stimulus-response functions in auditory processing. To this end, we developed a novel psychometric event-related auditory oddball task where oddball salience was parametrically manipulated to derive a stimulus-response function. To accomplish this, different oddballs deviated from a 1000 Hz standard with frequency offsets ranging from 1004 to 1128 Hz. A 3 Tesla Siemens MRI scanner was used to collect structural and functional data from participants as they were performing the oddball task. In addition behavioral data were collected. After pre-processing, fMRI data alongside task-based regressors were fed into the general linear model (GLM) to estimate β values. We utilized a hybrid (structural-functional) region of interest (ROI) to investigate the neurometric function. The goal was to understand the relationship between behavioral and neurometric stimulus-response functions. Behavioral results showed the approach yielded a psychometric function that monotonically increased as a function of oddball frequency offset. Further, the BOLD response in auditory-responding voxels increased with the oddball offset. These results demonstrate the viability of this novel psychometric oddball paradigm in characterizing both behavioral and neuronal stimulus-response functions of auditory processing. Future research investigating how these may be dependant upon fluctuations in the phasic activity of the Locus Coeruleus (LC) will be discussed.

Disclosures: **M. Alizadeh Shalchy:** None. **K.C. Yaghoubi:** None. **X. Chen:** None. **J. Langley:** None. **I.J. Bennett:** None. **X.P. Hu:** None. **M.A.K. Peters:** None. **A.R. Seitz:** None.

Poster

166. Human Perception and Imagery I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 166.05/Y21

Topic: H.02. Human Cognition and Behavior

Support: NVIDIA GPU Grant

Title: Exploring steady-state visual evoked potentials with video stimuli

Authors: A. C. LAKMAZAHERI, A. DATAR, *S. W. MICHALKA;
Olin Col. of Engin., Needham, MA

Abstract: Steady-state visual evoked potentials (SSVEPs) are robust responses that can be detected using electroencephalogram (EEG). These SSVEPs are often used in brain-machine interfaces and as a method for studying cognition. When used in brain-machine interfaces, the stimuli to generate SSVEPs are typically flashing LEDs or checkerboards, which may be uninteresting or unpleasant to view for sustained periods of time. In this work, we investigated if videos could be combined with semi-transparent flashing checkerboards in a more ergonomic SSVEP paradigm. We tested if these video-checkerboard stimuli could be used to effectively classify which stimulus a person was attending to. In the primary experiment, participants were instructed to attend to one of two videos presented on a computer screen. A semi-transparent checkerboard was overlaid on both of the videos, each flashing at a unique frequency (e.g. 6 Hz for the left video and 15 Hz for the right video). In each trial, the participants were cued to attend to a video at a specific location, then the stimuli were presented for 60 s, followed by a short break (self-paced). Data were preprocessed by bandpass filtering (0.5 Hz to 50 Hz), epoching into 10 s periods, and rejecting artifacts using visual inspection and independent component analysis. Classification analysis attempted to determine which of the two videos the participant attended to using frequency-domain information across EEG electrodes. In preliminary testing (n=2, 2 female, ongoing data collection), we were able to classify attention location with >70% accuracy within each subject. Additionally, we explored various checkerboard sizes and opacities to improve the stimulus ergonomics. This preliminary work suggests that video-checkerboard stimuli may be a feasible alternative to traditional checkerboard stimuli for SSVEP studies, and this technique may be applied to study other aspects of cognition by varying the video stimuli and tasks.

Disclosures: **A.C. Lakmazaheri:** None. **A. Datar:** None. **S.W. Michalka:** None.

Poster

166. Human Perception and Imagery I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 166.06/Y22

Topic: H.02. Human Cognition and Behavior

Support: Finding a Cure for Epilepsy and Seizures

Title: Electrocorticography (ECoG) high gamma modulation from the depth inversion illusion

Authors: *J. JESCHKE, D. FRIEDMAN;
NYU Langone Med. Ctr., New York City, NY

Abstract: Objective: Explore High Gamma (70-150 Hz) modulation differences between differing depth cues and patient responses during a simple Depth-Inversion Illusion (DII) task. Background: Previous research shows cohorts at risk for exhibiting psychotic symptoms such as schizophrenia patients and individuals at high risk for psychotic disorder have reduced susceptibility to depth inversion illusions (DII), where ambiguous concave surfaces appear convex. The classic example of this is the Hollow Mask Illusion where the concave side of a face mask will appear to “pop out.” In order to better understand the cortical mechanisms we explored this in ECoG. Methods: N=3 patients undergoing intracranial ECoG presurgical monitoring participated in the task, with electrode coverage largely over the left hemisphere and some coverage on the right hemisphere. Patients observed stimuli and reported “poping out” or “caving in” for between 96 and 192 trials. The 4 conditions for comparison were veridical and illusory responses for convex and concave stimuli compared by analyzing percentage change of high gamma amplitude compared to baseline, within subjects. Results: High gamma change from baseline was observed in both hemispheres, particularly in left parietal areas, temporal and occipital areas, but only the maximal illusory condition where patients reported convexity from concave cues showed significant difference in the left anterior mesial temporal region.

Disclosures: J. Jeschke: None. **D. Friedman:** None.

Poster

166. Human Perception and Imagery I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 166.07/Y23

Topic: H.02. Human Cognition and Behavior

Support: JSPS 18F18008
KAKEN-HI 19H01771

Title: Brain pattern in V1 changes after receiving social information

Authors: *W.-J. LIN[1,2], Y. YOTSUMOTO[1];
[1]The Univ. of Tokyo, Tokyo, Japan; [2]Japan Society for the Promotion of Sci., Tokyo, Japan

Abstract: People make various decisions every day. From what to eat, where to visit for vacation, which job offer to take, and so on. Social information, no doubt, has a certain degree of influence on the decisions we make. The impact of social information also exists when people perform perceptual decision-making tasks, as Asch showed in his essential works in the 1950s. A question has been raised but remains unanswered: when people change their answers in perceptual decision-making tasks after receiving social information, does the perceptual representation in the brain change along with their behavioral responses? We combined functional MRI and multivoxel pattern analysis (MVPA) to tackle this issue.
Forty-four undergraduates participated and performed a random dot motion task in the present study. Every two of them were paired as 'partners' while one performed the task inside the MRI scanner and the other performed it outside the scanner. Thus, we collected fMRI data from twenty-two participants (8 females). In the main task, dots moving to either 45° or 135° in the coherence of participants' threshold level. In each trial, an identical group of moving dots appeared twice. Once before participants provided their first answer, and once after they received social information (partner's answer). Participants also reported the main moving direction of the dots twice, both before and after they received social information. We informed participants that 'partner's answer' were answers from their partner for estimating the same moving dots, but a computer program assigned all the answers. In the control condition, the partner's answers were replaced by random answers. The logic behind this design is, if brain pattern within V1 for the first and the second stimuli are the same, then the classifier should not be able to decode these two patterns. On the other hand, if we can classify these two patterns, which would imply that there are different brain patterns in V1 for these two physically identical stimuli. In other words, social information would be able to change both behaviors and neuronal responses.
First of all, participants changed their answers to agree with partner's answers more frequently than when they changed their response to agree with random answers. This result indicates that participants did involve social information for visual perceptual decision making in this task. Furthermore, the classifier successfully classified the brain pattern of the first stimuli and that of the second stimuli when participants changed their answers to agree with partner's answers. The result suggests that social information may be able to alter people's visual perception.

Disclosures: W. Lin: None. **Y. Yotsumoto:** None.

Poster

166. Human Perception and Imagery I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 166.08/Y24

Topic: H.02. Human Cognition and Behavior

Title: Pre-stimulus alpha oscillatory mechanisms affecting objective and subjective perceptual decision making

Authors: *H. ELSHAFEI[1], L. IEMI[2], A. SHARMA[1], S. HAEGENS[1,2];
[1]Ctr. for Cognitive Neuroimaging, Donders Inst. For Brain, Cognition & Behaviour, Nijmegen, Netherlands; [2]Dept. of Neurolog. Surgery, Columbia Univ., New York, NY

Abstract: Alpha oscillations are proposed to reflect a mechanism of active inhibition that regulates cortical excitability in task (ir)relevant regions and networks, with high alpha power reflecting low excitability. According to this framework, alpha activity preceding a sensory stimulus affects its subsequent perception. However, there is no consensus regarding the nature and precise underlying mechanisms of these effects, with pre-stimulus alpha affecting objective perceptual measures (e.g., accuracy) in some studies, and subjective measures (e.g., detection bias and confidence) in others. In addition, most previous work has focused on the visual modality, while in the auditory modality results are scarce and mixed, with no work to date directly comparing perceptual effects across modalities. In a series of behavioral and MEG studies, we aimed to investigate whether pre-stimulus oscillations affect subjective or objective measures of perception, and whether these effects are comparable across visual and auditory modalities. We developed a novel paradigm in which participants are presented with either one of two alternative sensory stimuli embedded in noise. Participants have to report the identity of the stimulus (objective task) as well as their confidence (subjective task). We used spatially cued and non-cued variations of this paradigm, in both the visual and auditory modalities. Participants were more accurate and more confident when responding to noisy auditory stimuli in comparison to visual ones. In addition, in the spatially cued task, only in the visual modality did participants demonstrate lateralized alpha activity; i.e. more alpha power in the non-cued/irrelevant ipsilateral visual cortex relative to the cued/relevant contralateral visual cortex. Finally, for the non-cued experiment, binning analysis are employed using single-trial estimates of both periodic and aperiodic signals. For each bin, we calculate both objective and subjective perceptual measures, in order to compare these measures across bins, allowing us to establish whether lowest alpha power leads to more correct or confident discriminations. This work sheds light on the precise behavioral consequences of pre-stimulus alpha activity and addresses some of the inconsistencies in the literature. In addition, it provides a direct comparison between auditory and visual perception to identify modality-specific and/or supra-modal mechanisms.

Disclosures: **H. Elshafei:** None. **L. Iemi:** None. **A. Sharma:** None. **S. Haegens:** None.

Poster

166. Human Perception and Imagery I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 166.09/Y25

Topic: H.02. Human Cognition and Behavior

Support: JSPS KAKENHI Grant Number JP 19K11482

Title: Cortical structures associated with multiple object tracking performance

Authors: *K. KAMIBAYASHI, Y. NARAI, A. OSHIMA;
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Abstract: Athletic performances require quick and accurate perception and cognition of visual information. High-level athletes perform well in three-dimensional multiple object tracking (3D-MOT), which requires dynamic visual information processing, analogous to sports. Structural changes in the human brain from training and experience have been detected on magnetic resonance imaging (MRI). Hence, the purpose of the present study was to investigate whether regional variations in the brain cortex are associated with the 3D-MOT performance, using voxel-based morphometry (VBM) of the MRI data. The study included 51 adult subjects. Three-dimensional T1-weighted images of the whole brain (voxel size: 1 × 1 × 1 mm) were acquired on a 1.5-Tesla MRI scanner. Images were analyzed using the VBM toolbox in the SPM 12 software package. The images were segmented into gray matter, white matter, and cerebrospinal fluid. Spatial normalization of the images to a customized Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL) template, nonlinear modulation to preserve the relative differences in the regional volumes of the gray matter, and spatial smoothing using a Gaussian kernel were performed. In 3D-MOT, subjects were required to track 4 out of 8 spheres within a volumetric 3D cube space with virtual walls. The spheres moved in a linear direction randomly in the virtual 3D cube for 8 s and either bounced off or occluded one another. After 8 s, the spheres halted and were individually tagged with a number. The subjects pointed out the 4 spheres that were to be tracked. If the 4 targets were correctly identified, the speeds of the spheres were increased in the next trial. If the response was incorrect, the speeds in the subsequent trial were decreased. After 20 trials, each subject's threshold speed for effective perception and processing of visual information was designated as their task performance. Covariation of gray matter volume values with task performance was estimated at each voxel using the general liner model. As a result, the range of the threshold speed was 0.79 to 2.91. The whole-brain VBM analysis revealed that the gray matter volume in the areas of precuneus and visual cortex correlated positively with the 3D-MOT performance. Our data suggested that

superior visual processing ability might be related to the structural characteristics of the cortical areas implicated in visual information processing.

Disclosures: K. Kamibayashi: None. **Y. Narai:** None. **A. Oshima:** None.

Poster

166. Human Perception and Imagery I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 166.10/Y26

Topic: H.02. Human Cognition and Behavior

Support: ANR MySPACE 2015-2020 (PI Fadila Hadj-Bouziane)

Title: Social information in peripersonal space impact perceptual discrimination and internal state

Authors: *A. DUREUX, E. BLINI, L. GRANDI, C. DESOCHE, A. FARNE, F. HADJ-BOUZIANE;
IMPACT - Lyon Neurosciences Res. Ctr. - INSERM U1028, CRNS UMR 5292 - Lyon 1 Univ., Lyon, France

Abstract: In everyday life, humans and animals evolve in an environment composed of various objects with which they dynamically interact. The peripersonal space (PPS), defined as the region of space in close proximity to our body, constitutes a privileged area for the processing of external stimuli. The representation of this space is flexible, allowing us to adapt quickly and optimally to our environment, for example in order to grasp a cup of coffee or avoid sudden threats. However, our world is not only made of objects: navigating in a social world also requires a flexible adjustment according to the distance from our peers. The present study aimed to understand how the social context (i.e. the presence of another individual) modulates behavioral and physiological signatures of PPS representation. Participants were asked to discriminate male from female faces (gender discrimination task) presented at different distances (50 cm and 300 cm) in a virtual reality environment. The faces displayed different emotional expressions (happiness, anger or neutral). We measured the participants' accuracy and response times as well as their pupillary responses and heart rate. Our results show that participants are faster to discriminate faces when they are presented close to the body, even when farther ones appear bigger. Moreover, we found that participants' performances are influenced by the emotions displayed by the faces. Finally, these behavioral effects were accompanied by a modulation of participants' autonomic responses. Specifically, close faces induced an increase of the heart rate and a constriction of the pupil diameter. Altogether, these results suggest that the fastest processing classically found for objects in PPS extends to social stimuli. They also highlight physiological changes during the processing of social stimuli in the PPS.

Disclosures: **A. Dureux:** None. **E. Blini:** None. **L. Grandi:** None. **C. Desoche:** None. **A. Farne:** None. **F. Hadj-Bouziane:** None.

Poster

166. Human Perception and Imagery I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 166.11/Y27

Topic: H.02. Human Cognition and Behavior

Support: BNI ICM P09-015-F
CONICYT PFCHA/DOCTORADO BECAS CHILE/2016 - 21160388

Title: Ocular movements are involved in perceptual change in bistable stimuli

Authors: ***E. LORCA-PONCE**[1,2], D. ZENTENO[1], S. MADARIAGA[1,2], M. A. CONCHA[1,2], C. DEVIA[1,2], P. E. MALDONADO[1,2];
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Abstract: Bistable perception is a complex visual perceptual phenomenon where a visual input remains invariant, while our ensuing perception alternates between two different states. It has been proposed that eye movements increase the probability of experiencing these perceptual changes. However, the neural and behavioral mechanisms, in particular how the eye movements would be linked to the act of transiting between two perceptual states, are still unknown. We conjecture that features of the eye movements performed before the perceptual switch may impact the likelihood of perceptual alternations in bistable stimuli. We recorded perceptual switch reports, electroencephalographical activity and ocular behavior from 20 subjects who observed three bistable stimuli (plaids, moving dots, and the Necker's cube) in two conditions: free exploration and eye visual fixation. We found that perceptual changes occur in the same frequency for the free exploration and of the eye movement restriction conditions. We found that subjects exhibited stereotyped eye movement patterns for every three bistable stimuli, independent of size or whether they were static or dynamic. Moreover, the direction of the eye movement was a key feature related to the reversal events and was also present in the microsaccades patterns in the fixation condition. Finally, we found that the frequency of saccades presents time-modulation pattern, with a maximum occurring mainly between 800 - 600 ms before the reversal report. This time coincides with the stereotypical changes of powers spectrum associated with these reversals. These results suggest that ocular movements participate in neural mechanisms related to the perceptual switch, modulating the perception in an invariant visual stimulus.
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Disclosures: **E. Lorca-Ponce:** None. **D. Zenteno:** None. **S. Madariaga:** None. **M.A. Concha:** None. **C. Devia:** None. **P.E. Maldonado:** None.

Poster

166. Human Perception and Imagery I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 166.12/Y28

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01EY14681
NIH Grant 2T32EY018080-11
Carney Institute Center for Vision Research Pilot Grant
EPSCoR Grant 1632738

Title: Cross species neural correlates of visual simulation

Authors: *A. AHUJA, D. L. SHEINBERG;
Neurosci., Brown Univ., Providence, RI

Abstract: We regularly interact with moving objects in our environment. Yet, little is known about how we extrapolate the future movements of visually perceived objects. One possibility is that movements are experienced by a mental visual simulation, allowing one to internally picture an object's upcoming motion trajectory, even as the object itself remains stationary. To test whether visual simulation might be a reasonable strategy for predicting object movement, we designed a novel task in which human subjects were asked to make judgements about the trajectory of a falling ball on an obstacle-filled display. We found that subjects' behavioral metrics on this task were predicted by properties of the ball's trajectory as determined by a physics-engine driven simulation (for example, uncertainty as defined as robustness to noise). We also observed that subjects' eye movements made while attempting to ascertain the ball's trajectory bore significant spatiotemporal overlap with eye movements made while perceiving the same motion trajectory. Finally, we created a control condition by developing a convolutional neural network model for this task. We found that the model's outputs did not align with human behavior, further pointing to simulation as the likely strategy being employed (Ahuja & Sheinberg, 2019). Since completing this study, we have successfully trained a rhesus macaque monkey (GM) to perform the previously described task. We have uncovered striking behavioral parallels between GM and our human subjects, suggesting that monkeys might also be capable of engaging in this form of simulation. Notably, GM's success at the task is not accounted for by a variety of potential alternate strategies (such as low-level feature analysis of the scene). To provide additional concrete evidence, we have designed a cross-species comparative fMRI study involving both humans and macaques as they perform this task in an

MRI scanner. We thus aim to highlight the neural correlates of visual simulation, and pinpoint the role visual areas might play in facilitating simulation of motion.

Disclosures: A. Ahuja: None. **D.L. Sheinberg:** None.

Poster

166. Human Perception and Imagery I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 166.13/Y29

Topic: H.02. Human Cognition and Behavior

Title: Influence of attention on binocular information-processing in motion aftereffect investigated by frequency tagging technique

Authors: M. YAMASHITA[1], N. KOGO[2], N. NAKAJIMA[1], H. HAYAKAWA[1], ***T. AIHARA**[1]; [1]Tamagawa Univ., Tokyo, Japan; [2]Donders Inst. for Brain, Cognition and Behaviour, Radboud Univ., Nijmegen, Netherlands

Abstract: In our daily life, the visual system detects motion caused by two different sources: the wide-field motion associated with one's own movement and the local motion associated with the movement of object in the outside world. For the visual system to function properly, it must integrate such information in multiple stages of signal processing. Bottom-up visual information from both eyes is thought to be treated unconsciously in the brain and motion aftereffect has been studied in the context of bottom-up information processing in the wide-field motion. However, its modification by top-down mechanisms such as attention has not been elucidated. We investigated the influence of the attention-based top-down mechanism to the bottom-up information processing of two inputs from both eyes in the wide-field motion. The experiment was designed based on the recent finding that the motion aftereffect appears in the reverse direction of the addition of individual vectors of the translational motions given in the adaptation stimulus. Random-dot patterns moving upward and rightward were simultaneously and independently presented to the left eye and the right eye using a virtual reality headset and the characteristics of the motion aftereffect were measured. To measure the level of attention given to the two images, we added frequency-tagged “dynamic noise” to their background: sinusoidally modulated contrast of noise at 7.5Hz for the right eye and at 5.45Hz for the left eye images. The neural signals were monitored with EEG and were analyzed using Fast Fourier Transform (FFT) and Wavelet Transform (WT) during the presentation of the random dots superimposed with the dynamic noise. The responses at the tagging frequencies, their harmonics, and the intermodulation frequencies were detected by the FFT and WT analyses. The intermodulation frequency components emerge as the result of interactions between neural signals carrying different tagging frequencies and, hence, they signify neural integrations of the signals from the left and the right eyes. When attention was paid, for example, to the left eye

image, the response of the frequency used for tagging the left eye increased and the motion aftereffect sifted reflecting the amount of attention. Hence, it showed that the motion aftereffect was influenced by increasing vector of the opposite direction for the visual field motion to which attention was paid. Our results suggested that binocular information-processing is modified by attention (consciousness) through top-down feedback mechanisms.

Disclosures: M. Yamashita: None. **N. Kogo:** None. **N. Nakajima:** None. **H. Hayakawa:** None. **T. Aihara:** None.

Poster

166. Human Perception and Imagery I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 166.14/Y30

Topic: H.02. Human Cognition and Behavior

Title: Effect of inhalation of aroma of pelargonium graveolens compound essence on human electroencephalographic activity

Authors: *K. HYUN[1], G. LEE[2];
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Abstract: Pelargonium graveolens is an uncommon Pelargonium species native to the Northern Provinces of South Africa. As a flavoring, the flowers and leaves are used as edible additive and a flavoring agent. This study was carried out to investigate the response of human to aroma of Pelargonium graveolens compound (PGC) and changes in electroencephalography (EEG). A total of 30 persons were allowed to inhale the prepared PGC fragrance for 5 minutes. EEG electrodes were attached to the frontal (F3, F4), temporal (T3, T4), parietal (P3, P4) and occipital lobes (O1, O2) by the International 10 and 20 System. The results of the study showed that the EEG frequency and amplitude was decreased at all electrodes compared to the resting state after the PGC perfume exposure. The asymmetry index A2, which explains the difference in the relative activity of the left and right hemispheres in all the electrodes, also significantly changed. The highest alpha wave (7.5 to 13Hz) rate was observed at the occipital electrode before the fragrance exposure. After the scent exposure, the parietal electrode and occipital electrode increased significantly compared between PGC and before exposure. The asymmetry index A2 is shown by using the difference of the relative alpha wave(8 to 13Hz) activity of the left and right brain, and the closer to "0", the more stable. Compared with the PGC group and the placebo group, there was significant differences from 3 minutes after the perfume exposure. The inhalation of PGC aroma was found to be the result of the increase of total alpha wave and the decrease of A2 asymmetry index. These results suggest that the scent of PGC may help maintain a more stable psychological state of the subject.

Disclosures: **K. Hyun:** None. **G. lee:** None.

Poster

166. Human Perception and Imagery I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 166.15/Y31

Topic: H.02. Human Cognition and Behavior

Title: Electroencephalographic markers of body ownership illusion in virtual reality

Authors: *N. GOLDWAY[1,5], G. GUREVITCH[5,2], Y. ZAMIR[5,1], A. AZAMY[6], T. VAKNIN[1], I. GEFFEN[3], G. RAZ[1,4];
[1]Sagol Sch. of Neurosci., [2]Sch. of Psychological Sci., [3]Adi Lautman Interdisciplinary Program for Outstanding Students, [4]Steve Tisch Sch. of Film and Television, Tel Aviv Univ., Tel Aviv, Israel; [5]Sagol Brain Inst., Tel Aviv Sourasky Med. Ctr., Tel Aviv, Israel; [6]Fac. of Med. Sci., Radboud Univ., Nijmegen, Netherlands

Abstract: In recent years, the implementation of new virtual reality (VR) technologies enables to unveil the cognitive mechanisms that govern bodily self-consciousness (BSC). In order to enable reliable manipulation of BSC using VR it is crucial to maintain the feeling of immersion and presence. Unfortunately, however, common methods of assessing BSC during VR experiences rely on techniques that suffer from two main limitations; they impair the feeling of immersion and presence while overlooking the neural process underlying BSC. In order to overcome these shortcomings, we examined two electroencephalographic (EEG) components: Mu-suppression, and error positivity (Pe) in the context of non-volitional movements performed by virtual hands that participants perceived as their own.
Twenty-one healthy volunteers took part in an EEG-VR experiment that included five experimental sessions, varied by the method employed to induce virtual body-ownership illusion. Induction conditions included (1) passive synchronous visuotactile stimulation (SYN); (2) asynchronous stimulation (ASYN); (3) active hand (AH); where participants moved their hands volitionally, (4) Active cursor (AC); where the participant's hands were represented as cursors and (5) flipped hand (FH) in which the participant's virtual hands were rotated in 180°. During each session, self-report and proprioceptive drift were evaluated as well as EEG responses to nonvolitional hand movements that occurred in a designated task implemented in each experimental session.
Results revealed a main effect for condition for both the self-report and the drift test. Mu suppression was stronger during SYN and AH when compared to the rest of the conditions and Pewas significantly higher when the hand representation was altered (i.e, in the AC and FH conditions). Interestingly, Pe and proprioceptive drift were correlated.
We conclude that Mu suppression may be used to assess the virtual hand illusion strength and

that Pe can possibly be affected by “semantic violation” of body shame. We believe that both of these EEG components may be used as implicit indices for the sense of embodiment, and thus significantly contributing to the field of investigating BSC using VR.

Disclosures: **N. Goldway:** None. **G. Gurevitch:** None. **Y. Zamir:** None. **A. Azamy:** None. **T. Vaknin:** None. **I. Geffen:** None. **G. Raz:** None.

Poster

166. Human Perception and Imagery I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 166.16/Y32

Topic: H.02. Human Cognition and Behavior

Title: Correspondence competition in motion brings to implicit time perception

Authors: *M. GIRELLI;
Univ. of Verona, Verona, Italy

Abstract: Correspondence strength governs our perception of moving objects as simple as bright lines on a dark background. Though it is derived from the affinities between elements such as length and orientation and competitions among them such as split and fusion. The time separation of the two frames is also a crucial parameter to obtain a Beta motion (apparent motion) and it influences the correspondence strength. By manipulating the spatial distance between the elements the time separation is implicitly judged of a different length, although it remains constant, i.e. large distances lead to longer judged times, small distances lead to shorter judged times (Kappa effect). Taking advantage of these spatial and temporal interactions between physical parameters and cognitive operations, I tested observers performing in a visual discrimination task where they were asked to judge which bright bar of a first frame fused or split into a bright bar in a successive frame separated in time from the first one by a fixed ISI. Crucially I also manipulated another physical parameter which lead to different affinities, namely the orientation of the bright bars with respect to the direction of motion which could be collinear or orthogonal to it. The results showed that the orientation of the visual elements generating the motion, modulated the competitions of both split or fusion between the affinities. The study crucially also showed that the modulations generated by the orientation of the visual elements on the perceived direction of motion, led to a time illusion according to the Kappa effect. In order to better understand the correspondence problem in motion, therefore, we must take in account not only the affinities of spatial features and their configurations in a fixed temporal interval between two successive frames but also the illusory time perception generated by these particular spatial configurations which modulates the perception of visual motion.

Disclosures: **M. Girelli:** None.

Poster

166. Human Perception and Imagery I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 166.17/Y33

Topic: H.02. Human Cognition and Behavior

Support: National Science Foundation Graduate Research Fellowship under Grant No. DGE – 1147470
Training grant from the National Institutes of Health under award number T32MH020016

Title: Hysteresis in the transition between fusion and rivalry can be reversed as predicted by a tristable zone

Authors: *G. RIESEN[1], A. M. NORCIA[2], J. L. GARDNER[3];
[1]Stanford Univ. Dept. of Neurol. and Neurolog. Sci., Stanford, CA; [2]Dept. of Psychology, [3]Psychology, Stanford Univ., Stanford, CA

Abstract: Previous work has shown that the transitions between fusion and rivalry show hysteresis: as stimuli are made increasingly discrepant, fusion breaks at a different point than it is regained when stimuli are made less discrepant, suggesting that there is a variable, stimulus history-dependent threshold between fusion and rivalry (Buckthought et al, 2008). The existence of such a threshold is challenged by our recent finding that fusion and rivalrous states can coexist in some static stimuli within a 'tristable' zone. Here we reproduced the apparent hysteresis found in a prior study and demonstrated that its sign can be reversed when stimulus discrepancy is slowly changed so that more time is spent passing through the tristable zone. We first mapped the range of orientation disparities for small dichoptic gratings (0°, 90°, and 20°- 40° in 2° increments) that resulted in tristable percepts. We then rotated the stimuli (in or out from 10°-50° of disparity at 0.25 or 2 degrees per second (dps)), to increase or decrease orientation disparity in separate trials. We then located the first transitions from fusion to rivalry and rivalry to fusion, respectively. 14 observers reported their percepts as left/right-eye dominant, fused or uncertain. Three subjects were excluded due to incomplete or outlying responses. As in our earlier findings, non-rotating stimuli with ~25°-35° of orientation disparity were tristable. For rotating stimuli, the first transitions between fusion and rivalry were 5.6 ± 1.2° of disparity apart in the 2 dps condition, reproducing the apparent hysteresis seen previously. At 0.25 dps, 'reverse hysteresis' appeared; transitions occurred earlier than expected with a total difference of -7.0 ± 1.4° of disparity.
By considering the existence of a tristable zone, we can account for earlier findings ascribed to hysteresis in a single threshold, and we can also predict the reversal of the hysteresis effect for slowly rotating stimuli. This reversal and the existence of the tristable zone itself provide

important constraints for future models of binocular vision, as well as existing models which exhibit threshold hysteresis for rotating stimuli.

Disclosures: G. Riesen: None. **A.M. Norcia:** None. **J.L. Gardner:** None.

Poster

166. Human Perception and Imagery I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 166.18/Y34

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant ZIA MH002920-08
Canada First Research Excellence Fund, Vision: Science to Applications

Title: Scene-selective brain regions show a posterior-anterior gradient for representing perceptual and conceptual information

Authors: *N. KHAN[1], C. MULLIN[1], A. MARTIN[2], W. D. STEVENS[1];
[1]Psychology, York Univ., Toronto, ON, Canada; [2]Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Humans are able to rapidly and accurately identify real-world scenes, despite high complexity and variability. This requires rapid integration of incoming perceptual information with prior conceptual knowledge. However, little is known about how and where in the brain the neural signals associated with perceptual and conceptual processes are integrated in scene identification. Using univariate fMRI analysis, we previously demonstrated that scene-selective regions, including the parahippocampal place area (PPA) and occipital place area (OPA), were activated by both pictures of real-world scenes (scene-pictures) and words denoting scenes (scene-words; e.g., farm, skyline, lake, courtyard). Importantly, within these regions of interest (ROIs), there was a consistent posterior-to-anterior dissociation, such that the posterior aspect of these ROIs responded most strongly to scene-pictures, the mid aspect responded to both scene-pictures and scene-words, and the anterior aspect responded most strongly to scene-words. Here, we sought to further assess the nature of information represented across these category-specific regions – i.e., the extent to which they represent perceptual and/or conceptual information – using the multivoxel pattern analysis technique representational similarity analysis (RSA). RSA was conducted within the sub-regions of these scene-selective ROIs that were selective for scene-pictures, scene-words, or both. Results revealed a perceptual-to-conceptual representational gradient along the posterior-anterior axis of each ROI, such that perceptual similarity among scene-pictures was most strongly represented in the posterior aspect of the ROIs, while conceptual similarity among scene-words was most strongly represented in the anterior aspect. While the nature of the representations in scene-selective cortex has been debated (e.g., categorical, contextual, spatial, navigable, etc.), our results demonstrate that scene-

selective regions are functionally heterogeneous, showing a perceptual-to-conceptual dissociation along a posterior-to-anterior gradient. These findings suggest that integration of perceptual and conceptual information may occur across multiple distributed category-related cortical regions that represent category-relevant properties.

Disclosures: **N. Khan:** None. **C. Mullin:** None. **A. Martin:** None. **W.D. Stevens:** None.

Poster

167. Human Long-Term Memory: Medial Temporal Lobe I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 167.01/Y35

Topic: H.02. Human Cognition and Behavior

Support: St Hugh's College University of Oxford Middle Common Room Award

Title: A hippocampal subfield model of category learning

Authors: J. SUCEVIC[1], *A. C. SCHAPIRO[2];
[1]Univ. of Oxford, Oxford, United Kingdom; [2]Univ. of Pennsylvania, Philadelphia, PA

Abstract: According to the Complementary Learning Systems theory, the hippocampus is responsible for encoding memories of individual episodes, while the cortex extracts regularities across episodes (McClelland, McNaughton, & O'Reilly, 1995). Emerging data suggests that the hippocampus itself is capable of rapid statistical learning across episodes, and we have previously proposed that there may be complementary learning systems within the hippocampus supporting its episodic and statistical learning functions (Schapiro et al., 2017). This work has focused on statistical learning of temporal sequences, but the ability to extract regularities over multiple instances is also essential for novel category learning, and neuroimaging studies provide evidence for the involvement of the hippocampus in this form of learning as well (e.g., Zeithamova et al., 2008). The present modeling work investigates the role of the hippocampus and its subfields in category learning. We performed simulations of several category learning tasks using a neural network model of the hippocampus. The model consists of dentate gyrus (DG), CA3, and CA1 hippocampal subfields which map input from superficial to deep layers of the entorhinal cortex (EC). There is a trisynaptic pathway (TSP) connecting DG and CA1 via CA3 and a monosynaptic pathway (MSP) connecting EC directly with CA1. The former is largely responsible for pattern separation due to high inhibition and sparse connectivity; the second allows for more overlapping representations. We exposed the model to different types of categories, such as family-resemblance and probabilistic categories, and then assessed the model's ability to provide category labels for old and new category exemplars. The model was able to learn categories and generalize to novel category instances. The simulations highlighted the role of specific hippocampal subfields in category learning: the MSP was critical for

extracting the regularities underlying category structure, consistent with its use of overlapping representations and its previously described role in temporal statistical learning. Our work thus characterizes a potential mechanism and specific anatomical substrate for the involvement of the hippocampus in novel category learning.

Disclosures: J. Sucevic: None. **A.C. Schapiro:** None.

Poster

167. Human Long-Term Memory: Medial Temporal Lobe I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 167.02/Y36

Topic: H.02. Human Cognition and Behavior

Title: Grid-like hexadirectional modulation of theta oscillations in human ventromedial prefrontal cortex

Authors: *D. CHEN[1], L. KUNZ[2], H. ZHANG[3], N. AXMACHER[4], L. WANG[1];
[1]Inst. of Psychology, Chinese Acad. of Sci., Beijing, China; [2]Dept. of Epileptology, Univ. of Freiburg, Freiburg, Germany; [3]Ruhr University, Bochum, Bochum, Germany; [4]Inst. of Cognitive Neuroscience, Ruhr Univ. Bochum, Bochum, Germany

Abstract: Grid cells and theta oscillations are fundamental components of the brain's navigation system and are believed to support a wide range of spatial behaviors (Hafting et al., Nature, 2005; Buzsaki & Moser, Nature Neuroscience, 2013). Using human intracranial EEG, recent studies have demonstrated that entorhinal theta oscillations exhibit hexadirectional modulation by movement direction during virtual navigation, which is presumably linked to key properties of grid cells (Chen et al., Current Biology, 2018; Maidenbaum et al., PNAS, 2018). However, it remains elusive how this grid-like hexadirectional modulation of theta power is distributed across the whole brain. To answer this question, we performed intracranial EEG recordings in medically refractory epilepsy patients (n = 18; 1,345 electrodes in total) during an object-location memory task in a virtual environment. We performed an electrode-wise permutation procedure to detect putative electrodes that carried grid-like representations by comparing the observed strength of hexadirectional modulation against surrogate distributions. Electrodes were considered significant at an alpha level of $p < 0.05$, and binomial tests were employed to determine whether the percentages of significant electrodes within each region were higher than chance level (5%). Our results showed that 11% and 13% of the electrodes exhibiting grid-like hexadirectional modulation of theta power were found in the entorhinal cortex and ventral medial prefrontal cortex (vmPFC)(binomial tests, both $p < 0.02$). These results are consistent with human fMRI studies, which revealed grid-like representations in entorhinal cortex and medial prefrontal cortex (Doeller et al., Nature, 2010; Constantinescu et al., Science, 2016). To verify the specificity of hexadirectional modulation (i.e., six-fold rotational symmetry), we also

examined four-, five-, seven- or eight-fold rotational symmetry and did not find significant numbers of electrodes exhibiting those types of symmetry. Taken together, the current study suggests that grid-like hexadirectional modulation of theta power is distributed across a network of brain regions including both entorhinal cortex and vmPFC.

Disclosures: D. Chen: None. **L. Kunz:** None. **H. Zhang:** None. **N. Axmacher:** None. **L. Wang:** None.

Poster

167. Human Long-Term Memory: Medial Temporal Lobe I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 167.03/Y37

Topic: H.02. Human Cognition and Behavior

Support: NRF-2018R1A4A1025616

Title: Differential effect of human hippocampal stimulation on memory enhancement with theta rhythm

Authors: *S. JUN[1], S. LEE[3], J. KIM[2], C. CHUNG[1];
[1]Brain and Cognitive Sci., [2]Res. Inst. of Basic Sci., Seoul Natl. Univ., Seoul, Korea, Republic of; [3]Dept. of Bio and Brain Engin., Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: How the direct hippocampal stimulation affects episodic memory has not been well characterized. To characterize it, we applied 50 Hz electrical stimulation to the hippocampus during the encoding phases of each task, and recorded intracranial EEG (iEEG) from 10 epilepsy patients who performed two different verbal memory tasks, including paired associative memory and single item memory. Hippocampal stimulation modulated memory performance in a task-dependent manner: associative memory performance was enhanced, while item memory performance was impaired. In addition, subjects with poorer baseline memory improved much more by stimulation. On iEEG from the hippocampus during non-stimulation encoding blocks, the associative memory task elicited stronger theta oscillations than item memory. Also during retrieval, stimulation-induced memory enhancement was linked to increased theta power. These results suggest that: 1) hippocampal stimulation enhances associative memory but not item memory because it engages greater hippocampal theta activity and that 2) increased hippocampal theta may be a neural mechanism for memory enhancement.

Disclosures: S. Jun: None. **S. Lee:** None. **J. Kim:** None. **C. Chung:** None.

Poster

167. Human Long-Term Memory: Medial Temporal Lobe I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 167.04/Y38

Topic: H.02. Human Cognition and Behavior

Title: Spatiotemporal spiking patterns in human medial temporal lobe support successful episodic encoding and recognition

Authors: *Y. REN, T. I. BROWN;
Psychology, Georgia Inst. of Technol., Atlanta, GA

Abstract: The medial temporal lobe is well known to support memory encoding processes and successful episodic remembering. However, due to methodological constraints, very few studies have investigated medial temporal lobe (MTL) encoding-retrieval dynamics in humans at the level of single-cell signals. In this study, we reanalyzed intracranial electroencephalography spiking data from 1576 human hippocampal and amygdala neurons during a recognition memory task. The previously published analysis (Faraut, et.al., 2018) discovered 118 neurons that individually fired differently for correct recognition of familiar image stimuli or correct rejection of new stimuli. We hypothesized that MTL neurons carrying a veracious memory signal should spike differently for remembered and forgotten information. Moreover, this mnemonic information should emerge from neural firing patterns and arise from both successful encoding and retrieval processes. To test these predictions, we adopted a supervised learning memory pattern decoding approach. In our analysis, we included both correct and incorrect trials to test if neurons spike patterns can predict memory success. Our results showed that 497 neurons carried information predicting memory accuracy in at least one post-stimulus time window during encoding or retrieval. These neurons were equally distributed across the hippocampus and amygdala. The model could predict memory performance with high accuracy. Importantly, the information about memory success was represented in both decreasing and increasing spiking patterns across different post-stimulus time windows. This provided evidence for our prediction that neurons could show complex and divergent spiking profiles that collectively predict memory responses. We found memory-related clustering in the neuron spike profiles - neurons that showed similar temporal spiking pattern were also predictive during the same time window. This supports the prediction that neurons inside the hippocampus and amygdala support memory through functional connectivity and grouping. Collectively, our results provide new insight into how encoding and retrieving episodes arises from complex spatiotemporal firing patterns in the human MTL.

Disclosures: Y. Ren: None. **T.I. Brown:** None.

Poster

167. Human Long-Term Memory: Medial Temporal Lobe I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 167.05/Y39

Topic: H.02. Human Cognition and Behavior

Support: NIMH R01 MH100121
NIH R01 MH096914-05

Title: Developmental differences in sensitivity to naturalistic event boundaries

Authors: *A. DUTCHER[1], A. R. PRESTON[2];
[1]Neurosci., [2]The Univ. of Texas at Austin, Austin, TX

Abstract: To make sense of the world, it is necessary to segment our ongoing stream of experience into unique events i.e. event segmentation. One approach to studying event segmentation uses functional magnetic resonance imaging (fMRI) responses while participants view naturalistic stimuli (e.g. movies) to identify event boundaries from continuous input. Previous work has implemented both data driven computational models and subjective ratings to identify shifts in neural response that correspond to event boundaries during moving viewing. This work indicates that both parietal cortex and hippocampus play important roles in segmenting naturalistic input into discrete episodes. Given the protracted development of these structures into adulthood, here we test whether the neural mechanisms that support event segmentation demonstrate age-related differences. In particular, hippocampus-mediated novelty and pattern differentiation processes are thought to develop through middle childhood; these mechanisms are hypothesized to be critical in detecting and representing event boundaries. Using an open-source dataset (Richardson et al. 2018) in a developmental sample (children 3-12 years and adults 25-30 years), we tested whether neural response in hippocampus shows the same sensitivity to event boundaries in children and adults. Neural event boundaries were defined using a data-driven approach that detected when the neural pattern of activation shifted in parietal cortex during movie viewing. We then further classified these event boundaries as either aligned or misaligned to behaviorally-defined event boundaries derived from an independent sample of participants. We found that adults and children 5 years and older showed increased hippocampal activation at event boundaries that were aligned across both the data-driven and behaviorally-defined methods. In contrast, children 3-4 years failed to show increased hippocampal engagement at event boundaries. These results suggest that neural event segmentation mechanisms are still developing in early childhood and may underlie the differences in episodic memory performance observed at this developmental stage. In particular, immature event segmentation during early childhood may lead to less differentiation among memories, making it more difficult to remember detailed information about individual episodes.

Richardson, H., Lisandrelli, G., Riobueno-Naylor, A., & Saxe, R. (2018). Development of the social brain from age three to twelve years. *Nature Communications*, *9*(1), 1-12.

Disclosures: A. Dutcher: None. **A.R. Preston:** None.

Poster

167. Human Long-Term Memory: Medial Temporal Lobe I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 167.06/Y40

Topic: H.02. Human Cognition and Behavior

Support: NSF CAREER Award BCS-1844241

Title: Cholinergic modulation enhances hippocampally dependent spatial relational attention

Authors: *N. RUIZ, M. ALY;
Psychology, Columbia Univ., New York, NY

Abstract: Attention stabilizes representations in the human hippocampus (Aly & Turk-Browne, 2016a,b). We explore a mechanism by which this might occur: cholinergic modulation. Acetylcholine enhances afferent input from entorhinal cortex and suppresses recurrent connections in CA3 (Newman et al., 2012); this biases hippocampal processing toward environmental input, as must occur for externally-oriented attention. We examined cholinergic modulation on a modified version of a task we previously used to demonstrate hippocampal representations of attentional states (Aly & Turk-Browne, 2016a,b). On each trial, participants viewed two images (rooms with paintings). On 'room relational' trials, they judged whether the rooms had the same spatial layout from a different perspective. On 'art relational' trials, they judged whether the paintings could have been painted by the same artist. 'Control' trials had no demands on relational processing: participants simply had to detect identical paintings or rooms. We predicted that cholinergic modulation would enhance room relational attention, given our past findings that hippocampal representations correlated with behavior on this task. Cigarette smokers came in for two sessions: once after they had abstained from nicotine for 12 hours, and once after they had just ingested nicotine. Nicotine enhanced performance on room relational trials, and had no effect on the other tasks. If nicotine enhances room relational attention via the hippocampus, performance on this task should be selectively impaired following hippocampal lesions. This was confirmed in a group of patients with hippocampal damage. These results suggest that cholinergic modulation enhances hippocampally-dependent spatial relational attention, perhaps by sharpening input from entorhinal cortex.

Disclosures: N. Ruiz: None. **M. Aly:** None.

Poster

167. Human Long-Term Memory: Medial Temporal Lobe I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 167.07/Y41

Topic: H.02. Human Cognition and Behavior

Support: Wellcome principal research fellowship (202805/Z/16/Z)
ERC advanced grant NEUROMEM

Title: Are grid-like codes used to remember long lists of words?

Authors: *A. O. CONSTANTINESCU, E. PATEL, J. BISBY, A. CASTEGNARO, N. BURGESS;
UCL Inst. of Cognitive Neurosci., London, United Kingdom

Abstract: Spatial and non-spatial memories are organized into 2-d cognitive maps using a grid cell-like code. Here we ask whether remembering long lists of words can also make use of this mechanism. We trained human participants with the 'memory palace' technique, a well-known and efficient mnemonic strategy, using the same square room containing 36 unique landmarks presented in virtual reality (VR) for all participants. We then scanned their brains with functional magnetic resonance imaging (fMRI) as they learned to remember two sequences of 36 words in specific orders, by associating each of them with unique landmarks along two well-known routes through the room, and as they navigated between randomly scattered landmarks or words. We found that the fMRI activity in the entorhinal and medial prefrontal cortices when learning the lists along the two routes could be predicted from the grid angle alignment with one of the room walls during random navigation in VR. In these brain regions, we also found a repetition suppression effect as a function of distance: the suppression effect was stronger between words corresponding to locations that were closer in space. Our results suggest that grid-like representations can support memory for long lists of words by taking advantage of the spatial structure of routes embedded within a cognitive map.

Disclosures: A.O. Constantinescu: None. **E. Patel:** None. **J. Bisby:** None. **A. Castegnaro:** None. **N. Burgess:** None.

Poster

167. Human Long-Term Memory: Medial Temporal Lobe I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 167.08/Y42

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R21MH115366
NIH Grant UL1TR001422
NIH Grant T32NS047987

Title: Hippocampal theta coordinates memory processing during visual exploration

Authors: *J. E. KRAGEL[1], S. VANHAERENTS[2], J. W. TEMPLER[2], S. SCHUELE[2], J. M. ROSENOW[3], A. S. NILAKANTAN[1], D. J. BRIDGE[1];
[1]Med. Social Sci., [2]Neurol., [3]Neurolog. Surgery, Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: The hippocampus supports memory encoding and retrieval, with distinct phases of theta oscillations modulating the amplitude of gamma-band activity during each process. Encoding and retrieval operations dynamically interact over short timescales, especially when sensory information conflicts with memory. The ability to link hippocampal dynamics to specific memory-guided behaviors has been limited by experiments that lack the temporal resolution to segregate when encoding and retrieval occur. To resolve this issue, we simultaneously tracked eye movements and direct hippocampal recordings while neurosurgical patients performed a spatial memory task. Novelty-driven fixations increased phase-locking to the theta rhythm, which predicted successful memory performance. Theta to gamma phase amplitude coupling increased during these viewing behaviors and predicted forgetting of conflicting memories. In contrast, theta phase-locking preceded fixations initiated by memory retrieval, indicating that the hippocampus coordinates memory-guided eye movements. These findings suggest that theta oscillations in the hippocampus support learning through two interleaved processes: strengthening the encoding of novel information and guiding exploration based on prior experience.

Disclosures: J.E. Kragel: None. **S. VanHaerents:** None. **J.W. Templer:** None. **S. Schuele:** None. **J.M. Rosenow:** None. **A.S. Nilakantan:** None. **D.J. Bridge:** None.

Poster

167. Human Long-Term Memory: Medial Temporal Lobe I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 167.09/Y43

Topic: H.02. Human Cognition and Behavior

Title: Hippocampal spatial memory retrieval effects may be less localized than encoding effects

Authors: *H. A. FRITCH, S. D. SLOTNICK;
Boston Col., Chestnut Hill, MA

Abstract: There are multiple hypotheses regarding specialization of function along the long axis of the hippocampus. One hypothesis is that the anterior hippocampus and posterior hippocampus are associated with memory encoding and memory retrieval, respectively, while another hypothesis is that the anterior hippocampus and posterior hippocampus are associated with other cognitive processes and spatial memory, respectively. The current fMRI study investigated these two hypotheses using a spatial memory task to determine whether patterns of activity in the anterior and posterior hippocampus are associated with spatial memory encoding and spatial memory retrieval. During the encoding phase, participants were instructed to remember the location of abstract shapes that were sequentially presented in each quadrant of the visual field. During the retrieval phase, the same shapes from encoding were presented at fixation and participants identified the quadrant in which the shape had previously appeared. Multi-voxel pattern analysis (MVPA) was used to determine whether patterns of activity in the anterior and posterior hippocampus were associated with the retrieval of shapes from each visual field quadrant. For each retrieval quadrant, patterns of activity in the anterior and posterior hippocampus were split in half by run. The patterns of activity from half of the runs were then used to classify the patterns from the other half of the runs. A previous MVPA of the encoding data revealed that patterns of activity in the anterior hippocampus but not posterior hippocampus could classify encoding quadrants at above-chance levels. In the current analysis of the retrieval data, although MVPA classification accuracy was not significantly greater than chance in either the anterior or posterior hippocampus, there was a significant correlation between each participant's retrieval MVPA accuracy and behavioral memory accuracy in both regions ($r(14) = .66$, $R^2 = .43$, $p < .01$ for the anterior hippocampus; $r(14) = .74$, $R^2 = .55$, $p < .001$ for the posterior hippocampus), which suggests that MVPA was more accurate for participants with better memory retrieval. These results, in contrast to the significant classification accuracy in the anterior but not posterior hippocampus for encoding patterns, suggest that retrieval effects in the hippocampus may be less localized than encoding effects, possibly due to re-encoding during the retrieval phase.

Disclosures: H.A. Fritch: None. **S.D. Slotnick:** None.

Poster

167. Human Long-Term Memory: Medial Temporal Lobe I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 167.10/Y44

Topic: H.02. Human Cognition and Behavior

Support: ERC 819814 - RememberEx

Title: Connectivity dependent prediction of DBS induced memory improvement

Authors: *S. TREU[1], J. A. BARCIA[2], A. BIERBRAUER[4], L. KUNZ[5], C. NOMBELA-OTERO[2], N. LI[6], A. HORN[6], B. RENESES-PRIETO[3], N. AXMACHER[4], B. A. STRANGE[1]; [1]Lab. for Clin. Neurosci., Univ. Politecnica De Madrid, Pozuelo DE Alarcon, Spain; [2]Dept. of Neurosurgery, Hosp. Cl. San Carlos, [3]Dept. of Psychiatry, Hosp. Cl. San Carlos, Univ. Complutense de Madrid, Madrid, Spain; [4]Inst. of Cognitive Neurosci. - Fac. of Psychology, Ruhr Univ. Bochum, Bochum, Germany; [5]Dept. of Epileptology, Univ. of Freiburg, Freiburg, Germany; [6]Movement Disorders & Neuromodulation Unit, Dept. for Neurol., Charité Univ. Med. Berlin, Berlin, Germany

Abstract: There is cross-species evidence from animal models and human neuroimaging studies for a role for the nucleus accumbens (Nac) in modulating episodic memory. Deep-brain stimulation (DBS) of the rat Nac with simultaneous functional magnetic resonance imaging has been shown to evoke hemodynamic responses in the entorhinal cortex and the hippocampus, two structures widely associated with memory. In humans, Nac-DBS has become a common target for treating psychiatric diseases and cognitive improvements have been observed in these patients as well. To elucidate this beneficial effect, we tested 7 patients suffering from treatment-resistant obsessive-compulsive disorder (OCD) in a spatial navigation task. In each trial, they had to move to a target object within a virtual environment and then return to their start position. In two out of 6 experimental sessions, NAc-DBS was applied using standard clinical settings (130 Hz, 3.5 V, pulse-width 60µs). Patients were blind about the stimulation order. Recall accuracy was measured as the distance between the chosen and the correct location. We observed significant improvement during sessions of active stimulation. After precise postoperative electrode localization, connectivity seeding from each patient's volume of tissue activated (VTA) was calculated based on a normative connectome and correlated with stimulation-induced memory benefits. In a leave-one-out design, this optimal connectivity profile was subsequently predictive of patients' increase in performance. Plotting those fiber tracts, which were positively discriminative of memory improvement, supports previous observations of a structural connection of the Nac to the hippocampus via both the fornix and a ventral pathway. Given its relative preservation in dementia, the Nac might thus have the potential of a possible DBS site for treating memory-related disorders, by indirectly modulating medial-

temporal memory-related structures. Our findings further stress the value of the preoperative assessment of connectivity profiles to guide the targeting of DBS surgery.

Disclosures: S. Treu: None. **J.A. Barcia:** None. **A. Bierbrauer:** None. **L. Kunz:** None. **C. Nombela-Otero:** None. **N. Li:** None. **A. Horn:** None. **B. Reneses-Prieto:** None. **N. Axmacher:** None. **B.A. Strange:** None.

Poster

167. Human Long-Term Memory: Medial Temporal Lobe I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 167.11/Z1

Topic: H.02. Human Cognition and Behavior

Support: SFB 874, DFG

Title: A novel account of recognition memory based on generic memory storage and retrieval

Authors: *O. HAKOBYAN, S. CHENG;
Inst. for Neural Computation, Ruhr Univ. Bochum, Bochum, Germany

Abstract: Recognition memory refers to the ability to distinguish novel from previously encountered information. Mounting evidence suggests that there is much diversity in recognition performance and its underlying neural structures. The well-established, yet controversial, dual-process theory of recognition memory posits that there are two qualitatively different processes for recognition judgments. In particular, perirhinal cortex is associated with the vague feeling of familiarity, while hippocampus underlies recollection, i.e. the retrieval of specific details surrounding the previous encounter. Operationally, the separate contributions of perirhinal cortex and hippocampus to recognition memory are measured by the curvilinearity and the y-intercept of the receiver operating characteristic (ROC) curve, respectively. Many existing computational models of recognition memory follow the dual-process framework by implementing two qualitatively different memory operations for the perirhinal and hippocampal modules.
Here, we propose an alternative account, which is both parsimonious and in agreement with a wide range of empirical evidence. Our crucial hypothesis is that the differences between the perirhinal and hippocampal contributions to recognition memory arise from the quantitative rather than qualitative differences between these memory modules. Specifically, we assume that both memory modules engage in generic memory storage and retrieval and that recognition judgment is a decision process that evaluates the similarity between the retrieved pattern and the cue. Our results show that the features of the ROC-curve need not arise from two different processes as suggested by dual-process models. In fact, both can be generated by a single simple memory module and the relative expression of the two features depends on the parameters of the memory module, such as pattern separation and robustness to noise. We can therefore use the

same memory model with two different parameter settings to obtain modules that apparently exhibit very different recognition performance. In its current form, the model accounts for the influence of multiple factors on recognition performance and ROC-shape, such as input statistics, lesions, list length and retention time.

Disclosures: O. Hakobyan: None. **S. Cheng:** None.

Poster

167. Human Long-Term Memory: Medial Temporal Lobe I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 167.12/Z2

Topic: H.02. Human Cognition and Behavior

Support: U01NS103792
R01MH110831
Simons Collaboration on the Global Brain

Title: Task-dependent population-level representations of choice and memory in human medial frontal cortex

Authors: *J. MINXHA[1], A. MAMELAK[3], R. ADOLPHS[2], U. RUTISHAUSER[4];
[1]Caltech, Los Angeles, CA; [2]Caltech, Pasadena, CA; [3]Neurosurg., Cedars Sinai Medial Ctr., Los Angeles, CA; [4]Dept. of Neurosurg., Cedars-Sinai Med. Ctr., Los Angeles, CA

Abstract: Humans are capable of flexible, context-dependent behavior, but the nature of the underlying neural representations remains poorly understood. Most studies of context-dependent behavior in nonhuman primates have focused on task-switching paradigms where the subject attends to different sensory features of a stimulus to produce the relevant choice on a given trial. Here, we focus on choices to identical stimuli that instead depend on switching between two types of an *internal representation*: (1) image categorization (requiring high-level semantic knowledge), and, (2) recognition memory (requiring declarative memory). Our goal in this study was to evaluate how the neural representations of the relevant variables (image category, familiarity, and choice) are affected by the task the subject performs on a given trial. We recorded from 663 neurons in amygdala and hippocampus (MTL), and 767 neurons in dorsal anterior cingulate cortex and pre-supplementary motor area (MFC) in thirteen neurosurgical epilepsy patients implanted with depth electrodes for localization of focal seizures. Subjects were shown single images of objects from 4 categories and asked to respond to one of the above two questions (in blocks of 40 trials). Subjects gave yes/no responses with one of two types of choice actions: button press or a saccade. Population-level analyses were able to decode both familiarity (i.e. new vs. old) and image category in both the MFC (**image category:** accuracy = 60%, $p<0.001$, chance = 25%; **new/old:** accuracy = 82%, $p<0.001$, chance = 50%) and in the MTL

(i**mage category:** accuracy = 100%, p<0.001, chance = 25%; **new/old:** accuracy = 79%, p<0.001, chance = 50%). The information available at the population level generalized across conditions in the MTL (**image category could be decoded even during the memory task**: 100%, p<0.001, chance = 25%; **new/old could be decoded even in the categorization task**: 78%, p<0.001, chance = 50%). In contrast, the representations for both variables were strongly task-dependent in the MFC (**image category decoded during memory task**: 53%, p<0.001, chance = 25%; **new/old decoded during categorization**: 64%, n.s., chance = 50%). Decoding of choice in the MFC reveals a similar task-dependent pattern, generalizing across effector (accuracy = 84.5%, p<0.001, chance = 50%) but not task (accuracy = 46%, n.s., chance = 50%). Taken together, these results argue that MTL representations continue to learn about the stimuli independent of the task, whereas MFC representations dynamically represent the current task demands and the variables that are necessary to carry out that task.

Disclosures: J. Minxha: None. **A. Mamelak:** None. **R. Adolphs:** None. **U. Rutishauser:** None.

Poster

167. Human Long-Term Memory: Medial Temporal Lobe I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 167.13/DP12/Z3

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: H.02. Human Cognition and Behavior

Support: Marcus and Amalia Wallenberg Foundation
Stanford Center for Cognitive and Neurobiological Imaging

Title: Building integrated and structured cortical and medial temporal lobe representations during goal-directed navigation

Authors: *C. FERNANDEZ, J. JIANG, A. D. WAGNER;
Stanford Univ., Stanford, CA

Abstract: Interactions between the medial temporal lobe and neocortex allow us to both recall specific episodes from the past and abstract relationships across experiences when memories share common elements. Over learning, dynamic memory processes integrate new experiences with existing memory representations, building structured knowledge about the world. As the emergence of structured knowledge is crucial for planning and decision-making, delineation of the neural and psychological processes underlying integration and abstraction will advance understanding of how memory guides behavior. Towards this goal, we developed an experimental paradigm that leverages immersive virtual navigation, fMRI, and neural pattern

similarity analyses to (a) investigate how memory representations evolve across learning in complex environments and (b) examine the ways in which the structure of experience links and transforms these representations. We assayed memory representations across a three-day period during which participants (n=20) learned to navigate a virtual environment that included goal locations and proximal landmarks. Participants first learned to navigate between cued-goal locations on three distinct paths in the virtual environment (within-context navigation). The separately learned paths were then connected, and participants learned to navigate across the paths to reach cued goals (across-context navigation). To assay learning of the goal locations, we computed the path efficiency for each trial, quantifying the extent to which participants navigated to goal locations via the shortest route. Findings from these analyses revealed that all participants demonstrated knowledge of within- and across-context spatial relationships, despite variability in both the rate and overall magnitude of learning between individuals. To characterize experience-driven changes in memory representations across learning, participants completed an fMRI scan each day while viewing images of the landmarks and goals found within the virtual environment. We leveraged neural pattern similarity analyses to quantify the ways in which experienced trajectories through virtual space transform the similarity between these features (i.e., locations and goals) of the environment. Initial findings reveal that pattern similarity (at both the stimulus and category-level) changes over the three days of goal-directed navigation, reflecting mnemonic integration processes that facilitate the emergence of structured knowledge.

Disclosures: C. Fernandez: None. **J. Jiang:** None. **A.D. Wagner:** None.

Poster

167. Human Long-Term Memory: Medial Temporal Lobe I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 167.14/Z4

Topic: H.02. Human Cognition and Behavior

Support: NIA RF1AG039103

Title: Hippocampal BOLD recollection effects predict longitudinal change in memory performance in healthy older adults

Authors: *M. A. DE CHASTELAINE, M. HOU, M. D. RUGG;
Univ. of Texas At Dallas, Richardson, TX

Abstract: We previously reported a positive relationship between recollection-related BOLD activity in the hippocampus and memory performance across 136 young, middle-aged and older adults. Here, we investigated the relationship between these hippocampal ‘recollection effects’ and longitudinal change in memory scores (over 3 years) in that older adult sample (n=53;

female=27; age range=63-76 yrs; mean (sd) age= 68 (3.6) yrs). Participants completed a neuropsychological test battery (Session 1) which they repeated a month (Session 2) and 3 years (Session 3) later. Functional scans were acquired during an associative recognition test at Session 1 following a study phase in which word pairs were visually presented in the context of an elaborative encoding task. Test items comprised studied, rearranged (items studied on different trials) and new pairs. BOLD recollection effects were operationalized as greater activity for studied test pairs correctly identified as studied relative to those incorrectly identified as rearranged. Neuropsychological test performance across sessions was reduced to four constructs after applying principal components analysis to a larger older adult sample (n=69), all of whom were tested at Session 1. We focused on the latent construct that received heavy loadings on tests of episodic memory, including the California Verbal Learning Test and the Wechsler Memory Scale. Mean memory performance across Sessions 1 and 2 served as a baseline for assessing change in memory performance at Session 3. After controlling for chronological age, BOLD recollection effects in the hippocampus were found to predict longitudinal change in memory performance. This relationship was unmodified by the inclusion of a number of covariates including gender, years of education and APOE status. The results indicate that hippocampal recollection effects in older adults are predictive both of concurrent memory performance and of medium-term memory change.

Disclosures: M.A. De Chastelaine: None. **M. Hou:** None. **M.D. Rugg:** None.

Poster

167. Human Long-Term Memory: Medial Temporal Lobe I

Location: Hall A

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Program #/Poster #: 167.15/Z5

Topic: H.02. Human Cognition and Behavior

Support: European Commission/H2020-MSCA-IF-2016 to HS (752557)
Wellcome Trust/Royal Society Sir Henry Dale Fellowship to B.P.S. (107672/Z/15/Z)

Title: Memento - memory for objects and scenes in the human medial temporal lobe at 7 tesla

Authors: *H. SCHULTZ[1], A. GREVE[2], C. RUA[3], D. BERRON[5], R. HENSON[4], B. STARESINA[1];
[1]Sch. of Psychology and Ctr. for Human Brain Hlth., Univ. of Birmingham, Birmingham, United Kingdom; [2]MRC Cognition and Brain Sci. Unit, [3]Wolfson Brain Imaging Ctr., [4]MRC Cognition and Brain Sci. Unit and Dept. of Psychiatry, Univ. of Cambridge, Cambridge, United Kingdom; [5]Dept. of Clin. Sci. Malmö, Lund Univ., Lund, Sweden

Abstract: The human medial temporal lobe (MTL) is essential for episodic memory. A central connecting hub within the MTL is the entorhinal cortex. Its anterolateral and posteriormedial subregions relay object and scene information from visual and association cortices to the hippocampus during memory encoding, and back during memory retrieval. Superficial and deep layers of the entorhinal cortex may be differentially connected to input and output regions of the hippocampus, suggesting differential functional roles of these layers during memory encoding and retrieval. Here, we test encoding and retrieval for objects and scenes in subregions of the human medial temporal lobe, utilising the ultra-high spatial resolution afforded by 7T fMRI technology. Healthy volunteers (n=16) performed a memory task while undergoing fMRI (Siemens 7T, 0.8mm isotropic voxels, TR=1.78s, MB factor 3/iPAT factor 2, FLEET reference, 48 slices oriented in parallel to the longitudinal hippocampus axis). Additionally, we acquired high-resolution T1 (0.8mm isotropic) and T2-weighted structural images (0.4mm in-plane, 1.1mm slices). In each of 4 functional runs (2 object runs, 2 scene runs), participants first encoded either word-object or word-scene pairs. After a short active rest phase, they were cued with the word to recall objects or scenes from memory, and gave subjective "remember" and "forgotten" responses. These in-scan memory responses were validated in a post-scan explicit recall test. Behaviourally (n=16), 29 objects and 31 scenes were recalled on average out of 64 trials, indicating successful balancing of remembered versus forgotten trials. Functionally (n=15), analyses of the entire scanned volume (MNI-normalised and smoothed at 2.4mm, $p<.001$ uncorrected) showed effects of object vs. scene perception in extrastriatal regions (LOC, PPA/retrosplenial cortex) and MTL (perirhinal, entorhinal, and parahippocampal cortex). Importantly, successful recall was associated with elevated activity throughout MTL, including hippocampus, perirhinal, entorhinal, and parahippocampal cortex, with scene recall additionally supported by parahippocampal cortex and posterior-medial entorhinal cortex, and object recall by perirhinal cortex and anterior-lateral entorhinal cortex. Our results support and extend previous findings of material-sensitive and material-independent memory processing in the MTL.

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Poster

167. Human Long-Term Memory: Medial Temporal Lobe I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 167.16/Z6

Topic: H.02. Human Cognition and Behavior

Support: NIH R01 MH069456

Title: Stimulus prediction in the hippocampus resulting from rapid statistical learning

Authors: *E. A. MCDEVITT[1], G. KIM[3], N. B. TURK-BROWNE[4], K. A. NORMAN[1,2]; [1]Princeton Neurosci. Inst., [2]Psychology, Princeton Univ., Princeton, NJ; [3]Korea Brain Res. Inst., Daegu, Korea, Republic of; [4]Psychology, Yale Univ., New Haven, CT

Abstract: The hippocampus is involved in learning regularities from the environment. As these implicit associations are learned, the hippocampus can begin to retrieve predictions based on contextual cues. However, prior work has not examined how early in learning these predictive representations emerge. During high-resolution fMRI, we exposed participants to temporal regularities and tested for item-specific, predictive representations in the hippocampus after as few as three learning exposures. Participants viewed a continuous stream of scenes that, unbeknownst to them, followed a pair structure. Each pair had one scene as the first item (scene A) and a different scene as the second item (scene B). Each AB pair was shown three times, inserted in the stream amongst other pairs without breaks or segmentation cues. Prior to this AB learning, the A and B members of each pair were shown once separately (i.e., B did not follow A). This first presentation allowed us to estimate the multivoxel pattern of BOLD activity evoked by each scene ("pre-learning snapshot"). We then correlated these pre-learning snapshots with the voxel pattern from each AB learning trial, at each 1.5 s timepoint in a window spanning -1.5 to +10.5 s around trial onset (0 s = A, 3 s = B). We compared pattern similarity (PS) for the corresponding A and B pre-learning snapshots, baselined by the average pattern similarity to all other pre-learning scenes. We focused on two regions of interest: the parahippocampal place area (PPA) and the hippocampus. In the PPA, which is thought to track scene perception, we predicted and found in preliminary results that A and B scenes showed a similar time course of peak PS following their respective image onsets (4.5 s); this was true for all three learning exposures. We hypothesized that the hippocampus would represent prediction, not perception, which would be reflected in peak PS for the pre-learning snapshot of B following perception of A. The CA2/3/DG subfields of the hippocampus showed peak PS for B at 4 TRs (6 s) following A onset during the second learning exposure; A pre-learning snapshots did not show any peaks. This slightly faster time course, compared to the perception-related response in the PPA, could reflect the hippocampus generating a prediction of B in response to A. Together, these data suggest that patterns of neural activity in the hippocampus can reflect stimulus-specific predictions that arise from rapid statistical learning.

Disclosures: E.A. McDevitt: None. **G. Kim:** None. **N.B. Turk-Browne:** None. **K.A. Norman:** None.

Poster

167. Human Long-Term Memory: Medial Temporal Lobe I

Location: Hall A

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Program #/Poster #: 167.17/Z7

Topic: H.02. Human Cognition and Behavior

Support: CIHR to MM and MDB
James McDonnell Foundation to MDB

Title: Goal changes during navigation change hippocampal representations of space and time

Authors: *N. R. BOUFFARD[1,2], I. K. BRUNEC[1,2], J. D. OZUBKO[3], J. ROBIN[2], M. MOSCOVITCH[1,2], M. D. BARENSE[1,2];
[1]Psychology, Univ. of Toronto, Toronto, ON, Canada; [2]Rotman Res. Institute, Baycrest, Toronto, ON, Canada; [3]Psychology, SUNY Geneseo, Pittsford, NY

Abstract: A critical feature of goal-directed navigation is the maintenance and implementation of extended spatial and temporal representations. Research in humans and animals has shown that the hippocampus has complex neural mechanisms that support these types of spatiotemporal representations. Little is known, however, about how changes in navigational goal might affect the representations of space and time during navigation. In the present study, we investigated whether hippocampal representations of spatial and temporal distances in a well-known environment are modulated by the specific goal participants have in mind while navigating. Nineteen participants were scanned while navigating Google Street View routes to four different goals around the University of Toronto campus. Participants navigated a series of consecutive routes to these goal locations, where each route was unique and adjacent routes had different goal locations. If the current goal has no effect on spatiotemporal representations, the distance between two locations in space should be represented similarly, regardless of whether they were linked with the same goal or not (e.g., from the same route or different routes). Alternatively, a change in goal might transform the representations of otherwise equal distances, depending on whether the locations were linked with the same goal or separated by different goals. Using representational similarity analysis, we correlated patterns of hippocampal activity between pairs of timepoints from within the same route (same goal) or across adjacent routes (different goals). We found that points from the same route that had a short spatial distance between them were represented less similarly than points separated by a longer spatial distance, regardless of temporal distance. In other words, as the spatial distance between two points increases, the hippocampal pattern dissimilarity decreases. Moreover, we found this effect to be strongest for scene-based navigators compared to map-based navigators. We next compared pairs of timepoints from adjacent routes with different navigational goals. We found that points from different routes were represented dissimilarly, regardless of spatial or temporal distance. These results suggest that during navigation, the hippocampus uses orthogonalized codes to represent nearby locations that are linked by the same goal, and that the presence of a goal change attenuates pattern similarity changes across spatial and temporal distances.

Disclosures: N.R. Bouffard: None. **I.K. Brunec:** None. **J.D. Ozubko:** None. **J. Robin:** None. **M. Moscovitch:** None. **M.D. Barense:** None.

Poster

167. Human Long-Term Memory: Medial Temporal Lobe I

Location: Hall A

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Program #/Poster #: 167.18/Z8

Topic: H.02. Human Cognition and Behavior

Support: Deutsche Forschungsgemeinschaft SFB 1315

Title: Mapping memory consolidation in humans with propofol

Authors: D.-U. MOON[1], N. ESFAHANI-BAYERL[1], C. FINKE[1], D. SALCHOW[1], S. BAYERL[1], R. KEMPTER[2], ***C. J. PLONER**[1];
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Abstract: The stabilization and transformation of memory traces across time is crucial for adaptive behavior. Current theories posit that these consolidation processes depend on the reorganization of distributed brain circuits with interactions between hippocampus and neocortical regions. However, conflicting hypotheses have been advanced concerning a possible time-limited role of the hippocampus during consolidation. Translational research on this issue is challenged by the paucity of techniques to transiently interfere with hippocampal neural activity during consolidation in humans. Here, we report a new neuro-pharmacological approach on consolidation with the GABA-ergic anesthetic propofol in neurologically normal human participants performing a memory task sensitive to hippocampal dysfunction. Patients undergoing minor surgery learned word lists at two timepoints before injection of an anesthetic dose of propofol. Results show that administration of propofol 14 minutes after learning significantly impairs recall after awakening but spares recognition. By contrast, administration 104 minutes after learning had no effect. These findings indicate that memory consolidation is a highly dynamic process and suggest that the involvement of the hippocampus in this process changes significantly over time. Propofol anesthesia provides a novel tool to map the temporal properties of hippocampus-dependent memory consolidation.

Disclosures: **D. Moon:** None. **N. Esfahani-Bayerl:** None. **C. Finke:** None. **D. Salchow:** None. **S. Bayerl:** None. **R. Kempter:** None. **C.J. Ploner:** None.

Poster

167. Human Long-Term Memory: Medial Temporal Lobe I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 167.19/Z9

Topic: H.02. Human Cognition and Behavior

Support: NSF GRFP
NIH R01 MH069456
Swebilius Foundation

Title: Dynamic interactions between statistical learning and episodic memory

Authors: *B. SHERMAN[1], C. ELLIS[1], C. F. BENJAMIN[2], J. L. GERRARD[2], D. D. SPENCER[2], N. B. TURK-BROWNE[1];
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Abstract: Any experience contains information both specific to that instance (episodic details), as well as common across similar experiences (statistical regularities). Computationally, the encoding of details and learning of regularities are incompatible: episodic memories are formed rapidly using separated representations to minimize interference, whereas statistical learning occurs gradually using overlapping representations to identify commonalities. Despite this conflict, the hippocampus has been implicated in both computations, raising questions of how they might interact. Indeed, we recently demonstrated a behavioral trade-off between statistical learning and episodic memory: items that generated learned predictions in the hippocampus were worse remembered episodically (Sherman & Turk-Browne, 2018, *SfN*). However, the temporal dynamics of this trade-off — both at the behavioral level and within the hippocampus — are poorly understood. To address this question, we recorded neural activity from epileptic patients with intracranial electrodes. We first replicated a prior study (Henin et al., 2019, *bioRxiv*) to establish a neural correlate of statistical learning: patients listened to a stream of syllables that were — unbeknownst to them and without explicit segmentation cues — temporally grouped into “words”, such that given one syllable, the next two syllables were predictable; as was found previously, electrodes over superior temporal gyrus exhibited increased power not only at the frequency of syllable presentation (reflecting neural entrainment), but also at the word frequency, providing an online signature of learning. To assess the interaction between statistical learning and episodic memory, patients viewed a continuous stream of images containing temporal regularities followed by a memory test. That is, analogous to the words in the auditory task, the stream contained category pairs, whereby images from one category (e.g., beach) were always followed by images of another category (e.g., mountain). Critically, each image was trial unique, thus allowing for simultaneous learning of category regularities and idiosyncratic image details.

Consistent with the auditory results, electrodes in occipital cortex exhibited increased power at the pair frequency, reflecting the learned structure. We are relating this online measure of statistical learning to episodic memory over time, thus probing how the trade-off between learning and memory evolves in time. Further work will assess the relation between cortical learning signals and hippocampal activity and will examine how these interactions arise via hippocampal circuit dynamics.

Disclosures: B. Sherman: None. **C. Ellis:** None. **C.F. Benjamin:** None. **J.L. Gerrard:** None. **D.D. Spencer:** None. **N.B. Turk-Browne:** None.

Poster

167. Human Long-Term Memory: Medial Temporal Lobe I

Location: Hall A

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Topic: H.02. Human Cognition and Behavior

Support: NINDS Grant R01-NS078396
NSF Grant BCS-1358907
Marcus and Amalia Wallenberg Foundation

Title: Hippocampal pattern separation and associative memory: Distinct intracranial EEG temporal encoding patterns predict subsequent memory

Authors: *S. HSU[1], K. F. LAROCQUE[2], O. RACCAH[3], A. GONZALEZ[4], J. PARVIZI[5], A. D. WAGNER[2];
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Abstract: Episodic memory critically depends on mnemonic representations encoded in the hippocampus. From one perspective, the hippocampus is theorized to encode pattern separated conjunctive representations that support later memory for co-occurring event elements. While extant data in humans have predominantly focused on assaying the relationship between the similarity of spatial patterns at encoding and later memory performance, emerging data suggest that distinctiveness in the temporal domain may also reveal encoding computations predictive of future memory. To examine the link between overlap in temporal patterns of encoding-period hippocampus activity and later associative memory performance, human participants (n=7) with intracranial electrodes implanted in the hippocampus performed a paired-associate cued-recall task. Subsequent memory analyses revealed a relationship between global temporal pattern similarity in the hippocampus during encoding -- that is, the similarity of the gamma power (70-180Hz) time series elicited by a given stimulus pair to that elicited by all other stimuli pairs -- and later memory performance. Stimuli that elicited distinctive temporal patterns in the

hippocampus during encoding predicted a higher likelihood of correct associative recall at test. These data suggest that the distinctiveness of hippocampal traces may be particularly important for subsequent retrieval of an ensemble of co-occuring event elements.

Disclosures: S. Hsu: None. **J. Parvizi:** None. **O. Raccah:** None. **A.D. Wagner:** None. **K.F. Larocque:** None. **A. Gonzalez:** None.

Poster

167. Human Long-Term Memory: Medial Temporal Lobe I

Location: Hall A

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Program #/Poster #: 167.21/Z11

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R00AG036845
NIH Grant 1UL1TR001430

Title: Hippocampal subfield volumes, cardiorespiratory fitness, and pattern separation task performance in older adults

Authors: *K. L. KERN[1], R. K. NAUER[2], T. W. STORER[3], K. SCHON[1];
[1]Dept. of Anat. and Neurobio., Boston Univ. Sch. of Med., Boston, MA; [2]Dept. of Psychological and Brain Sci., Boston Univ., Boston, MA; [3]Brigham and Women's Hospital, Harvard Med. Sch., Boston, MA

Abstract: Cognitive aging is associated with reduced integrity of the medial temporal lobe memory system. Accurate episodic memory formation requires the computational process of pattern separation (PS), or the disambiguation of similar stimuli during encoding. PS is supported by the dentate gyrus (DG), the hippocampal subfield that primarily demonstrates adult hippocampal neurogenesis (AHN) throughout the lifespan. In rodents, aging is associated with downregulated AHN, as well as impaired PS. Comparatively, in rodents, exercise upregulates AHN, which is associated with enhanced PS. While AHN cannot be measured directly in humans *in vivo*, behavioral and neuroimaging studies suggest that greater cardiorespiratory fitness (CRF) is associated with better PS task performance in young adults and greater hippocampal volume in older adults. However, the relationship between CRF, DG volume, and PS in older adults remains unclear. Given the relationship between exercise, AHN, and PS in rodents, we predicted that greater CRF and larger DG volume would be associated with better PS task performance in older adults. Additionally, we sought to extend previous work in our lab that demonstrated a relationship between CRF and volume of the right entorhinal cortex and left anterior DG/CA3 in young adults to the aging brain. Twenty-five participants aged 55-85 years underwent a submaximal treadmill test to estimate maximal oxygen uptake (VO_{2MAX}), which operationally defined CRF, an MRI scan to examine volume of the hippocampal subfields and

medial temporal lobe cortices, and a behavioral task designed to tax PS at parametrically varying levels of stimulus similarity (10, 30, 50%). There was a significant effect of similarity level on PS task performance, with participants demonstrating lowest task accuracy in the condition theoretically placing the highest taxation on PS (50%). Left DG body volume significantly predicted performance in the 50% condition. However, there was no relationship between CRF and PS task performance. Furthermore, contrary to our prediction, CRF significantly negatively predicted right entorhinal cortex volume. Altogether, these findings provide support for a role of the DG in PS in older adult humans while also suggesting that further research is necessary to examine if CRF modulates PS task performance in older adults.

Disclosures: **K.L. Kern:** None. **R.K. Nauer:** None. **T.W. Storer:** None. **K. Schon:** None.

Poster

167. Human Long-Term Memory: Medial Temporal Lobe I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 167.22/Z12

Topic: H.02. Human Cognition and Behavior

Support: EPSCoR-1632738
Diamond Foundation Research Development Award

Title: Impact of interictal epileptiform discharges on short timescale cortical activity

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Abstract: We investigated how interictal epileptiform discharges (IEDs) changes cortical activity on a short timescale as human subjects participated in a free recall working memory task. Previously, we showed that IEDs can momentarily impair cognitive function in a free recall task, and that circadian trends in IEDs are related to memory performance. However, the mechanism by which IEDs actually bring about changes in cognition is poorly understood.
It is also not fully understood whether IEDs are a pathological symptom of epilepsy or an attempt by the brain to restore healthy brain activity.
We conducted this study with subjects who were undergoing intracranial monitoring as part of treatment for epilepsy. We measured changes in cortical activity in the time periods surrounding IEDs using electrocorticography while subjects participated in a free recall task, and sought to determine whether these changes in activity promote improvement or decline of memory performance. We found changes in cortical activity in the vicinity of IEDs, which may help explain their impact on cognition.

Disclosures: **S. Meisenhelter:** None. **B.C. Jobst:** None.

Poster

168. Human Long-Term Memory: Encoding and Retrieval I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 168.01/Z13

Topic: H.02. Human Cognition and Behavior

Support: the National Science Foundation of China (31730038)
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Title: Individual-specific and shared representations during episodic memory encoding and retrieval

Authors: *Y. ZHOU[1], X. XIAO[1], Z. YE[1], L. YAO[2], C. CHEN[3], G. XUE[1];
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Abstract: Human memories are both unique and shared across individuals. Although subject-specific and shared representations during memory encoding retrieval have been separately reported in different studies, how shared memories are formed from encoded representations is not clearly understood. Using a unique fMRI design involving memory encoding and retrieval, and second-order representational similarity analysis to link representations from different individuals, brain regions and processing stages, the current study revealed that distributed brain regions showed both subject-specific and shared neural representations during memory encoding and retrieval. The visual cortex showed greater unique and shared representations during encoding, whereas the angular gyrus showed greater unique and shared representations during retrieval. The neural representations during encoding were transformed during retrieval, as shown by smaller cross-subject encoding-retrieval similarity (ERS) than cross-subject similarity either during encoding or during retrieval. Simulation analysis further suggests that these patterns could be achieved by incorporating stage-specific representational strength, and subject-specific cross-region reinstatement from encoding to retrieval, but not by common transformation from encoding across subjects. Together, our results shed light on how memory presentations are encoded and transformed to maintain individual characteristics and at the same time to facilitate interpersonal communication.

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Poster

168. Human Long-Term Memory: Encoding and Retrieval I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 168.02/Z14

Topic: H.02. Human Cognition and Behavior

Support: NSFC Grant31730038

Title: The neural pattern similarity between working memory maintenance and retrieval was associated with successful long-term memory

Authors: ***J. LIU**, G. XUE;
State Key Lab. of Cognitive Neurosci. and Learning and IDG/McGovern Inst. of Brain Reasearch, Beijing, China

Abstract: Working memory serves as an important bridge to link perception and long-term memory, and recent evidence from both fMRI and EEG found that activities during the working memory maintenance could predict long-term memory formation. By examining the neural pattern similarity between encoding and retrieval (ERS), mounting studies have revealed significantly greater ERS for successfully retrieved items than forgotten items, providing strong evidence to support the view that memory retrieval is achieved by reactivating the content-specific neural patterns during encoding. Despite the general similarity between encoding and retrieval, recent evidence carefully examining the nature of representation during encoding and retrieval have revealed significant differences between the representations in these two stages, suggesting transformed neural patterns from memory encoding and retrieval. To further understanding how this transform is achieved, it is thus important to examine the nature of representation at both encoding, working memory maintenance and retrieval. To achieve this goal, the present study used intracranial EEG recordings and a paradigm that combined encoding, working memory and long-term memory retrieval tasks. Participants were asked to learn associations between Chinese words and pictures during encoding, immediately followed by a short-term maintenance phase, which required participants maintain these associations for a few seconds in each trial. After each session of short-term memory task, participants were tested for their long-term memory retrieval of these associates. We applied spatiotemporal pattern similarity analysis across electrodes and frequencies to compare the neural representation across encoding, working memory maintenance and long-term memory retrieval systematically. The results revealed greater pattern similarity between encoding and retrieval (ERS), as well as between working memory maintenance and retrieval (MRS) for successful retrieved items. These

results indicate that the neural representations during working memory maintenance was reinstated during memory retrieval.

Disclosures: **J. Liu:** None. **G. Xue:** None.

Poster

168. Human Long-Term Memory: Encoding and Retrieval I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 168.03/Z15

Topic: H.02. Human Cognition and Behavior

Support: National Institute of Mental Health (NIMH) Intramural Research Program

Title: Temporal distance modulates functional-anatomic correlates of overt autobiographical memory retrieval

Authors: *A. QUACH[1], A. W. GILMORE[1], S. KALINOWSKI[2], S. J. GOTTS[1], D. L. SCHACTER[2], A. MARTIN[1];
[1]Lab. of Brain and Cognition, NIMH/NIH, Bethesda, MD; [2]Dept. of Psychology, Harvard Univ., Cambridge, MA

Abstract: An ongoing debate in neuroscience concerns the degree to which episodic memories become independent of the hippocampus over time. A "standard" model assumes that memories for past events are initially reinstated by the hippocampus but are consolidated to the neocortex over time (Squire et al., 1984). An alternative model, termed "multiple trace theory" posits that the hippocampus is always necessary to vividly remember past episodes (Nadel & Moscovitch, 1997). Support from neuroimaging studies has been equivocal. One potential source of difficulty in their disentanglement is that memories typically change and become less detailed over time (Eustache et al., 2003), particularly when combined with the covert retrieval periods employed in fMRI studies of autobiographical memory (e.g., Söderlund et al., 2011). Thus, the content and age of memories are often confounded, and Likert-type ratings of subjective vividness or detail provide only an impoverished view into the nature of how detailed a recollection may or may not have been. In this experiment, we addressed this standing concern by asking human participants (N = 40) to engage in 2-minute periods of continuous overt (spoken) autobiographical recall while undergoing multi-echo fMRI. Participants were cued with photographs of complex scenes to recall memories from earlier in the same day, memories approximately 1 year old, or 5-10 years old. As a control task, participants were shown the same types of cues and were asked to describe the contents of each. Overt retrieval allowed for the contents of memories to be analyzed using an adaptation of the Autobiographical Interview (Levine et al., 2002). Effects of temporal distance were observed primarily along the parietal midline, including mid/posterior cingulate cortex and the precuneus, as well as the left angular gyrus. Targeted ROI analysis

further identified temporal distance effects in the posterior hippocampus. In all cases, a graded pattern was observed such that increased distance was associated with less activity. These results appear broadly consistent with a standard model of memory consolidation and suggest that even when accounting for differences in retrieved content, the hippocampus becomes less involved in memory retrieval over time.

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Poster

168. Human Long-Term Memory: Encoding and Retrieval I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 168.04/Z16

Topic: H.02. Human Cognition and Behavior

Support: NIMH Division of Intramural Research Grant ZIAMH002920

Title: In-scanner spoken recall identifies dynamic, content-specific retrieval of autobiographical memories

Authors: *A. W. GILMORE[1], A. QUACH[2], S. KALINOWSKI[3], S. J. GOTTS[1], D. L. SCHACTER[3], A. MARTIN[1];
[1]Lab. of Brain and Cognition, NIMH/NIH, Bethesda, MD; [2]Lab. of Brain and Cognition, NIMH/NIH, Rockville, MD; [3]Harvard Univ., Cambridge, MA

Abstract: FMRI experiments of autobiographical memory typically require participants to covertly recall specific episodes in response to a word or picture cue (e.g., Addis et al., 2007). Phenomenological ratings, if collected, are reported either after a trial or at the end of an experiment. This approach has yielded informative and consistent results, but it comes at a cost—the specific order in which different details are recalled is obscured and information associated with phenomenological reports is relatively sparse (typically reduced to a Likert-type rating). Here, we extend this prior literature by asking human participants (N = 40) to engage in 2-minute periods of continuous overt recall while undergoing multi-echo fMRI. In response to photographic retrieval cues, participants recalled and described specific memories from three different time periods, including earlier in the same day, a period approximately 1 year prior, or a period of 5 to 10 years prior. As a control task that engaged narrative processes without also requiring an autobiographical element, participants described the events being depicted in the photographic cue images. FMRI data were denoised using a multi-echo ICA approach to reduce spurious signals relating to speech-related subject motion (Kundu et al., 2011). Regions associated with autobiographical retrieval relative to the picture description control included medial prefrontal and posterior cingulate cortex, whereas lateral parietal, occipitotemporal, and

dorsolateral prefrontal cortex were associated with the picture description task. Activity during naturalistic speech periods in which participants discussed various detail categories recapitulated known divisions along the ventral stream and elsewhere in the cortex. For example, activity comparisons during descriptions of people and places found that recalling people activated anterior temporal and posterior cingulate cortex whereas recall of places was associated with parahippocampal cortex, ventral parietal occipital sulcus, and angular gyrus/transverse occipital sulcus. These results provide a glimpse into dynamic retrieval activity associated with the reinstatement of specific episodic details and highlight the benefits of implementing overt spoken recall in an fMRI environment.

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Poster

168. Human Long-Term Memory: Encoding and Retrieval I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 168.05/Z17

Topic: H.02. Human Cognition and Behavior

Support: NIH F32AG056080
NIH R21AG058111
Marcus and Amelia Wallenberg Foundation
Wallenberg Network Initiative

Title: Prefrontal reinstatement of contextual task demand is mediated by repulsion in hippocampal activity patterns between contexts

Authors: *J. JIANG[1], S.-F. WANG[1], W. GUO[2], A. D. WAGNER[1];
[1]Dept. of Psychology, Stanford Univ., Stanford, CA; [2]Psychology, Univ. of Oregon, Eugene, OR

Abstract: Cognitive control is crucial for humans to adaptively adjust mental states and behavior to task demands. In addition to reacting to current task demands, cognitive control can be regulated proactively based on predictions of forthcoming demands. In real life, a major predictor of task demands is often the spatial context in which tasks are performed. In the present fMRI study, we investigated the neurocognitive mechanisms underlying the retrieval of contextual task demand (CTD). The experiment consisted of 6 runs of 8 blocks each, during which participants needed to draw on selective attention to make perceptual decisions. In each block, participants (N = 33) were cued to navigate to one of four buildings (i.e., contexts) in a 3D virtual environment. After entering each building, its interior was shown for 7.75s, followed by 8 perceptual decision-making trials. Each trial started with a task cue, followed by two overlapping translucent images (one face and one object image); based on the task, participants indicated

either the gender of the face or the type of tool. To manipulate the CTD, participants performed 75% face judgments in two buildings and 75% object judgments in the other two buildings. Initial fMRI analyses revealed that: (1) right dorsolateral prefrontal cortex (dlPFC) reinstated the CTD at the onset of the context (i.e., when the interior was shown); and (2) the degree of CTD reinstatement modulated decision reaction times in the block. (3) Moreover, similarity analyses of hippocampal fMRI activity patterns at context onset were consistent with 'repulsion' accounts of pattern separation, as activity patterns were less similar for different contexts associated with the same CTD than different contexts associated with different CTDs. Surprisingly, (4) hippocampal activity pattern similarity between different contexts sharing the same CTD positively correlated with the amount of CTD reinstatement in dlPFC, perhaps marking recurrent inputs to the hippocampus of the retrieved CTD. These findings shed light on the neural computations underlying the retrieval of CTDs. Future analyses will focus on the temporal dynamics of the repulsion, examining how it occurred across the learning of CTDs.

Disclosures: J. Jiang: None. **S. Wang:** None. **W. Guo:** None. **A.D. Wagner:** None.

Poster

168. Human Long-Term Memory: Encoding and Retrieval I

Location: Hall A

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Program #/Poster #: 168.06/Z18

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant F32-AG059341
NIH Grant R56-MH111672

Title: Pre-trial fluctuations in alpha and theta oscillatory power affect goal-state orienting and accuracy during episodic remembering

Authors: *K. P. MADORE[1], A. M. KHAZENZON[1], C. W. BACKES[2], J. M. QI[1], A. M. NORCIA[1], A. D. WAGNER[1];
[1]Psychology, [2]Symbolic Systems, Stanford Univ., Stanford, CA

Abstract: Moment-to-moment interactions between goal states, attention, and episodic memory retrieval may influence when individuals remember and when they forget. We recorded concurrent scalp EEG and pupillometry during an encoding/retrieval paradigm with 80 young adults to examine how (a) multimodal indices of attentional lapses (e.g., pre-trial increases in alpha power and decreases in theta power) relate to goal-state representation and episodic memory, and how (b) trait differences in sustained attention, media multitasking, and related constructs may contribute to these relationships. At encoding, participants performed two tasks, classifying individual objects on either a conceptual or perceptual dimension. At retrieval, participants oriented to one of three retrieval goals (concept before? vs. percept before? vs. new

item?) and then were cued with an old or new object and made the retrieval judgment. We examined how trial-by-trial tonic fluctuations in alpha (8-12Hz) and theta (4-7Hz) oscillatory power (a) before orienting to the retrieval goal and (b) before viewing the object and making the retrieval judgment affect accuracy. Across goal states, retrieval performance was predicted by fluctuations in alpha power and theta power during the 1000ms before goal orienting and during the 1000ms before the retrieval cue. Pre-goal and pre-retrieval mean alpha power were significantly higher for misses relative to hits, and pre-retrieval mean alpha power also was significantly higher for false alarms relative to correct rejections. In addition, mean pre-goal theta power was significantly lower for misses relative to hits, and mean pre-retrieval theta power was significantly lower for false alarms relative to correct rejections. Individual difference analyses further revealed that (a) self-reported levels of media multitasking, self-reported rates of spontaneous mind wandering, and behaviorally assayed commission error rates on the gradual-onset continuous performance test (gradCPT) significantly positively related to subject-level mean alpha power during the pre-goal and pre-retrieval epochs, and (b) self-reported attentional control was significantly negatively related to mean alpha power. Media multitasking, commission error rates on the gradCPT, and mean alpha power were also significantly negatively related to d' (overall memory performance), and attentional control was significantly positively related to d'. These results highlight how preparatory attention and goal-state representation impact episodic remembering.

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Poster

168. Human Long-Term Memory: Encoding and Retrieval I

Location: Hall A

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Program #/Poster #: 168.07/Z19

Topic: H.02. Human Cognition and Behavior

Support: LUCI grant from Air Force Research Lab and Vannevar Bush Faculty Fellowship

Title: Community detection of the default mode network in resting fMRI reveals anterior temporal, posterior medial, and lateral subnetworks

Authors: *A. J. BARNETT, W. REILLY, C. RANGANATH;
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Abstract: Functional imaging in episodic memory and neuropsychological assessments of brain disorders have suggested that a posterior medial network (PMN)—composed of the posterior cingulate cortex, parahippocampus, and lateral parietal cortex—and an anterior temporal network (ATN)—composed of the perirhinal cortex, anterior temporal lobe, and orbitofrontal cortex—

play an important role in memory for contextual and semantic information, respectively. Many regions in the PMN and ATN have been collectively identified as parts of the "Default Mode Network" (DMN), a large-scale network identified in resting state functional MRI (fMRI) studies. Here, we tested whether the PMN and ATN are subnetworks within the DMN. We collected 25 minutes of resting fMRI from 42 healthy young adults. Using the HCP-MMP atlas, we calculated pairwise connectivity between every node in the brain to create connectivity matrices. A group averaged matrix was calculated and Louvain community detection algorithm was iterated 1000 times to produce a consensus clustering solution which contained canonical brain networks that have been previously shown in the literature. Of these networks, the DMN and a community of medial temporal regions was identified, each of which included regions in the PMN and ATN. Community detection analysis on this set of regions identified three subnetworks—the PMN, ATN, and a third network we are calling the lateral network (LN). The LN closely abuts language-affiliated regions in the temporal, parietal, and frontal cortex, and, like the PMN, and ATN, is closely affiliated with the hippocampus. Ongoing analyses will examine age-related changes in these networks and their relation to cognition.

Disclosures: **A.J. Barnett:** None. **W. Reilly:** None. **C. Ranganath:** None.

Poster

168. Human Long-Term Memory: Encoding and Retrieval I

Location: Hall A

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Program #/Poster #: 168.08/Z20

Topic: H.02. Human Cognition and Behavior

Support: ONR/DoD N00014-17-1-2961

Title: Sleep modulates a delay-dependent retrieval benefit for events which form a coherent narrative

Authors: *A. I. DELARAZAN, B. I. COHN-SHEEHY, C. RANGANATH;
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Abstract: Although many studies have shown that people segment continuous experiences into discrete events, we can recollect events as integrated narratives. For example, if a friend mentions that he is auditioning for a play and later invites you to support him during opening night, information from these separate events can be integrated, even though they were encoded at separate times. We previously discovered that people can show enhanced recall of temporally separated events in narratives if they can be integrated into a coherent narrative, but this effect was only evident after a 24-hour delay (Cohn-Sheehy, Delarazan, et al., in prep). Some theories propose that sleep may facilitate integration of information across different events. Here, we sought to test whether the time dependent memory benefit for coherent narratives was driven by

sleep, rather than differing retrieval processes that depend on a retention interval. Participants encoded four fictional audio stories that each included "sideplot" events involving characters that were unrelated to the surrounding main stories. Two sideplot events could be integrated into a larger narrative ("*coherent"*), and two sideplot events were unrelated to one another ("*incoherent"*). After encoding, we tested their recall after a 12-hour retention interval. Critically, half of the participants had an overnight retention interval (*sleep*), and half of the participants had a daytime retention interval (*wake*). Preliminary analyses indicated that participants recalled more information from coherent sideplot events than from incoherent sideplot events—this effect was not observed in the wake group. Further analyses will adjudicate whether a delay-dependent retrieval benefit for events which form coherent narratives is due to sleep-dependent integration across events versus sleep-associated reduction of interference between events in memory.

Disclosures: A.I. Delarazan: None. **B.I. Cohn-Sheehy:** None. **C. Ranganath:** None.

Poster

168. Human Long-Term Memory: Encoding and Retrieval I

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ONR Grant N00014- 17-1-2961

Title: Structured representations of characters, contexts, and event schemas in cortico-hippocampal networks

Authors: *Z. M. REAGH, J. MACALUSO, R. BUGSCH, C. RANGANATH;
Univ. of California, Davis, Davis, CA

Abstract: Real-world events are complex, featuring many people and places that may be unique to, or shared across, multiple situations. Moreover, complex events have a temporal structure in addition to a content-based structure. In the present study, we used fMRI to identify how different aspects of events are represented in real time, and during memory retrieval. Twenty participants were scanned while viewing eight video clips depicting real-world events. Clips were filmed to systematically combine specific people and spatial contexts in rich, dynamic situations. Moreover, multiple exemplars of the same *type* of event were depicted (i.e., tapping into a common event schema despite low-level perceptual differences). Participants viewed each clip three times and performed cued recall during fMRI scanning, and finally performed an out-

of-scanner recognition memory task. Recognition was highly accurate across video clips, with no significant memory bias for any character or context, and considerable detail was retrieved during recall. Multi-voxel pattern similarity analyses revealed evidence for stable representations of specific people in an anterior-temporal (AT) network that included perirhinal cortex and temporal poles, and these representations appeared to be stable across different contexts. Conversely, we found evidence for representations of specific contexts in a posterior-medial (PM) network that including parahippocampal and medial parietal cortex that appeared to be stable across films with different characters. We also found schema-like merging across related contexts in medial prefrontal cortex (mPFC), and episodic specificity in the hippocampus. In the mPFC, AT, and PM networks, representations of schemas, characters, and contexts were reinstated during spoken recall. Preliminary analysis of the temporal dynamics of activity patterns during encoding revealed evidence for distinct timescales of representational stability between the hippocampus, PM, and AT networks. Together, these results reveal how different aspects of complex events are processed in real time, and are reinstated during episodic memory retrieval by different cortico-hippocampal networks.

Disclosures: **Z.M. Reagh:** None. **J. Macaluso:** None. **R. Bugsch:** None. **C. Ranganath:** None.

Poster

168. Human Long-Term Memory: Encoding and Retrieval I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 168.10/Z22

Topic: H.02. Human Cognition and Behavior

Support: ONR/DoD N00014-17-1-2961
Floyd and Mary Schwall Medical Research Fellowship (UC Davis)

Title: Cortical representations that cross events facilitate the integration of events which form coherent narratives in memory

Authors: *B. I. COHN-SHEEHY, A. I. DELARAZAN, J. E. CRIVELLI-DECKER, W. B. REILLY, Z. M. REAGH, C. RANGANATH;
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Abstract: Most research on episodic memory has focused on recall or recognition of specific items from lists of words or pictures. Recall of real-life events, however, typically does not involve literal reproduction of items. Instead, people often construct narratives to recapitulate information from past events, and these narratives can become more integrated or schematized over time (Bartlett, 1932). Here, we used functional magnetic resonance imaging (fMRI) to identify how different cortico-hippocampal networks support recollection of complex events, and integration of information across different events that form a coherent narrative. We

hypothesized that regions within a posterior medial cortical network would support situational representations that span multiple, distinct events, and which could enable these events to become integrated by the time of recall, even if these events were experienced at different times. On Day 1, participants were scanned while listening to fictional stories that included a primary storyline as well as "sideplot" events involving characters that were unrelated to the surrounding main stories. Two of these sideplot events could be integrated into a larger narrative ("coherent"), and two sideplot events were unrelated to one another ("incoherent"). On Day 2, participants were scanned as they recalled these stories, and then as the original stories were presented again. Preliminary analyses replicated our previous behavioral findings: coherent sideplots were more successfully recalled than incoherent sideplots after a 24-hour delay. During encoding, ventromedial parietal cortex activity patterns tracked information which crossed multiple main protagonist events within a given primary storyline. Interestingly, we also observed that during re-presentation of the original stories after delayed recall, ventrolateral parietal cortex activity patterns were more highly similar across coherent sideplot events than incoherent sideplot events. These initial findings suggest that ventral parietal cortical areas play a critical role in both encoding and post-encoding integration processes which support the formation of a coherent narrative across distinct events. Further analyses will assess how neocortical areas and the hippocampus differentially contribute to the dynamic evolution of memory for complex events.

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Poster

168. Human Long-Term Memory: Encoding and Retrieval I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 168.11/Z23

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01 DC009209

Title: Semantic knowledge distorts episodic memory: Behavioral and neural investigations

Authors: *A. TOMPARY, A. XIA, S. L. THOMPSON-SCHILL;
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Abstract: Category members that are more typical, or perceived to better represent their category, benefit from enhanced semantic processing and resistance to disruption by brain damage. Furthermore, features of typical (compared to atypical) items are more often generalized to other category members. Although prior knowledge is known to influence episodic encoding, it remains unclear how the semantic properties of typical and atypical category membership bias

encoding of new information. Here we investigated whether new episodic memories of typical category members are more influenced by items from the same category (category neighbors) relative to memories of atypical category members. We further tested whether such influences are driven solely by properties of our semantic knowledge, or if they extend to stimuli organized by visual similarity. Participants on Amazon Mechanical Turk encoded and retrieved novel item-location associations for typical and atypical category members. Critically, item locations were spatially clustered by category membership (e.g., many images of birds were located near each other), but typical and atypical items (e.g. cardinal and ostrich) were randomly located far from category neighbors. In a control condition, participants encoded the locations of objects that were artificially colored (e.g., chairs with varying shades of blue) such that typical and atypical objects could be likewise compared, but based on visual rather than semantic information. We used a continuous retrieval measure to disentangle biases in spatial memory driven by category neighbors from errors due to forgetting. In both conditions, retrieval of locations paired with typical (relative to atypical) category members was biased towards the locations of category neighbors. This effect was observed when participants completed the memory experiment with images clustered by semantic category (N = 35), or by color (N = 35), and when 50% of the images were clustered by semantic category and 50% by color (N = 70). Taken together, this suggests that category typicality dictates the extent to which new encoding is biased both by semantic knowledge and by visual similarity. Despite the consistency of these similarity-based distortions, we hypothesize that semantic and visual biases are supported by different neural mechanisms. For example, the anterior temporal lobes (ATL) may support distortions in memory due to semantic knowledge but not for color organization. An ongoing experiment applying transcranial magnetic stimulation to the left ATL will help clarify how similarity-based computations from semantic knowledge and visual similarity distort new encoding.

Disclosures: **A. Tompary:** None. **A. Xia:** None. **S.L. Thompson-Schill:** None.

Poster

168. Human Long-Term Memory: Encoding and Retrieval I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 168.12/Z24

Topic: H.02. Human Cognition and Behavior

Support: NINDS Intramural Funding
NIGMS T32 GM007171

Title: Replay of neural sequences underlies human memory retrieval

Authors: ***A. VAZ**[1], J. WITTIG, Jr[2], S. INATI[2], N. BRUNEL[3], K. ZAGHLOUL[2];
[1]Duke Univ. Sch. of Med., Durham, NC; [2]Natl. Inst. of Neurolog. Disorders and Stroke (NINDS), Bethesda, MD; [3]Duke Univ. Dept. of Neurobio., Durham, NC

Abstract: Episodic memory retrieval is thought to rely on the replay of past experiences. This has been demonstrated robustly in rodent models where spatial memories are replayed and consolidated concomitant to fast frequency oscillations known as ripples. Strikingly, these memory representations take the form of highly stereotyped sequences of neural activity that are replayed with variable speeds. However, it remains unknown how human single unit activity is organized during episodic memory encoding and retrieval. Here, we simultaneously recorded cortical macro-iEEG, micro-LFP, and single unit data as patients performed a verbal episodic memory task. We found that ripple oscillations in the human cortex reflect bursting of local spiking activity. During the memory task, this spiking activity organized into memory-specific sequences that were then replayed during successful retrieval. Our findings demonstrate that associative memories in humans are encoded by specific sequences of neural activity and memory recall involves reinstating this temporal order of activity. In a broader sense, this data connects recent findings of coupled ripple oscillations between the human MTL and neocortex to a general mechanistic understanding of how information is represented in the human brain.

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Poster

168. Human Long-Term Memory: Encoding and Retrieval I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 168.13/Z25

Topic: H.02. Human Cognition and Behavior

Support: JSPS KAKENHI Grant Number JP16H01727

Title: Representing myself in multiple ways: Dissociable activation between different levels of abstractness for self in autobiographical memories

Authors: *A. KATSUMI, S. IWATA, T. TSUKIURA;
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Abstract: Self representations in autobiographical memory are modulated by different levels of abstractness for self in personally experienced events (for review, see Martinelli et al., 2013). Previous functional neuroimaging findings suggest that the ventromedial (vmPFC) and anteromedial (amPFC) prefrontal cortices, the right anterior temporal lobe (ATL), and the precuneus could contribute to the processing of more concrete self represented in a special event including socioemotional interactions with personally familiar individuals, whereas the dorsomedial prefrontal cortex (dmPFC) and the left ATL could be involved in the processing of more abstract self represented in social contexts. However, little is known about the neural correlates modulated by the different self representations in autobiographical memories. The

present study investigated this issue. In the present study, 40 right-handed and college-aged healthy participants were recruited from the Kyoto University community (18 females, mean age: 21.67, SD: 1.60). All participants were presented with sentences describing autobiographical events in three conditions of Interpersonal Self (IS), Social Self (SS) and Non-Social Self (NS), and were required to judge whether they really experienced each event in their lives by choosing either "Remember", "Know", or "New". Sentences in IS described autobiographical events reflecting specific situations which include social interactions with an intimate friend, and sentences in SS showed autobiographical events related to situations of being praised or criticized by a group of surrounding people in social contexts. In NS as a control condition, participants remembered autobiographical events of doing something alone described by sentences. Neural activation was measured during making judgment of sentences related to these autobiographical memories. Behavioral results confirmed that participants remembered their autobiographical memories and generated subjective feelings appropriately in each condition. In fMRI data, activation in the vmPFC, amPFC, right ATL, and the precuneus significantly increased in IS than SS and NS, whereas the dmPFC and the left lateral temporal regions including ATL showed significantly greater activation in SS than IS and NS. In addition, compared to the "Know" responses, significant activation related to the "Remember" responses was found in the bilateral hippocampi and several other regions. These findings suggest that self representations modulated by different levels of abstractness for self could be involved in different cortical regions during the retrieval of autobiographical memories.

Disclosures: A. Katsumi: None. **S. Iwata:** None. **T. Tsukiura:** None.

Poster

168. Human Long-Term Memory: Encoding and Retrieval I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 168.14/Z26

Topic: H.02. Human Cognition and Behavior

Support: 31671161

Title: Why the expert more concentrating than novice: Attentional superiority and memory trace on scene information

Authors: *M. HE, C. QI;
Psychology, Wuhan Sports Univ., wuhan, China

Abstract: Sports experts are notably more skilled in visually perceptual-cognitive processes of skill- related scene information. However, the attentional and memory basis of it has not been thoroughly explored. **Objective**: The present study examined whether a sport expert has the attentional superiority on scene information relevant to his/her sport skill, and explored how the

memory trace relevant with the sport expertise affect the current attention distribution. **Method**: **1.Participants** Nineteen Chinese varsity tennis players(10 male,8 female) were recruited as expert and nineteen Chinese recreational tennis players(10 male,8 female) were recruited as novice(mean age 22.06 ±2.36 and20.89 ±1.99,p>.05). The sample size was decided by prior power analysis. An expert vs. non-expert effect size for processing speed on interceptive sport information, which was Hedges's g=.96 reported in a previous meta-analysis (Voss et al.,2010), was used here as the reference to calculate the sample size. It turned out that a particular N =19 sample size for each group was required to reach the level of a =.05 and b=.08. **2.Design** The present study employed a 2 (scene: tennis vs. nontennis) by 2 (stimulus type: overlap vs. non-overlap) by 2 (group: expert vs. novice) mixed experimental design. The scene and the stimulus were within-subject factors and group was between-subject factor. **3.Analysis** We additionally applied Bayes Factor (B.F., for calculation see http://pcl. missouri.edu/bayesfactor) to make a statistical decision about the tested effects. The different ERP wave method, frequency analysis and LORETA technique were utilized to study brain and cortical electrical activities related to attentional competition and memory trace. **Results:** 1. For expert participants, tennis scene elicited a significantly larger potential of attentional competition than non-tennis scene, while this site difference was not significant in the novice group. 2.The attentional competition effect was observed to be strongest in theta band in posterior sites(POz,PO2, PO4,PO8,P6 and O2) around 200 ms . 3. The generators of the theta band of expert were consistently located in the precuneus, ROI analyses showed significant difference in the expert across the time 170-210ms, but the effect was not significant in novices **Conclusion:** Experts showed an increased attentional processing on skill-related scene information compared to novices. This attentional function was observed to be strongest in theta band and dynamically driven by the precuneus. This attentional superiority is likely due to memory trace related to their skill.

Disclosures: M. He: None. **C. Qi:** None.

Poster

168. Human Long-Term Memory: Encoding and Retrieval I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 168.15/Z27

Topic: H.02. Human Cognition and Behavior

Title: Recognition memory performance can be estimated based on few robust functional brain networks

Authors: *J. PETROVSKA, E. LOOS, D. COYNEL, T. EGLI, A. PAPASSOTIROPOULOS, D. J. DE QUERVAIN, A. MILNIK;
Univ. of Basel, Basel, Switzerland

Abstract: Recognition memory can result from familiarity or recollection. Deficits in both recognition qualities have been found in neurodegenerative and psychiatric disorders, such as Alzheimer's disease or schizophrenia. Establishing robust brain networks linked to familiarity and recollection can help understanding the neural basis of recognition memory performance in health and disease.
We used whole-brain fMRI data from 1'410 healthy young adults that performed a picture-recognition task. We decomposed the fMRI signal for previously seen vs. new pictures (old-new) into few brain networks by performing independent component analysis (ICA) in two independent samples (training sample *N*=645, replication sample *N*=665). Next, we investigated the relationship between the identified brain networks and interindividual differences in familiarity and recollection memory performances by conducting prediction analysis. Predication accuracy was estimated in a third independent sample (test sample *N*=100).
We identified 12 robust and replicable brain networks by applying ICA to the old-new fMRI signal. Based on these networks we were able to estimate familiarity and recollection performance with high accuracy (familiarity: $r = 0.68$, $p = 7.13 \times 10^{-15}$; recollection: $r = 0.45$, $p = 2.07 \times 10^{-06}$). Three of these networks, including frontal, frontal-parietal and occipital regions, were highly associated with both recognition qualities, additional two frontal networks specifically with familiarity and two occipital networks specifically with recollection.
Given the high prediction accuracy, the identified brain networks may be considered as potential biomarkers of familiarity and recollection performance in healthy young adults and can be further investigated in the context of neurodegenerative and psychiatric disorders.

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Poster

168. Human Long-Term Memory: Encoding and Retrieval I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 168.16/Z28

Topic: H.02. Human Cognition and Behavior

Support: University of Missouri Research Board

Title: Voluntary and involuntary autobiographical memory retrieval across the lifespan

Authors: *A. STROUD, R. TWOHY, E. BAI, C. NEILL, M. GUGLIANO, A. BELFI; Missouri Univ. of Sci. and Technol., Rolla, MO

Abstract: The ability to recall specific, vivid autobiographical memories tends to decline with age and in age-related neurological disorders. For example, autobiographical memories recalled by older adults contain fewer episodic details than those recalled by younger adults, resulting in

memories that are less episodically rich. However, such age-related differences in autobiographical memory have typically been found using tests of voluntary, or effortful, memory retrieval. Here, we investigated whether involuntary memory retrieval is more resistant to age-related decline. Involuntary autobiographical memories are those that come to mind without any effort, and are often evoked by sensory cues, such as music and images. In particular, music is often used as an effective cue for autobiographical memory retrieval in patients with dementia. We tested the prediction that autobiographical memories evoked involuntarily would show less age-related decline in episodic richness. Younger (n=30; 21-30 years old) and older adults (n=30; 65+ years old) completed the following task. Participants were shown two categories of stimuli: music and pictures. After each stimulus, participants stated whether the stimulus evoked an involuntary memory. If so, they verbally described the memory. If not, they were asked to retrieve a voluntary memory. In addition to these stimulus-cued memories, a standard voluntary autobiographical memory task, the Autobiographical Interview, was used. Younger (n=30; 21-30 years old) and older adults (n=30; >65 years old) completed the task. All memory descriptions were transcribed and coded to identify the number of internal and external details, a measure of episodic richness. We conducted a mixed-ANOVA to compare the within-subjects variables of retrieval effort (involuntary, voluntary), cue type (music, pictures, Autobiographical Interview), and the between-subjects variable age (younger, older). Results indicated that older adults showed significantly poorer performance for voluntary memories, but not for involuntary memories, as compared to younger adults. These results indicate that involuntary memory retrieval may be preserved in healthy aging, and may rely on different neural and cognitive mechanisms than those underlying voluntary memory retrieval. Finally, these results have important implications for the use of music listening as an intervention that may help improve quality of life in healthy aging and dementia.

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Poster

168. Human Long-Term Memory: Encoding and Retrieval I

Location: Hall A

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Program #/Poster #: 168.17/Z29

Topic: H.02. Human Cognition and Behavior

Support: AA005965
AA010723
AA013521

Title: Frontal correlates of verbal and visual memory in alcoholism: A double dissociation

Authors: R. FAMA[1,2], A.-P. LE BERRE[1], S. A. SASSOON[2], N. M. ZAHR[2], K. M. POHL[1], A. PFEFFERBAUM[2], *E. V. SULLIVAN[1];
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Abstract: Memory deficits occur in chronic heavy drinkers. Although global amnesia is a hallmark of alcoholic Korsakoff's syndrome (KS), an outcome of severe thiamine deficiency and untreated or undertreated Wernicke's encephalopathy (WE), alcoholics without a history of WE/KS can express impairment, albeit less profound, in verbal and nonverbal explicit memory processes. There is marked heterogeneity in the pattern and severity of memory deficits among alcoholics related to neurological and nutritional deficits, notably ataxia and dietary deficiency. Frontal brain regions, often reported to be affected in alcoholism, may contribute to impairment in explicit mnemonic processes.

Here we examined relations between regional frontal gray matter volumes and verbal and visual episodic memory performance (narratives and complex figure recall: immediate and delayed conditions) in 91 alcoholics (ALC, age 25-70 yrs) and 37 controls (CTRL, age 25-73 yrs). We also tested brain-behavior relations in the context of WE/KS-related signs, namely nystagmus, cognitive impairment, ataxia, and dietary deficiency modeled on Caine et al. (1997) who based these criteria on chart review of postmortem cases of non-WE/KS alcoholism and reflected the severity of brain lesions observed.

In the current study, the ALC group was impaired on verbal and visual memory tests compared with CTRL. Regression analyses indicated that frontal orbital volume was a unique predictor of verbal memory scores in ALC, accounting for 15.4% of variance in immediate recall and 18.6% for delayed recall scores. By contrast, frontal inferior volume was a unique predictor of visual memory scores, accounting for 9.8% and 6.1% of the variance in immediate and delayed recall scores. Analyses considering Caine criteria (no sign, 1 sign, 2 or more signs) indicated that this double dissociation occurred specifically in ALC who showed signs of ataxia and reported dietary deficiency: frontal orbital volume accounted for up to 23.6% of the variance in verbal memory scores, whereas frontal inferior volume accounted for <1%; conversely, frontal inferior volume accounted for up to 27.1% of the variance in visual memory scores, whereas frontal orbital volume accounted for ≤4.2% of the variance.

These results demonstrate that dissociable frontal regions contribute to verbal and visual episodic memory impairment in alcoholism, especially in the context of concurrent signs of ataxia and self-report of dietary deficiency. These findings provide further evidence for an association between orbitofrontal volume and verbal memory and suggest that inferior frontal volume may be associated with visual memory in alcoholism.

Disclosures: R. Fama: None. **A. Le Berre:** None. **S.A. Sassoon:** None. **N.M. Zahr:** None. **K.M. Pohl:** None. **A. Pfefferbaum:** None. **E.V. Sullivan:** None.

Poster

169. Human Long-Term Memory: Encoding and Retrieval II

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Program #/Poster #: 169.01/Z30

Topic: H.02. Human Cognition and Behavior

Support: NSF CAREER Award BCS-1844241

Title: Memory-guided attentional states are established by the hippocampus and medial prefrontal cortex

Authors: *E. GÜNSELI, M. ALY;
Psychology, Columbia Univ., New York, NY

Abstract: Attention is usually studied by telling individuals, with explicit external instructions, what they should attend to. But in daily life, we often have to call on memories of past experience to direct our attentional states. Based on previous evidence for higher activity levels in the hippocampus prior to memory-guided attention, and given the importance of the hippocampus and medial prefrontal cortex (mPFC) in memory-guided behaviors, we hypothesized that these regions would represent attentional states that are informed by retrieved memories even before those attentional states are used. To test this, we compared brain activity in two tasks that differed only in the demand to use memory to guide attention. In one task (memory-guided), participants selected an attentional goal based on memory for images in the preceding trial. In the other task (explicitly instructed), the attentional goal was randomly assigned on each trial. Activity levels in the hippocampus and mPFC were higher for memory-guided vs. explicitly instructed attention. The activity enhancement in these regions was correlated across individuals, suggesting that hippocampus and mPFC might be working together to coordinate memory-guided attention. Representational similarity analyses revealed that the hippocampus and mPFC support memory-guided attention by anticipating upcoming attentional states even before those states have to be deployed. Together, this work highlights the mechanisms by which the hippocampus and mPFC use memory to prepare for, and guide, goal-directed attention.

Disclosures: E. Günseli: None. **M. Aly:** None.

Poster

169. Human Long-Term Memory: Encoding and Retrieval II

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Program #/Poster #: 169.02/Z31

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R21HD083785-01A1
NIH Grant R01MH100121-06

Title: Evidence for differential neural reinstatement of associative memories in children and adults

Authors: *N. L. VARGA[1], H. E. ROOME[1], R. J. MOLITOR[1], L. MARTINEZ[1], E. M. HIPSKIND[1], A. R. PRESTON[1], M. L. SCHLICHTING[2];
[1]The Univ. of Texas at Austin, Austin, TX; [2]Univ. of Toronto, Toronto, ON, Canada

Abstract: Optimal decision making depends on retrieval and consideration of past experiences to guide behavior in new contexts. Neuroimaging evidence in adults has shown that, when making decisions about past experiences, hippocampus works in concert with sensory regions to reinstate prior relevant memories. Behavioral evidence further indicates that adults can retrieve information from partially overlapping cues, whereas children require greater overlap with the original experience to effectively cue retrieval. In the present research, we used pattern similarity analysis of functional magnetic resonance imaging (fMRI) data to provide a representational account of developmental differences in memory retrieval. Children (7-10 years) and adults (18-30 years) learned a series of object-scene and object-face associations to criterion. Following learning, fMRI activity was measured while participants retrieved the target associates (faces or scenes) in preparation for a match/mismatch decision. Decision accuracy was high in both groups, suggesting that participants were able to retrieve prior memories to guide decision making. To decode neural reinstatement during the preceding retrieval period, participants were pre-exposed to the individual faces and scenes in isolation prior to associative learning, thereby enabling estimation of the pattern of activity associated with perceiving each item. To control for between-group differences in neural patterns elicited during item perception, we identified sensory regions that exhibited equivalent, above-chance face and scene selectivity to the pre-exposure items in children and adults, including the inferior temporal gyrus (ITG; face), lingual gyrus (scene), and parahippocampal gyrus (PHG; scene). Within these regions, we then tested for reinstatement of the pre-exposure neural patterns during the retrieval task. Specifically, neural reinstatement was evidenced when the fMRI activation patterns associated with retrieval of a target item exhibited stronger correlations with activation patterns evoked during pre-exposure for items from the same versus a different stimulus category. Above-chance reinstatement of both faces (in ITG) and scenes (in lingual and PHG) was observed in adults. In contrast, children

showed significant reinstatement of scenes (in lingual) but not faces. Although children showed scene reinstatement, it was less robust than that observed in adults. These findings highlight key developmental differences in neural reinstatement and have implications for our understanding of how neural representations may influence development of behaviors that rely on memory retrieval.

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Poster

169. Human Long-Term Memory: Encoding and Retrieval II

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Topic: H.02. Human Cognition and Behavior

Support: NIH/NIA R24-AG054355 (subaward thereof)
NIH MH098023

Title: Memory-based choices: Computational mechanisms and neural signatures

Authors: *Z. ZHANG[1], S. WANG[1], S. HRISTOVA[1], M. GOOD[1], A. S. KAYSER[2], M. HSU[1]; [1]Haas Sch. of Business, Univ. of California, Berkeley, Berkeley, CA; [2]Neurol., Univ. of California San Francisco, San Francisco, CA

Abstract: Most existing work on the neural basis of economic decision making to date has focused on "stimulus-based choices" (SB-C), in which the decision is made largely based on information available in the environment. Such focus may have overlooked important constraints that other cognitive processes, such as memory, impose on decisions. To fill this gap, we examine an important class of so-called "memory-based choices" (MB-C), such as choosing a restaurant from memory. In contrast with SB-C, in which an externally defined set constrains choices, MB-C require decision makers to retrieve a set of relevant choice alternatives. Similarly, while SB-C is driven largely by valuation, we hypothesize that MB-C require subjects to first construct an internal choice set based on memory, after which they choose based on valuation of the options in the set.
We collected independent behavioral datasets for brands from two categories (fast food restaurants and running shoes) to define three measures: semantic memory, measured by a brand-related semantic fluency task (N=240); valuation, measured by choices in SB-C (N=1405); and MB-C, measured by menu-free brand choices in the same category (N=1405). To understand these data, we developed a computational model in which the first stage of MB-C, choice set generation, is modeled as a random walk on an associative network inferred from fluency data. The second stage of the model uses SB-C data to predict a value-based choice from the options

comprising the internal choice set. This two-stage model of MB-C, based on fluency and SB-C data, achieves impressive out-of-sample prediction accuracy (R-squared > 0.9) and improves substantially over the baseline value-only model or memory-only model ($p < 0.001$), supporting the critical role of memory-preference interactions in MB-C.
We next sought to characterize the neural basis of MB-C with an ongoing fMRI study in which all subjects completed SB-C, fluency, and MB-C tasks. Initial findings (N=12) are consistent with our model-based hypotheses. While stronger activations in valuation-related areas (e.g. striatum and orbitofrontal cortex) are found in MB-C compared with the fluency task, increased activations in memory circuits (e.g. parahippocampal gyrus) are seen in MB-C compared with SB-C.
These findings demonstrate the behavioral relevance of semantic memory to decisions, and propose a neurally plausible cognitive mechanism by which semantic memory influences and constrains decision making. They also bear potentially important implications for suboptimal decision making in pathological conditions that impact semantic memory, such as Alzheimer's disease and semantic dementia.

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Poster

169. Human Long-Term Memory: Encoding and Retrieval II

Location: Hall A

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Program #/Poster #: 169.04/Z33

Topic: H.02. Human Cognition and Behavior

Support: NIH-NINDS (R01-NS107727) to B.A.K.
NSF CAREER Award (BCS-1752921) to B.A.K.

Title: Competition induces adaptive exaggeration of memories

Authors: ***Y. ZHAO**[1], A. J. CHANALES[2], B. A. KUHL[1];
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Abstract: Neural differentiation is thought to be an adaptive means for reducing interference between similar memories (Hulbert and Norman, 2015; Duncan and Schlichting, 2018). Recent fMRI research indicates that differentiation can render competing memories less similar (in terms of neural activity patterns) than completely unrelated memories (Chanales et al., 2017; Favila et al., 2016). However, it is unknown if this exaggeration in neural representations corresponds to an exaggeration in the features of events that are actually remembered. Here we tested for competition-induced exaggeration in memory for object colors. In our study, pairs of objects were generated where the only difference between the paired objects was a 24°

differencein color. However, each object was associated with a unique face. In an initial behavioral study, participants (n = 34) learned the face-object associations across multiple learning runs that included study rounds and associative memory tests. After 14 study-test runs, participants completed a color memory test during which they recalled the color for each object using a color wheel. Successful face-object learning (during the study-test runs) was associated with significant exaggeration in color memory as measured by the final test. Namely, the remembered color distance between paired objects was greater than the veridical color distance between these objects. The magnitude of this exaggeration was also predicted by the success of face-object learning (i.e., better associative learning predicted greater color exaggeration). Thus, competition between paired objects induced an adaptive exaggeration of remembered feature values. In a separate fMRI study, participants (n = 8) completed 14 study-test runs outside of the scanner on day 1 and then returned on day 2 for a perception task and retrieval task that were completed inside the scanner. After scanning, participants completed a final color memory test. Preliminary fMRI results indicate that greater dissimilarity of lateral parietal activity patterns during object retrieval predicts greater exaggeration of remembered color values during the final memory test. This result is consistent with previous findings of adaptive feature representations in lateral parietal cortex (Favila et al., 2018). Collectively, our results demonstrate that (1) overlap between memories triggers an exaggeration of remembered features, (2) this exaggeration plays an adaptive role in reducing competition between overlapping memories, and (3) activity patterns in lateral parietal cortex track these adaptive changes in remembered features.

Disclosures: **Y. Zhao:** None. **A.J. Chanales:** None. **B.A. Kuhl:** None.

Poster

169. Human Long-Term Memory: Encoding and Retrieval II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 169.05/Z34

Topic: H.02. Human Cognition and Behavior

Support: NIH-NINDS (R01-NS107727) to B.A.K.
NIH-NINDS (R01-NS089729) to B.A.K.

Title: Repulsion and sharpening along diagnostic feature dimensions support resolution of memory interference

Authors: *M. L. DRASCHER, A. G. TREMBLAY-MCGAW, B. A. KUHL;
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Abstract: When memories share overlapping features, this results in interference and, ultimately, forgetting. With practice, however, interference between overlapping memories

subsides (Anderson et al., 1994; Norman et al., 2007). These learning-related reductions in interference are thought to be supported, at least in part, by differentiation of neural representations (Chanales et al., 2017; Favila et al., 2016; Hulbert and Norman, 2015). However, it remains poorly understood how overlapping memories actually change as neural representations are differentiated or, more generally, as interference subsides. Here, using a continuous, multidimensional feature space, we tested for learning-related changes in feature memory induced by competition. Subjects first studied and practiced remembering a set of artificially-generated face stimuli in an extended learning session. Critically, the set of learned faces included pairs of highly similar faces that only differed on a single face dimension (the diagnostic face dimension). After the learning session, participants repeatedly 'reconstructed' each of the faces, from memory, by manipulating randomly-generated face images along two dimensions. The two dimensions included the diagnostic dimension that discriminated the paired faces and a non-diagnostic dimension that did not discriminate the paired faces. We found learning induced two distinct changes in feature memory that specifically occurred for the diagnostic dimension. First, participants' reconstructions revealed exaggerated differences between paired faces (repulsion) that were significantly stronger on the diagnostic dimension than the non-diagnostic dimension. Second, participants' reconstructions were significantly less variable (or sharper) along the diagnostic dimension than the non-diagnostic dimension. Critically, each of these mechanisms (repulsion and sharpening) is likely to be adaptive in that it improves discriminability between competing memories. Indeed, both the repulsion and sharpening effects were stronger for faces that were better learned, suggesting that these targeted changes in feature memory play a role in interference-resolution. Collectively, these findings provide a compelling account of how memory features change in order to reduce interference and suggest specific behavioral changes that may be related to differentiation of neural representations. Preliminary fMRI results that investigate the relationship between behavioral feature memory and neural representational changes will be discussed.

Disclosures: **M.L. Drascher:** None. **A.G. Tremblay-Mcgaw:** None. **B.A. Kuhl:** None.

Poster

169. Human Long-Term Memory: Encoding and Retrieval II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 169.06/Z35

Topic: H.02. Human Cognition and Behavior

Support: NIH-NINDS (R01-NS089729)

Title: Repulsion of competing hippocampal representations parallels learning-related reductions in memory interference

Authors: *W. GUO[1], G. KIM[2], S. E. FAVILA[3], B. A. KUHL[1];
[1]Psychology Dept., Univ. of Oregon, Eugene, OR; [2]Korea Brain Res. Inst., Daegu, Korea, Republic of; [3]Psychology, New York Univ., New York, NY

Abstract: In episodic memory, the hippocampus is believed to play a critical role in disambiguating overlapping events (Yassa & Stark, 2011). Indeed, recent fMRI studies have shown that event overlap triggers an active "repulsion" of hippocampal activity patterns (Chanales et al., 2017; Favila et al., 2016). Critically, these changes in hippocampal representations are thought to reduce memory interference (Favila et al., 2016; Hulbert and Norman, 2014). Here, we sought to "time lock" the repulsion of competing hippocampal representations to behavioral measures of interference resolution. To this end, we tracked hippocampal representations of overlapping events over the course of an extended learning task and compared these hippocampal measures to behavioral measures of memory interference. The experiment consisted of 6 runs conducted during fMRI scanning. Each run consisted of a study phase, test phase, and exposure phase. During each study phase, participants (N = 12) learned 36 scene-object associations. Critically, the 36 scenes were comprised of 18 pairs of highly similar images (e.g., two barns; Favila et al., 2016), but each scene was associated with a unique object. After each study phase, participants completed a forced-choice associative memory test, in which each scene was shown along with two objects: the 'target' object and the object that was associated with the scene's pairmate (i.e., an interfering association). After each test phase, participants completed an exposure phase during which all of the scene images were presented again. The exposure phase was used to index the hippocampal activity pattern associated with each scene. Behavioral performance from the test phase showed robust improvement in discriminating between scene pairmates over the course of learning. Preliminary fMRI analyses revealed that, within the hippocampus, there was a targeted decrease in the representational similarity of scene pairmates and that the timing of these hippocampal changes paralleled the timing of behavioral improvement. Strikingly, the learning-related changes observed in the hippocampus were fully absent in early visual cortex (EVC). In fact, by the end of learning, the representational structure in the hippocampus was opposite to the representational structure in EVC. Collectively, these findings indicate that learning induces targeted "repulsion" between hippocampal representations of overlapping events and that these representational changes in the hippocampus parallel behavioral interference resolution. Future analyses will focus on linking hippocampal and behavioral changes at the level of individual subjects and individual memories.

Disclosures: W. Guo: None. **G. Kim:** None. **S.E. Favila:** None. **B.A. Kuhl:** None.

Poster

169. Human Long-Term Memory: Encoding and Retrieval II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 169.07/Z36

Topic: H.02. Human Cognition and Behavior

Support: CIHR

Title: Hippocampal and cortical contributions to spatiotemporal context memory for a remote real-world event

Authors: *N. B. DIAMOND[1], B. LEVINE[2];
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Abstract: Episodic and autobiographical memory retrieval involve reinstating the spatiotemporal context of past experiences. This includes not only 'when' an event occurred with respect to one's autobiographical timeline, but also the order and location of different sub-events comprising extended episodes. The hippocampus is thought to mediate this process, or set of processes, in virtue of its interactions with the cortex. Autobiographical memory retrieval continues to recruit the hippocampus months and years after encoding to the degree that memories remain vivid and contextually rich. However, still little is known about explicit retrieval of spatiotemporal contextual information at such long delays remains because autobiographical events are typically uncontrolled and unverifiable.
In the present experiment, participants visited a real-world museum exhibit with a track-like layout. They were tested on their memory for spatiotemporal relations among items encountered in the exhibit along with discrimination of conceptually similar lure items (Diamond, Romero, Jeyakumar, & Levine, 2018). Two years post-encoding, a subset of these participants completed an expanded follow-up test during fMRI scanning. Using partial least squares (PLS), a multivariate data reduction technique, we found sensitivity to inter-item order in the hippocampus and cortical recollection network, and sensitivity to distance in the medial prefrontal cortex and regions associated with egocentric navigation. Further analyses will explore hippocampal-cortical interactions supporting spatiotemporal context retrieval versus lure discrimination. The present findings provide novel behavioural and neural evidence concerning our ability to retrace trajectories through extended real-world experiences years after they occurred.

Disclosures: N.B. Diamond: None. **B. Levine:** None.

Poster

169. Human Long-Term Memory: Encoding and Retrieval II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 169.08/Z37

Topic: H.02. Human Cognition and Behavior

Support: Mind Science Foundation Grant
Cambridge Trust Scholarship

Title: Identifying and characterising the supramodal inhibitory control network in the brain - and its role in the regulation of memories, emotion, and actions

Authors: *S. SANKARASUBRAMANIAN[1], D. APSVALKA[1], Y. GUO[1], M. ANDERSON[1,2]; [1]MRC-Cognition and Brain Sci. Unit, MRC-CBU, Univ. of Cambridge, Cambridge, United Kingdom; [2]Behavioral and Clin. Neurosci. Institute, Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Motor and memory inhibition can be seen as similar processes involving the stopping of prepotent responses. Studies have hinted at a supramodal inhibitory control network in the brain which is engaged in the suppression of both unwanted memories and actions. A recent meta-analysis study showed that there are overlapping cortical and sub-cortical regions which get activated in both memory and motor control tasks (Guo et al, 2018). Through this study, we chose to investigate the role of the potential key players in this control network namely the right dorsolateral prefrontal cortex (rDLPFC) and the basal ganglia, and to causally identify the rDLPFC as a key node in the network using non-invasive brain stimulation.
Study 1 was exploratory, aimed at using motor and memory inhibitory control tasks, along with fMRI, diffusion weighted imaging (DWI) and dynamic causal modelling (DCM), in an attempt to infer the causal interactions between the nodes in the inhibitory network. Study 2 is planned to be a confirmatory study, wherein we attempt to use transcranial magnetic stimulation (TMS) to establish causal necessity of the rDLPFC as a key node. We plan to use psychophysiological measures along with self-reports, to assess emotional regulation.
Study 1: 33 healthy young adults were recruited for the study, and they performed two sessions inside the 3T scanner-the stop signal task which is a standard motor inhibition task and a version of the Think No Think (TNT) memory inhibition task. DWI was also acquired using a multi-band sequence.
Study 2:Three groups of 30 healthy young adults will be recruited for two sessions of motor and memory control tasks. Two of these groups control for stimulation and the region of stimulation. Continuous theta burst stimulation (cTBS) protocol is to be used to temporarily disrupt the region in rDLPFC (anatomically at the intersection of BA46/9/10).
The univariate fMRI analysis revealed that the conjunction of regions of activation seen in both the right DLPFC and the basal ganglia for the two inhibitory tasks closely overlap with the regions previously identified in the meta-analysis. Preliminary DCM analysis show that the key nodes identified do casually interact with each other. Analyses looking at how DCM parameter estimates, tract strengths from DWI, and behaviour correlates, are linked to one another, will be done next.
We predict that for Study 2, disrupting the specific key region in rDLPFC would manifest in decreased motor and memory control, leading to higher stop signal reaction times,lower rates of suppression induced forgetting, higher intrusions and higher psychophysiological and self-report measures, compared to the control groups.

Disclosures: S. Sankarasubramanian: None. **D. Apsvalka:** None. **M. Anderson:** None. **Y. Guo:** None.

Poster

169. Human Long-Term Memory: Encoding and Retrieval II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 169.09/Z38

Topic: H.02. Human Cognition and Behavior

Title: Mechanisms of regulation of intrusive autobiographical memories: A cognitive neuroscience perspective

Authors: *G. BARSUOLA[1], S. SANKARASUBRAMANIAN[1], D. APSVALKA[1], H. ENGEN[2], J. M. FAWCETT[3], M. C. ANDERSON[1];
[1]MRC Cognition and Briain Sci. Unit, Univ. of Cambridge, Cambridge, United Kingdom; [2]Deutsches Resilienz Zentrum, DRZ, Mainz, Germany; [3]Mem. Univ. of Newfoundland, St. John's, NL, Canada

Abstract: Introduction The flow of human thoughts is frequently plagued by unwanted cognitive activity, which has a profound impact on many aspects of our lives, for example interfering with task performance, planning, social behaviour (Clark and Rhyno, 2005). Importantly, unwanted thoughts and memories also play a major role in psychopathology. Studies have shown that inhibitory deficits might play a key role in maintaining intrusive memories and thoughts across psychopathologies, especially in PTSD, OCD, anxiety, and depression (Fawcet et al., 2014). Drawing on Benoit's Imagine/No-Imagine study based on personally relevant future fears (Benoit et al., 2016), we developed the Autobiographical Think/No-Think paradigm, a modified version of Anderson's Think/No-Think task (Anderson & Green, 2001) based on autobiographically grounded word pairs to study recurrent upsetting personal memories. This study represents the first attempt to use intrusion ratings in an Autobiographical Think/No-Think study. This novel paradigm enables us to elicit and control the recall of autobiographical intrusive involuntary memories in the lab. **Methods** 40 participants were tested for this behavioural study at the MRC Cognition and Brain Sciences Unit, University of Cambridge, Cambridge, UK. Unlike most studies, no standardised materials were used; rather, participants generated their own materials because of our focus on autobiographical memories. Participants were instructed to generate a list of twenty-two negative intruding events happened in the past three years and to select two key words ("cue word" and "code word"). Then they completed the pre-TNT phase, the TNT phase, and the post-TNT phase. **Analysis and Results** We predicted that memories would frequently intrude into awareness involuntarily initially, but that with repeated attempts to stop retrieval, intrusion frequency would decline. Our one-way ANOVA analysis confirmed that intrusions declined significantly from the first block to the

fourth. This intrusion decline is seen for all the range of emotions (fear, dissociation, sadness, surprise, anger, helplessness, shame, guilt, horror, disgust) found in the reported autobiographical memories. **Conclusions** These preliminary results indicate that participants gained increasing control over the intrusions of unwanted memories. This improved regulation of intrusive memories may reflect both increasing success at controlling retrieval and inhibition of suppressed traces that make them less intrusive over trials. Skin conductance data and neuroimaging data will shortly be added to this study, providing novel and insightful views on this topic.

Disclosures: G. Barsuola: None. **S. Sankarasubramanian:** None. **D. Apsvalka:** None. **H. Engen:** None. **J.M. Fawcett:** None. **M.C. Anderson:** None.

Poster

169. Human Long-Term Memory: Encoding and Retrieval II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 169.10/Z39

Topic: H.02. Human Cognition and Behavior

Support: I-CORE program of the Planning and Budgeting Commitee (ISF Grant 51/11)
ISF Grant 526/17

Title: The effects of motivated forgetting on contextual memory

Authors: *S. KOZAK, N. HERZ, Y. BAR-HAIM, N. CENSOR;
Sch. of Psychological Sci. and Sagol Sch. of Neurosci., Tel-Aviv Univ., Tel-Aviv, Israel

Abstract: Contextual information was found to affect the ability to retrieve encoded memories, and to generalize the encoded information to new situations. In the clinical field, it is believed that maladaptive context encoding and generalization relates to mental disorders such as PTSD. Therefore, modification of memory for contextual information could influence impaired memory retrieval and generalization involved in these conditions. In humans, memory could be modified and forgotten through active voluntary process such as directed forgetting (DF). In the DF list-method paradigm, subjects study two lists of words and receive a cue to forget one of them. The forget cue was shown to both impair recall for the words in the 'forget' list and enhance recall for words in the subsequent list. While the mechanisms underlying this paradigm are not fully understood, studies suggest that the forget instruction influences memory by changing its context. Based on these studies, the list-method might prove effective in modifying contextual memory. In the current study, participants studied two lists of neutral words embedded within neutral pictorial context. The contextual images were shown before and after each word. Participant were instructed to study only the words, and as in previous DF experiments, received a cue to forget one of the lists. While participants were asked to forget or remember only the

words, memory for the words and pictorial context was tested at the end of the study session and on the following day. Preliminary results indicate that consistent with previous studies, the forget instruction modified the content of the memory (word recall). Interestingly, the forget instructions also affected recognition for the contextual memory (pictures). The results therefore suggest that motivated forgetting modifies not only the content of the memory, but also its context. Additional follow-up experiments will test these mechanisms in memories with emotional content. These findings expand our understanding of the effects of memory suppression on contextual memory and could prove relevant to clinical settings.

Disclosures: S. Kozak: None. **N. Herz:** None. **Y. Bar-Haim:** None. **N. Censor:** None.

Poster

169. Human Long-Term Memory: Encoding and Retrieval II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 169.11/Z40

Topic: H.02. Human Cognition and Behavior

Title: Memory guided preferential viewing behavior spans context changes

Authors: *M. R. DULAS, H. SCHWARB, N. J. COHEN;
Beckman Inst., Univ. of Illinois, Urbana, IL

Abstract: The hippocampus supports the reactivation of relational information when returning to a previously studied context, such as retrieving which person was previously associated with a location. Further, this reactivation guides eye-movements towards previously learned item-context associations (preferential viewing). But, contexts often change over time, requiring the integration and discrimination of information within that context across events (e.g. a person that works at a store during the day may not work there at night). It is unknown whether changes to a studied context impact relational reactivation and fast preferential viewing effects. Here, we assess whether visual/temporal context changes (day vs. night) impact memory-guided preferential viewing. Further, we evaluate whether memory behaviors differ depending on instructions to treat day vs. night variants of a location as the same or distinct contexts. Participants first studied face-scene pairs, and were asked to think of a job each person could do at that location. On each trial at test, participants were first presented with a scene and had to indicate whether the scene was exactly the Same as at study (same place, same time of day) or if it was Similar to a studied scene (same place, different time). Next, three faces appeared with the scene and participants had to select the appropriate face. For Same scenes, this was the studied face. However, for Similar scenes, half of the participants were instructed to treat day/night scene variants as the same context, and to select the studied face (Integrate group). The other group was told to treat these time-changed scenes as distinct contexts and to select a different face than the studied associate (Discriminate group). Results showed that both groups were

equally good at identifying Same vs. Similar scenes and at selecting studied faces for Same scenes. Both groups also showed fast (500-1000ms), preferential viewing to the studied face for Same scenes. For Similar scenes, the Discriminate group was significantly worse than the Integrate group at selecting the appropriate face. The Integrate group showed nearly identical preferential viewing effects for Same and Similar scenes. Interestingly, despite instructions, the Discriminate group also showed initial preferential viewing to the previously learned, but no longer valid face, before later shifting to the new, correct face. Results suggest that fast, preferential viewing behavior, previously tied to the hippocampus, is robust even in the face of visual/temporal context changes, and that this viewing behavior is obligatory, occurring even when we need to ignore previously learned associations.

Disclosures: M.R. Dulas: None. **H. Schwarb:** None. **N.J. Cohen:** None.

Poster

169. Human Long-Term Memory: Encoding and Retrieval II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 169.12/Z41

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant F99NS105223

Title: Incidental spatial encoding in human visual memory

Authors: *S. E. FAVILA, J. WINAWER;
Psychology, New York Univ., New York, NY

Abstract: Human neocortex is tiled with maps of the visual field. We previously demonstrated that these maps encode remembered spatial locations during cued long-term memory retrieval (Favila et al., SfN 2018). Here, we aimed to extend this work by addressing new questions: 1) Does spatial reactivation in these maps relate to memory fidelity on a trial-by-trial basis? 2) Does spatial reactivation occur when spatial information is irrelevant during memory encoding and retrieval? We designed an experiment motivated by both of these questions. We first identified a large set of object images that could be recognized in the near periphery. To do this, we exposed six human subjects to over 1400 normed stimuli made available in the Bank of Standardized Stimuli (BOSS). Stimuli were presented at 2 degrees eccentricity in one of four positions (45, 135, 225, or 315 degrees of polar angle). Subjects rated whether they could recognize each stimulus and indicated their confidence. Responses were sorted by recognition, confidence, and response time to identify the most easily recognized 480 stimuli. In the main experiment, a different group of subjects encountered 240 of these object images in a single-shot incidental memory encoding task. Each object was presented once in one of the four visual field locations used to identify recognizable stimuli. On each trial, subjects maintained central fixation and

made a judgment about whether the real-life size of the object was smaller or larger than a shoebox. The location of the object in the visual field was incidental to this task. Subjects then performed surprise recognition and source memory tasks. During the recognition memory task, subjects were presented with 480 images (240 old and 240 new) at central fixation and indicated whether each image was old or new. Recognition memory performance was robust, with an average hit rate of 79.4% and an average false alarm rate of 13.6%. For stimuli judged 'old', subjects were given a four alternative forced choice task that required them to choose the previous location of the stimulus. Subjects chose the correct location 58.9% of the time, and chose the correct hemifield 75.7% of the time. These behavioral results verify that human subjects can reliably report spatial information encountered incidentally, briefly, and only once, in a long-term memory task. fMRI data collected during the recognition memory task will examine the amplitude and precision of spatial reactivation within visual field maps, and relate this to recognition and spatial memory performance on a trial-by-trial basis.

Disclosures: S.E. Favila: None. **J. Winawer:** None.

Poster

169. Human Long-Term Memory: Encoding and Retrieval II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 169.13/Z42

Topic: H.02. Human Cognition and Behavior

Support: NIH R00MH103401

Title: Probing intra-network dynamics of the posterior medial system during episodic memory retrieval

Authors: *R. COOPER, M. RITCHEY;
Boston Col., Chestnut Hill, MA

Abstract: Episodic memory retrieval reliably engages a 'core recollection' network of medial temporal and posterior medial (PM) brain regions, which show concurrent increases in activity and functional communication during the reconstruction of rich, multi-sensory information. Despite the fact that much research has consistently demonstrated these neural effects, it remains unknown how intra-network dynamics of the hippocampal-PM system operate to support the retrieval of complex past events. Here, we probe the specific pattern of PM connections that change during memory retrieval and uncover the region and connection dependencies that shift when we remember previous experiences. We analyzed functional magnetic resonance imaging data from a study of 28 participants who encoded and retrieved a set of multi-featural events, each including an object-location-sound association. Analyses were conducted by first estimating the mean activity of each encoding and retrieval trial within a set of six PM brain regions.

Contrasting changes in intra-network functional communication revealed a more integrated network structure during memory retrieval compared to encoding, with disproportionate increases in connectivity of the parahippocampal cortex and precuneus with the rest of the network. Precuneus activity accounted for more variance in other PM connections, revealing it as a process-specific network hub during retrieval. We further found that connections involving posterior hippocampus increased their dependency to other network edges, in that hippocampal connectivity was associated with enhanced communication throughout the network, suggesting that these paths might be important for guiding the flow of information during episodic retrieval. Together, these analyses provide the first step towards a model of how complementary core recollection network processes facilitate the mental reconstruction of events.

Disclosures: R. Cooper: None. **M. Ritchey:** None.

Poster

169. Human Long-Term Memory: Encoding and Retrieval II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 169.14/AA1

Topic: H.02. Human Cognition and Behavior

Title: Unique and overlapping contributions of posterior medial network nodes to predicting recollection outcomes

Authors: *K. A. KURKELA, M. RITCHEY;
Boston Col., Chestnut Hill, MA

Abstract: Recollection-based memory has been associated with activity in a set of medial temporal and posterior medial (PM) regions collectively referred to as the core recollection network. Although the activity and connectivity of PM regions has been shown to reliably distinguish between successful and unsuccessful episodic retrieval, it remains unknown how these regions complement and/or interact with another to predict memory success. In this study, we aim to disentangle the unique and overlapping contributions of PM regions to predicting memory outcomes during retrieval. To do so, we performed a univariate decoding analysis by iteratively building logistic regression models and testing the accuracy of their predictions using a leave-one-trial-out cross validation procedure. Importantly, we performed the decoding analysis using a combinatorial region of interest (ROI) approach, testing the predictive power of mean BOLD activation in each PM region both in isolation and in all possible combinations. The combinatorial approach allowed us to test for both redundancy and complementary effects on prediction for all subsets of nodes within the network. That is, for each combination of regions, we tested whether the nodes were informationally redundant with one another (i.e., they made the same contribution to predicting recollection outcomes) or whether they were complementary to one another (i.e., each region conferred unique prediction benefits). Results suggest that PM

network regions play complementary roles in supporting memory recollection, such that mean activation across combinations of nodes supports the most accurate prediction of recollection success.

Disclosures: K.A. Kurkela: None. **M. Ritchey:** None.

Poster

169. Human Long-Term Memory: Encoding and Retrieval II

Location: Hall A

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Program #/Poster #: 169.15/AA2

Topic: H.02. Human Cognition and Behavior

Support: JSPS KAKENHI Grant Number JP18H04193
JSPS KAKENHI Grant Number JP18J20036

Title: Different activation patterns during the temporal-order judgment in memory for face-name associations between happy and neutral facial expressions

Authors: *S. IWATA[1,2], T. TSUKIURA[1];
[1]Cogn and Behav Sci., Kyoto Univ., Kyoto, Japan; [2]JSPS, Tokyo, Japan

Abstract: The contextual information of time is one of the essential components in episodic memories. Functional neuroimaging studies have demonstrated that the lateral prefrontal cortex and hippocampus (HC) play an important role in the temporal-order judgment (TOJ) between episodic events (Wang and Diana, 2017), and that the contribution of these regions to TOJ is dissociable between short- and long-lag events (Suzuki et al., 2002). In addition, there is fMRI evidence that the effects of emotion on episodic memory are involved in the interacting mechanisms between the amygdala (AMY) and HC (Dolcos et al., 2017). However, little is known about the neural correlates underlying the modulatory effect of emotion on TOJ in episodic memory, which has been found in psychological studies (Chiu et al., 2013). To investigate this issue, our fMRI study scanned 42 right-handed and college-aged healthy adults (20 females, mean age: 21.91, SD: 1.52) during the recency judgment as TOJ between long-lag events. All participants performed the 3 day-experimental sessions, which included the study sessions in Day 1 and 2 without fMRI and the recency judgment session in Day 3 with fMRI. In the study sessions, participants were randomly presented with face-name associations in a happy or neutral expression one by one, and were required to study them. In the recency judgment session, participants were randomly presented with sets of two names learned in Day 1 and 2 one by one, and were required to judge which name had been studied more recently. All trials in the recency judgment were categorized into successful (Correct) and unsuccessful (Incorrect) judgment trials, and were subdivided into the Happy and Neutral conditions decided by facial expressions associated in the recent day (Day 2). In addition, all analyses were focused only on

data from participants who showed better scores than -1SD of mean scores of face-name associations in Day 1 and 2. In behavioral results, there was no significant difference in the Correct rates and response times during the recency judgment between the Happy and Neutral conditions. In fMRI data, significant activation related to the successful recency judgment (Correct vs. Incorrect) in both Happy and Neutral was identified in the right HC and left middle frontal gyrus. In addition, successful recency judgment activation correlated with individual Correct rates was significantly identified in the right AMY and HC for Happy, and in the right middle temporal gyrus for Neutral. These findings suggest that the day-by-day TOJ in memory for face-name associations could be involved in dissociable neural correlates between happy and neutral facial expressions.

Disclosures: S. Iwata: None. **T. Tsukiura:** None.

Poster

169. Human Long-Term Memory: Encoding and Retrieval II

Location: Hall A

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R01AG054628

Title: Holistic recollection and incidental reinstatement of scene context

Authors: A. TAMM[1], K. CAMPION[1], R. HENSON[2], A. FORSBERG[1], S. COX[1], ***A. M. MORCOM**[3];
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Abstract: Computational theories of memory propose that recollection is supported by reinstatement of distributed cortical representations of events, triggered by hippocampal (HC) pattern completion. These theories predict that recollection is holistic, with simultaneous reinstatement of multiple dimensions of unique events (e.g., location, sensory modality). We report support for this prediction from a preregistered functional magnetic resonance imaging (fMRI) study (https://osf.io/hndbq). We aimed to use representational similarity analysis (RSA) to replicate and extend a previous study which found evidence for event-unique reinstatement. Staresina, Henson, Kriegeskorte & Alink (2012) showed that when people successfully

recollected the scene that a word had been studied with, multivoxel activity patterns in parahippocampal cortex (PHC) were more similar to the patterns observed during the original event than to patterns elicited by other words studied with that scene. The current study asked whether this reinstatement would also be found when recollection of the scenes was incidental to the memory judgement. Across 5 study-test cycles, men and women (N=28) encoded 120 items as auditory words or pictures, rating their fit with scene backgrounds. At test they judged whether visual words referred to items previously heard or items seen as pictures. Scene names were given as cues prior to test word presentation, but source memory for study modality did not require any recall of the scene context. Representational similarity between encoding and retrieval phases was computed using Fisher-transformed Pearson correlations of their voxel-wise regional activity patterns, after adjusting for cue-locked responses. As in the previous study we found evidence for event-unique scene context reinstatement in PHC during successful source recollection, even though now the source was the modality of the study episode, not the scene itself. We also replicated Staresina et al.'s finding that over trials, activation in HC activation predicted PHC reinstatement, with evidence also of scene reinstatement in HC. The data support the notion that the HC is involved in holistic cortical reinstatement with recovery of multiple information dimensions of a unique experienced event.

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Poster

169. Human Long-Term Memory: Encoding and Retrieval II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 169.17/AA4

Topic: H.02. Human Cognition and Behavior

Title: A role for schema in establishing rapid relational memory associations in the human brain

Authors: *J. P. PAULUS, M. N. COUTANCHE;
Psychology, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The integration of newly-learned information into memory is influenced by prior knowledge through the use of schemas. Schema-consistent information is more rapidly consolidated into cortical regions than schema-inconsistent information. Following prior studies that have found differences in univariate activity in regions such as the angular gyrus (AG) and medial prefrontal cortex (mPFC) between information that is consistent and inconsistent with a schema, this study investigates the multivariate patterns of schema consistent/inconsistent word pairs in an associative memory task that includes untaught relational associations. In a pre-scanning training session, participants first learned four words in pairs of occupations and locations (A-B, B-C, C-D) that were either consistent (teacher-classroom) or inconsistent (nurse-

bar) with a schema. Participants were then asked to retrieve explicitly taught (A-B, B-C, C-D) and untaught relational (A-C, A-D) associations while undergoing a functional magnetic resonance imaging (fMRI) scan. A machine learning classifier revealed multivariate differences in activity patterns between schema-consistent and inconsistent word pairs in the left and right AG, mPFC, Visual Word Form Area (VWFA), and hippocampus.

Disclosures: **J.P. Paulus:** None. **M.N. Coutanche:** None.

Poster

169. Human Long-Term Memory: Encoding and Retrieval II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

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Topic: H.02. Human Cognition and Behavior

Support: This research was supported by NRF-2019R1A2C2005213 and NRF-2018R1D1A1B07041409.
This research was supported by IBSR015-D1

Title: One year after long-term value learning: Remembering the historical values of visual objects both explicitly and implicitly

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Abstract: Long-term experience with object values leads to fast and accurate saccades to find learned good objects without reward outcome, called visual habit (Kim and Hikosaka, 2013). In previous study (Kim HZ et al., 2017 SfN poster), we found that 5-day of learning in object-reward association task generated visual habit after a 1-day retention period with human subjects. What we call the habit, however, are often sustained for more than a year in real life, and the retention period is not sufficient to be called the long-term. We thus have a question: Does our learning task generate a year-lasting long-term memory? If so, is there any difference in retention between explicit and implicit long-term memory?
To address the questions, we trained 23 normal participants for 5 days with the object-value associative learning task in which subjects chose one of two fractal objects by saccade and received monetary rewards according to the chosen object values. After more than 1 year, we tested the explicit and implicit memories of the same 9 subjects. In explicit memory task, subjects answered whether the objects were familiar, novel, good, neutral or bad without feedback. Subjects successfully discriminated learned objects from novel ones, and the discrimination rate of good objects (75%) is slightly higher than the rates of bad (50%) and neutral (29%) ones. The data show that subjects explicitly remembered the familiarity and values

of objects.
To test the implicit memory, we investigated the visual habit during free gazing and free decision tasks. In the free gazing task, subjects were instructed to look at 9 objects that were learned more than a year ago freely on the screen without reward outcome and feedback. Interestingly, rate of the first gaze to good objects (38%) was higher than to neutral (28%) and bad (33%) ones, indicating that the eyes were implicitly guided based on the 1-year retained value memory. However, we did not found differences in the number and duration of gaze, which showed the differences 1 day after learning. In free decision task, subjects were instructed to freely choose one of two objects that were learned more than a year ago without reward outcome and feedback. We found that subjects more chose good objects (62%) than neutral (42%) and bad objects (45%). Our data showed that the long-term learned values of visual objects faded but persisted more than 1 year after the last learning, and subjects explicitly discriminated the values and implicitly generated visual habit.

Disclosures: S. Hwang: None. **H.Z. Kim:** None. **H.F. Kim:** None.

Poster

169. Human Long-Term Memory: Encoding and Retrieval II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 169.19/AA6

Topic: H.02. Human Cognition and Behavior

Support: Geisel School of Medicine at Dartmouth, Research Stipend

Title: Accelerated long-term forgetting in patients with seizures

Authors: *R. L. TOM[1], E. D'AGOSTINO[2], B. C. JOBST[3];
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Abstract: Objective: To identify differences in memory among patients with epileptic or psychogenic seizures compared with healthy controls.
Background: Patients with epilepsy report that difficulty remembering impacts everyday life, causing significant distress. Studies have shown that short-term memory may not be impaired, while long-term memory degrades at a significantly higher rate. This phenomenon has been termed accelerated long-term forgetting.
Methods: Patients with seizures were admitted to the Dartmouth-Hitchcock Medical Center for video-EEG monitoring. Patients and healthy controls were asked to watch a 30-minute nature film. (Only patient EEG data were collected and synced with the film in 1 second intervals.) All participants were then asked to answer questions about the film at two time points: (1) immediately after watching, and (2) 24 hours after watching. Participants could choose to

provide their own response (free recall) or, if unsure, to choose the correct answer on the next screen (recognition). They then rated their confidence level in their chosen answer. Each participant received three questions per scene of the film targeting a distinct type of memory: verbal, episodic, and visual. After discharge, patients' seizures were characterized as epileptic or psychogenic using EEG data.
Results: Same-day recall is impaired in patients with epileptic and psychogenic seizures compared to controls. Those with epileptic seizures have a decreased confidence rating, potentially because of the stigma associated with their disorder. 24-hour recall is impaired in patients with epileptic seizures, which demonstrates accelerated long-term forgetting. There is no difference in the 24-hour recall of patients with psychogenic seizures and controls.
Conclusions: Our results indicate impairments in same-day and 24-hour tests of memory in patients with epilepsy. Patients seem acutely aware of their poor performance, as they consistently rate their confidence lower than controls. These results suggest that epileptic seizures alter cognition in a way that psychogenic seizures do not.

Disclosures: R.L. Tom: None. **E. D'Agostino:** None. **B.C. Jobst:** None.

Poster

169. Human Long-Term Memory: Encoding and Retrieval II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 169.20/AA7

Topic: H.02. Human Cognition and Behavior

Title: Interactive effects of endogenous testosterone and cortisol levels on emotional episodic memory performance in college aged men

Authors: *A. MARTINEZ TLATENCHI[1], J. D. PAYNE[2];
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Abstract: Human and animal research has indicated testosterone is involved in hippocampal functioning and development (Pike, 2001; Galea et al. 1999). However, studies that investigate the relationship between testosterone and hippocampal functioning have overwhelmingly focused on the effects of testosterone in isolation, with little consideration for other neuroendocrine factors. Yet evidence from the field of behavioral neuroendocrinology suggests cortisol and testosterone systems exhibit mutual inhibitory effects which modulate behavior (Mehta and Prasad, 2015). The aim of the present study was to examine the interactive effects of testosterone and cortisol in relation to emotional memory performance in a sample of college aged men. Participants (N = 53; Men; Age: 18 - 24; 29 control, 25 stress) provided saliva samples prior to and after a stressor or matched control condition, completed an encoding task in the afternoon, and were tested for memory performance the following morning. Area under the curve with respect to ground (AUCg; Pruessner et al., 2003) measures for cortisol and

testosterone were utilized. Cortisol and testosterone AUCg measures were positively correlated ($r = 0.296$, $p = .035$) regardless of stress condition. A MANOVA revealed a significant testosterone by cortisol interaction ($F_{(4, 48)} = 2.976$, $p = 0.033$), such that when cortisol was low (1 SD below the mean) and testosterone was high (1 SD above the mean) average emotional object memory was enhanced. These findings were also observed in the opposite direction such that when cortisol was high (1 SD above the mean) and testosterone was low (1 SD below the mean) average negative object memory performance was impaired. Our results, in conjunction with previous research, suggest a need to consider the interactive effects of multiple hormones in order to understand emotional episodic memory performance.

Disclosures: A. Martinez Tlatenchi: None. **J.D. Payne:** None.

Poster

169. Human Long-Term Memory: Encoding and Retrieval II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 169.21/AA8

Topic: H.02. Human Cognition and Behavior

Title: Patterns of spatial memory activity are more similar within than between sex

Authors: *D. S. SPETS[1], S. D. SLOTNICK[2];
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Abstract: In a previous fMRI investigation, we identified sex differences in the human brain during visual spatial long-term memory. During encoding, participants maintained central fixation while viewing abstract shapes in the left or right visual field. During retrieval, old shapes were presented at fixation and participants classified each shape as previously in the “left” or “right” visual field. We selected eighteen female participants (from forty) to match the behavioral spatial memory accuracy and standard error of the eighteen male participants. We found different patterns of activity for females and males during spatial long-term memory, including greater activity in language processing regions for females and greater activity in the hippocampus for males. It is possible that such differential brain activity may have reflected individual differences between the two groups rather than sex differences. To determine if this was the case, we ran within and between sex multi-voxel pattern correlation analyses on voxels contained in functional regions-of-interests (ROIs). The functional ROIs were defined as the union of activity produced from the contrasts of female spatial memory hits > misses and male spatial memory hits > misses ($p < .001$, corrected for multiple comparisons to $p < .05$). For each pair of female and male participants, all voxels within the functionally defined ROIs ($N = 3,096$) were correlated with the same set of voxels (using a Pearson correlation). The independent correlation matrix values were used to compute within and between sex correlations. If the observed differences in the group activation maps were due to other individual differences, rather

than sex, there should be no difference in the within and between sex correlational values. However, the correlation within sex ($t(305) = 4.83$, $p < 1x10^{-5}$) was significantly higher than the correlation between sex ($t(323) = -3.94$, $p < 1x10^{-4}$; $F(1, 628) = 37.87$, $p < 1x10^{-8}$). This indicates that the patterns of activity within sex were more similar during spatial long-term memory than patterns of activity between sex. Furthermore, the patterns of activity between sex were anti-correlated, which suggests that spatial memory for females and males often evoked activity that was opposite in sign. The current results suggest that the observed sex differences in patterns of cortical activity during spatial long-term memory are due to sex rather than other individual differences between the groups.

Disclosures: D.S. Spets: None. **S.D. Slotnick:** None.

Poster

170. Encoding and Retrieval in High-Level Content and Naturalistic Protocols

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 170.01/AA9

Topic: H.02. Human Cognition and Behavior

Support: Sloan Research Fellowship, Janice Chen

Title: Measuring behavioural and neural responses to fluctuations in real-world predictability

Authors: *B. BELLANA, H. LEE, X. ZUO, J. CHEN;
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Abstract: How does the human brain respond to predictability in the environment? Our continuous stream of experience has an ebb and flow of predictable and uncertain periods. This fluctuation may have a purpose: during uncertain times we gather new information for *building* internal world models, then during the more predictable periods we *test* and refine those models. Despite this prominent dynamic, the current paradigms for studying predictability incorporate the dimension of time in a highly artificial manner, if at all (e.g., gambling tasks). Therefore, we propose to study the dynamics of predictability, and its relationship to information-seeking, using audiovisual movies of the kind seen in theaters. Such narratives are a microcosm of real life, capturing fluctuations in predictability at the level of relationships between entities, actions and outcomes ostensibly similar to our daily lives. 160 human subjects watched an 11-minute clip of the movie “Catch Me If You Can” online. Viewing was interrupted periodically (~1 time per minute) by asking participants to type predictions about what they thought would happen in the next 30 seconds, followed by confidence rating on a 7-point scale. The onset of the interruptions was counterbalanced such that 20-25 participants generated predictions for every 10 seconds of the movie. Each prediction was then converted into a high dimensional vector using Google’s Universal Sentence Encoder (USE), where similar sentence vectors reflect similar semantic

meanings. An across-subject timecourse of predictability was calculated by averaging pairwise cosine similarity estimates across all predictions generated at each timepoint. A similar timecourse was calculated for confidence. Group-level prediction and confidence timecourses were positively correlated ($r = .34$, $p = .005$). A separate group of 23 human subjects watched the same movie clip while scanned (fMRI). The predictability timecourse was then up-sampled to match the brain data (TR = 1.5s) and regressed onto each voxel of the brain. Activity in regions of the default mode network (DMN) positively coupled with real-world predictability. This is consistent with previous work suggesting the DMN supports situation models of an unfolding narrative (e.g., Chen et al., 2017). Interestingly, regions of the superior temporal gyrus and visual cortex showed the opposite relationship, where activity tracked uncertainty. Overall, this paradigm provides unique insight into the neural correlates of real-world fluctuations in predictability. Future analyses will further examine how real-world predictability relates to information seeking in behaviour and the brain.

Disclosures: **B. Bellana:** None. **H. Lee:** None. **X. Zuo:** None. **J. Chen:** None.

Poster

170. Encoding and Retrieval in High-Level Content and Naturalistic Protocols

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 170.02/AA10

Topic: H.02. Human Cognition and Behavior

Title: Narratives as networks: Predicting memory from the structure of naturalistic events

Authors: *H. LEE, J. CHEN;
Johns Hopkins Univ., Baltimore, MD

Abstract: Much of our experience in real life consists of multiple inter-related events. However, little is known about whether and how the relationship between event components, or the 'structure' of an experience, affects the way we process and remember the experience, as traditional studies have mostly relied on trial-based paradigms using simple and highly controlled stimuli. In this study, we used a naturalistic movie-viewing and free recall paradigm with fMRI to investigate how the structure of complex realistic experiences relates to the behavioral and neural markers of memories for those experiences. Fifteen subjects watched a series of 10 short movie clips and then verbally recalled the movie plots while being scanned. To quantify and assess the structure of the movies, we employed a novel approach wherein we transformed a narrative into a graph/network. In this narrative network, the events that constitute a movie plot (nodes) form connections with each other (edges), and the connection strength between a pair of events is determined by their content similarity (edge weight). To build a narrative network for each movie, human annotators first segmented the movies into shorter events and provided written descriptions for each event. The text descriptions were in turn

encoded into high-dimensional vectors with Google's Universal Sentence Encoder (USE). The connection strength between event node pairs was computed by correlating their corresponding USE vectors. We first examined the relationship between behavioral memory performance for individual events and their relative importance in the narrative network (i.e., the overall quantity and quality of connections with all other events) measured as PageRank centrality, and found a positive correlation between the centrality and the recall probability/accuracy of events. Next, analyses of the neural data showed that higher centrality of events predicted more consistent multi-voxel patterns across subjects during recall in the posterior medial cortex (PMC), an area thought to represent abstract 'situation models' of events. This positive correlation was not observed in control sensory areas. Whole-brain representational similarity analysis revealed that the text-based narrative network structure was most similar to the neural pattern similarity structure across recalled events in the default mode network (DMN) regions including PMC and angular gyrus. Overall, our results suggest that rich connections between events in a complex narrative protect against forgetting, and neural patterns in the higher-level associative areas of the DMN can reflect the structure of narratives during memory recall.

Disclosures: H. Lee: None. **J. Chen:** None.

Poster

170. Encoding and Retrieval in High-Level Content and Naturalistic Protocols

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 170.03/AA11

Topic: H.02. Human Cognition and Behavior

Support: IBS-R015-D1

Title: Decoding narratives from fMRI responses of the present and causally related past events during movie-watching

Authors: *H. SONG[1], H. KO[2], J. PARK[3,1], J. LEE[2], W. SHIM[3,1];
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Abstract: Understanding a story involves continuous reinstatement of past events that are causally related to the present (Trabasso & Langston, 1998). Recent studies were successful in decoding the contents of the movie scenes represented in the brain, by linearly mapping fMRI responses of the movie-watching subjects to natural language annotations (Nishida & Nishimoto, 2017; Vodrahalli et al., 2017). Here, we ask if incorporating fMRI responses of causally related past events improves decoding of narrative contents of the movie. The narrative contents of the temporally scrambled silent films were annotated per time step and transformed into a high-

dimensional feature space using a GloVe word embedding model (Pennington et al., 2014). In a separate behavioral experiment, the causal relatedness of each pairwise moment in the films was rated. Multiple subjects' fMRI data were obtained during movie-watching and were aggregated into a group-level shared response (Chen et al., 2015). We employed regularized linear regression to predict temporally corresponding narrative content given shared fMRI response ('Present'). Crucially, we incorporated, as contexts, fMRI responses of past time points that were i) highest in causal relatedness ('Present + Causal Past'), ii) contiguous in time ('Present + Near Past'), or iii) randomly selected ('Present + Random Past'). The prediction accuracy improved considerably by combining the causally related past events with the present moment, compared to the 'Present + Near Past' and 'Present' conditions. There was significant decrease in accuracy for 'Present + Random Past,' indicating that merely adding past events did not increase the prediction performance. The performance improvement via combining causally related past events was most pronounced when the task was decoding the narrative contents, compared to decoding low-level visual features or semantic content of the movie. Our results suggest that the brain constructs coherent narratives by integrating information from the causally related past with the present moment.

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Poster

170. Encoding and Retrieval in High-Level Content and Naturalistic Protocols

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 170.04/AA12

Topic: H.02. Human Cognition and Behavior

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ESRC Wales Doctoral Training Partnership PhD studentship

Title: Connecting the past and the future: The role of the pre-commissural fornix in episodic autobiographical memory and simulation

Authors: A. N. WILLIAMS, S. RIDGEWAY, M. POSTANS, K. S. GRAHAM, A. D. LAWRENCE, ***C. J. HODGETTS**;
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Abstract: Neuropsychological and functional MRI evidence suggests that the ability to remember our personal past, and imagine future scenarios, involves two closely connected regions: the hippocampus and ventromedial prefrontal cortex (vmPFC). Despite the existence of direct anatomical connections between these regions, particularly via the fornix, it is unknown whether hippocampal-vmPFC structural connectivity supports both past and future-oriented

episodic thinking. To address this, we applied diffusion-weighted MRI and a novel deterministic tractography protocol to reconstruct distinct subdivisions of the fornix previously detected in axonal tracer studies, namely pre-commissural fornix (connecting the hippocampus to vmPFC) and post-commissural (linking the hippocampus and medial diencephalon) fornix, in a group of healthy individuals who undertook an adapted past-future autobiographical interview. As predicted, we found that inter-individual differences in pre-commissural - but not post-commissural - fornix microstructure (fractional anisotropy) was significantly correlated with the episodic richness of both past *and* future autobiographical narratives. Notably, these results remained significant when controlling for both non-episodic narrative content and grey matter volumes of the hippocampus and vmPFC. This study provides novel evidence that reconstructing events from one's personal past, and constructing possible future events, involves a distinct, structurally-instantiated hippocampal-vmPFC circuit.

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Poster

170. Encoding and Retrieval in High-Level Content and Naturalistic Protocols

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 170.05/AA13

Topic: H.02. Human Cognition and Behavior

Support: NIH IRP ZIA-MH-002909

Title: Differences in the neural representations of visual content between encoding and free recall across the brain

Authors: *W. A. BAINBRIDGE[1], E. H. HALL[2], C. I. BAKER[1];
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Abstract: Our recalled memories are impressively detailed, containing large amounts of visual information (Bainbridge et al., 2019). Prior studies comparing the neural representations during encoding and recall have revealed shared neural substrates, but have often focused on paired associate learning for individual images. Here, using ultra-high field 7T fMRI, we compared the neural representations of encoding and free recall across exemplars for different levels of stimulus content. 22 human participants performed a task in-scanner where they studied an image for 6s, completed a visual distractor task, and then freely recalled the preceding image for 6s. Participants were presented with 192 trial-unique stimuli that varied with nested content structure in terms of broad stimulus category (scenes/objects), categorical properties (big/small and tool/non-tool for objects, natural/manmade and open/closed for scenes), and individual stimulus types (e.g., cupcake, guitar, cave), with 8 exemplars each. We calculated

discriminability indices in visual and memory-related regions of interest for different stimulus content to see what is represented in these regions during encoding and recall. Discriminability was calculated across exemplars, minimizing the potential contribution of specific stimulus-unique visual properties. During encoding, early and late visual areas show discrimination of several stimulus properties, including tools vs. non-tools in lateral occipital complex (LOC) and natural vs. manmade scenes in medial place area (MPA). The hippocampus only shows discriminability of scenes vs. objects during encoding. During recall, visual areas (e.g., LOC, MPA) generally show coarse discriminability of scenes vs. objects. However, the parahippocampal cortex (PHC) uniquely shows representations of fine-grained stimulus content during both encoding and recall, with significant discrimination of scenes vs. objects, open vs. closed scenes, and individual scene individuation. The hippocampus does not show stimulus content representations during recall, but memory strength can be decoded. Collectively, these results provide new insight into the specific content represented across the brain during encoding vs. free recall.

Disclosures: **W.A. Bainbridge:** None. **E.H. Hall:** None. **C.I. Baker:** None.

Poster

170. Encoding and Retrieval in High-Level Content and Naturalistic Protocols

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Program #/Poster #: 170.06/AA14

Topic: H.02. Human Cognition and Behavior

Support: Office of Naval Research
NIH T32 NS047987

Title: Multivariate encoding-retrieval similarity in visual cortex during high-confidence memory

Authors: ***M. S. COHEN**, L. Y. CHENG, K. A. PALLER, P. J. REBER;
Dept. of Psychology, Northwestern Univ., Evanston, IL

Abstract: Confidence judgments on a memory test may be based on one's ability to recreate the encoding event at the time of retrieval. Specifically, participants may be sensitive to the richness of memory-driven representations in sensory cortex at test. This hypothesis predicts that confidence will be associated with the degree of study-test representational similarity, as long as representations at test faithfully recreate perceptual processing during study. Prior work has shown that successful memory retrieval is associated with multivariate fMRI encoding-retrieval pattern similarity (ERS) in high-level visual cortex (e.g., Ritchey et al., 2013). Here, we measured brain activity using fMRI in young adults during memory encoding and during two types of memory tests. Stimuli were abstract visual kaleidoscope images originally presented in one of four spatial locations. After each study set (16 items presented twice), a recognition test

with old and new items was administered. For items judged as old, memory for spatial location was also assessed, as was confidence in spatial location memory. After 6 study-test cycles, an unexpected forced-choice recognition test was administered, with each previously shown image paired with a highly similar foil image, and both memory and confidence assessed. Confidence in memory for spatial location was associated with greater ERS in a region of interest (ROI) in the cuneus, motivated by a univariate analysis and defined using Neurosynth. ERS was measured by computing Pearson correlations between encoding and retrieval activity for all voxels in the ROI, for each studied item. Logistic regression analyses showed that after controlling for mean signal intensities, ERS in cuneus for correct items was positively associated with the likelihood of a high-confidence spatial location judgment. The novel association between ERS in cuneus and confidence in memory for spatial location is consistent with this region's location early in the dorsal visual stream. ERS was also examined in an occipitotemporal ROI during the forced-choice test, and was positively associated with likelihood of a confident response, whether correct or incorrect. Occipitotemporal cortex is relevant as a high-level ventral visual region important for perceiving and remembering shapes, and also shows greater univariate activity during high-confidence responses on this test. Our data indicate that reactivation of relevant aspects of the encoding experience can yield a high confidence response even when the response itself is inaccurate. These results inform our understanding of the signals relied upon in making retrospective metamemory judgments.

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Poster

170. Encoding and Retrieval in High-Level Content and Naturalistic Protocols

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Title: Human hippocampus and medial prefrontal cortex represent hierarchical task schemas

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Abstract: The ability to connect new situations to prior experiences can facilitate understanding and speed learning. For example, if you have already had experience with how to behave at a party compared to how to behave at a workplace, adjusting to a new party or workplace will be much easier. The ability to generalize learning between different situations is thought to rely on neural schemas. In a recent set of studies where rats had to learn opposing reward contingencies that varied depending on spatial context, hippocampal neural populations developed a hierarchical response that represented multiple task features simultaneously. Population responses to the two distinct contexts were anticorrelated, suggesting hippocampus represents separate schemas that are split by context. In contrast, orbitofrontal cortex representations demonstrated a hierarchical response with anticorrelated responses to different reward valences. We hypothesized that, if the anticorrelated responses observed in these studies represent distinct schemas, then the other task-relevant features should be represented with distinct coding that does not generalize across schemas. Using a similar task in humans, we collected fMRI data while participants viewed objects within contexts that had been associated with distinct reward contingencies. We tested for representation of individual features of the task (context, object, position, and reward valence) while controlling for the other features. Consistent with the work in rats, we found that pattern similarity in hippocampus is greater for items in the same context, while medial prefrontal cortex exhibits similar patterns for items with the same reward valence. Furthermore, representations of these features are maintained when participants transfer their learning to a distinct pair of contexts with a similar structure to the first pair of contexts. Context representations in hippocampus and posterior medial prefrontal cortex were reinstated during transfer learning. In contrast, anterior medial prefrontal cortex reinstated patterns representing object-valence conjunctions. Our results suggest that hippocampus reactivates relevant contexts, providing constraint for medial prefrontal cortex to represent the correct object-valence conjunction code to guide the correct behavioral response. Furthermore, we find that context or valence changes may cause other features, such as object identity and position, to develop distinct representations that do not generalize across schemas. This process of splitting a task representation into distinct schemas may be critical for guiding context-specific behavior.

Disclosures: **N.W. Morton:** None. **R.J. Molitor:** None. **M.L. Schlichting:** None. **M.L. Mack:** None. **S.A. McKenzie:** None. **A.R. Preston:** None.

Poster

170. Encoding and Retrieval in High-Level Content and Naturalistic Protocols

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 170.08/AA16

Topic: H.02. Human Cognition and Behavior

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Title: Retrieval practice induces dynamic neural reorganization to boost long-term memory retention

Authors: *L. ZHUANG[1,2], J. WANG[1,2], B. XIONG[1,2], L. HAO[1,2], P. J. BAYLEY[3,4], S. QIN[1,2]; [1]State Key Lab. of Cognitive Neurosci. and Learning, [2]IDG/McGovern Inst. for Brain Res., Beijing Normal Univ., Beijing, China; [3]War Related Illness and Injury Study Ctr., VA Palo Alto Hlth. Care Syst., Palo Alto, CA; [4]Dept. of Psychiatry and Behavioral Sci., Stanford Univ., Stanford, CA

Abstract: One of the most effective learning strategies, known as retrieval practice, is that through actively recalling learnt information, memory for that information will become strengthened, especially after a period of consolidation. Although the merit of retrieval practice is well established in psychology and widely utilized in education, the underlying neurocognitive mechanisms of how retrieval practice reshapes memories to promote long-term retention after consolidation remains unclear. Using event-related fMRI and a prospective memory consolidation paradigm, we track the dynamic evolution of memory-related neural representations and network reconfigurations over the course of multiple retrieval practice runs and how these dynamic metrics predict subsequent memory outcomes. Behaviorally, retrieval practice boosted long-term retention across 24 hours but not 30 minutes, indicating that offline consolidation is required to produce enhanced memory performance. Retrieval practice also led to more false memories at both delay intervals, indicating that retrieval actively reshapes original memories. Critically, the benefits of retrieval practice on long-term retention were associated with the inter-trial distinctiveness of multivoxel representation patterns in the posterior parietal cortex that increased progressively across retrieval practice. False memory was predicted by unstable representations in the medial temporal lobe over retrieval practice. Further network analyses revealed that retrieval practice led to a gradual build-up of functional connections among memory-related neural networks consisting of the medial temporal lobe, prefrontal and posterior parietal regions. Moreover, brain-behavior prediction analysis revealed that dynamic reconfiguration of memory-related neural networks over retrieval practice was highly predictive of individual's long-term retention gains after consolidation. Specifically, the ventrolateral prefrontal cortex emerged as the most prominent predictor, acting as a hub to not only facilitate efficient information access and but also refine discriminative representations in the posterior parietal cortex across retrieval practice. Together, our findings suggest a neurocognitive mechanism of dynamic network neural reorganization during retrieval practice, through which memories are arranged into discrete yet malleable representations for subsequent consolidation.

Disclosures: L. Zhuang: None. **J. Wang:** None. **B. Xiong:** None. **L. Hao:** None. **P.J. Bayley:** None. **S. Qin:** None.

Poster

170. Encoding and Retrieval in High-Level Content and Naturalistic Protocols

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 170.09/AA17

Topic: H.02. Human Cognition and Behavior

Support: NIH NS003144

Title: Memorability of arbitrary verbal associations and its role during memory retrieval in the anterior temporal lobe

Authors: *W. XIE, W. BAINBRIDGE, K. ZAGHLOUL;
NIH, Bethesda, MD

Abstract: Although people's memory ability tends to vary, the degree to which a stimulus is likely to be later remembered - memorability - has been shown to be highly consistent for certain task content. However, moving beyond item-level memorability, what constitutes memorability for associative information remains largely unknown. Taking advantage of intracranial electroencephalogram (iEEG) recordings during a cued-recall verbal pair-associate task, this study investigates memorability for arbitrary verbal associations and its neural correlates during memory retrieval. First, in a group of 27 participants undergoing seizure monitoring with iEEG, we found that certain words in a pre-selected pool (n = 300) consistently led to successful recall when they were used as retrieval targets, paired with arbitrary retrieval cues, in the pair-associate task. This observation was replicated by an online crowd-sourced study with 2594 participants using the same word pool. Second, increases in the likelihood of remembering for certain retrieval targets could be partly explained by higher average semantic similarity between a given target word and arbitrary retrieval cues from the word pool. In contrast, single-dimension word properties (e.g., concreteness) could not account for this memorability phenomenon for associative concepts. Third, behaviorally, words with higher memorability scores tended to be recalled faster and, when intrusions occurred, intruded words also had higher memorability values, suggesting that memorable target words were more accessible during retrieval. Fourth, these behavioral effects were supported by decodable multivariate iEEG patterns at low frequency (4-16 Hz) activities in the anterior temporal lobe (ATL). Specifically, following the onset of retrieval cue, above-chance decoding accuracy for memorable versus forgettable target words emerged from 500 to1000 ms in the ATL. This decodable pattern, however, dissipated in the later stage of memory retrieval, starting from around 1000 ms after cue onset to until recall vocalization (~2500 ms), in contrast to the significantly increased decoding accuracy for successful recall at this later time period of memory retrial. Furthermore, these memorability-related neural effects were absent in other temporal lobe recording sites (e.g., medial and posterior temporal lobes). Together, with converging behavioral and neural evidence, this study

suggests that memorability for arbitrary verbal associations, partly driven by intrinsic semantic mapping between associative concepts, may modulate memory processes in the ATL semantic network at the initial stage of memory retrieval.

Disclosures: W. Xie: None. **W. Bainbridge:** None. **K. Zaghloul:** None.

Poster

170. Encoding and Retrieval in High-Level Content and Naturalistic Protocols

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 170.10/AA18

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant 1P01HD080679
Leverhulme Trust grant RPG-2014-075
Wellcome Trust Senior Investigator Award WT106931MA

Title: An abstract neural representation of category membership beyond information coding stimulus or response

Authors: *R. M. MOK[1], J. D. CARLIN[2], B. C. LOVE[1];
[1]Univ. Col. London, London, United Kingdom; [2]MRC Cognition and Brain Sci. Unit, Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Concepts organize knowledge to support categorization and inference. Category representations mediate between relevant inputs (stimulus) and outputs (motor response). On the input side, neural representations of categories are often analyzed in terms of stimulus structure. On the output side, one popular idea is that internal representations are grounded and situated in terms of action. In contrast to these views, we find abstract category signals throughout the brain (from early visual cortex to prefrontal cortex) that serve as markers, akin to a symbol, of category membership. These category signals carry information above-and-beyond what is reflected in the stimulus or motor response.
We conducted a functional magnetic resonance imaging (fMRI) study in which 33 participants mastered a probabilistic concept learning task in which category, stimulus, and motor variables could be de-correlated from one another. On each trial, participants were presented with a moving-dot stimulus moving in one direction and were required to judge whether it belonged to one category (face) or another (scene). The stimuli spanned 12 directions from 0°-330°, with the category determined by a category bound (i.e., opposite motion directions tended to be in contrasting categories). The corrective category feedback consisted of a face or house stimulus. The feedback was probabilistic such that the closer to the bound a stimulus was the more probabilistic the feedback was.
We estimated subjective category bounds from each individual's behavior. To examine the

neural representations of stimulus, motor, and category, we used multivariate pattern analysis across a broad range or brain areas from early sensory to prefrontal cortex. Although the category structure was entirely based on direction, we were unable to find activity that explicitly coded stimulus direction. Strikingly, despite difficulty decoding stimulus direction, which probabilistically defined the categories, we were able to decode abstract category signals across brain regions, including area V3A. These abstract representations did not appear to reflect motor plans, with no category signal present in motor cortex.
In sum, we found abstract category signals throughout the brain and absent decodable stimulus direction information, suggesting that the brain constructs an abstract code as an intermediary between stimulus and response. Because the motor response to indicate category response switched across runs, building an abstract representation would be useful, mirroring real-world tasks in which the meaning of a situation can remain constant even when the contextually appropriate response changes.

Disclosures: **R.M. Mok:** None. **J.D. Carlin:** None. **B.C. Love:** None.

Poster

170. Encoding and Retrieval in High-Level Content and Naturalistic Protocols

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 170.11/AA19

Topic: H.02. Human Cognition and Behavior

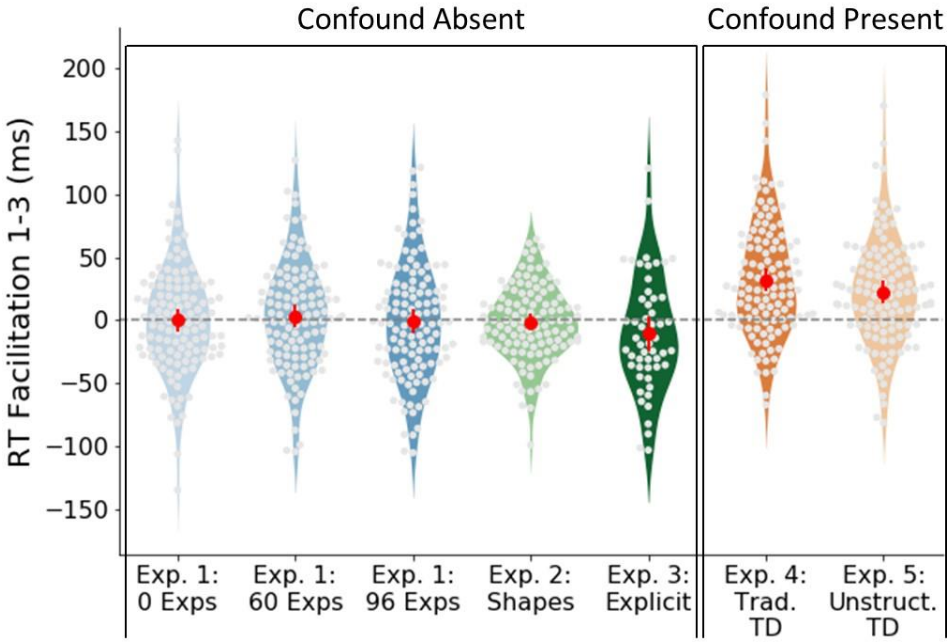
Support: Sloan Foundation

Title: No evidence for visual statistical learning in standard reaction time measures

Authors: *K. HIMBERGER[1], A. S. FINN[2], C. J. HONEY[1];
[1]Psychological & Brain Sci., Johns Hopkins Univ., Baltimore, MD; [2]Univ. of Toronto, Toronto, ON, Canada

Abstract: Standard visual statistical learning (VSL) paradigms in the temporal domain entail exposing participants to regularities and then assessing learning with direct measures like forced-choice recognition tests (Siegelman et al., 2017). However, because direct measures may be subject to influence from explicit knowledge (Bertels et al., 2015), it has been proposed that genuinely implicit aspects of VSL should be assessed indirectly (Turk-Browne et al., 2005). Across several experiments, we assess learning indirectly by calculating response time differences between perfectly predictable stimuli and pseudo-random stimuli. We found that predictable stimuli were not detected faster than non-predictable stimuli (Fig. 1, Experiments 1-3). At the same time, we identified a key measurement confound in standard reaction time paradigms which had shown speeding for predictable items. This confound is sufficient to account for previously reported reaction time facilitation effects, and may be necessary for

obtaining such effects (Fig. 1, Experiments 4 & 5). While finding no speeding for predictable items, we did find reliable, and small, VSL effects using two direct measures (forced-choice and recall). These results undermine the evidence that VSL can occur without any explicit knowledge. Additionally, we suggest that prior VSL work using reaction time measures should be re-interpreted.



Disclosures: **K. Himberger:** None. **A.S. Finn:** None. **C.J. Honey:** None.

Poster

170. Encoding and Retrieval in High-Level Content and Naturalistic Protocols

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 170.12/AA20

Topic: H.02. Human Cognition and Behavior

Support: EU-H2020-FET 1564
EU-M-GATE 765549

Title: Estimating Pi with memory recall experiments

Authors: ***M. TSODYKS**, M. KATKOV, M. NAIM;
Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Free recall is a standard paradigm to probe human memory. Previous studies showed that the number of words that can be recalled from a certain number of randomly assembled

words depends on how much time participants have for acquiring the presented list. Nevertheless, we showed that when independently estimating how many words were actually acquired during the presentation, the recall performance exhibits universal behaviour independently on the presentation rate. Moreover, the quantitative form of recall performance agrees remarkably well to an analytical prediction from a simple deterministic step-by-step recall algorithm based on random matrix of inter-item associations: R = sqrt(3pi/2)*sqrt(M), where M is the number of words acquired during presentation and R the number of words recalled, under identical conditions and for the same group of participants. We performed novel recall experiments with short sentences expressing well-known but unrelated facts, and found that also in this paradigm, performance obeys the same mathematical relation as for lists of words. Memory recall therefore appears to be a universal process that is common to different types of information as long as this information is unstructured. Since the law of recall contains Pi in it, another way of expressing these results is that one can use memory experiments to estimate the numerical value of Pi.

Disclosures: **M. Tsodyks:** None. **M. Katkov:** None. **M. Naim:** None.

Poster

170. Encoding and Retrieval in High-Level Content and Naturalistic Protocols

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 170.13/AA21

Topic: G.03. Emotion

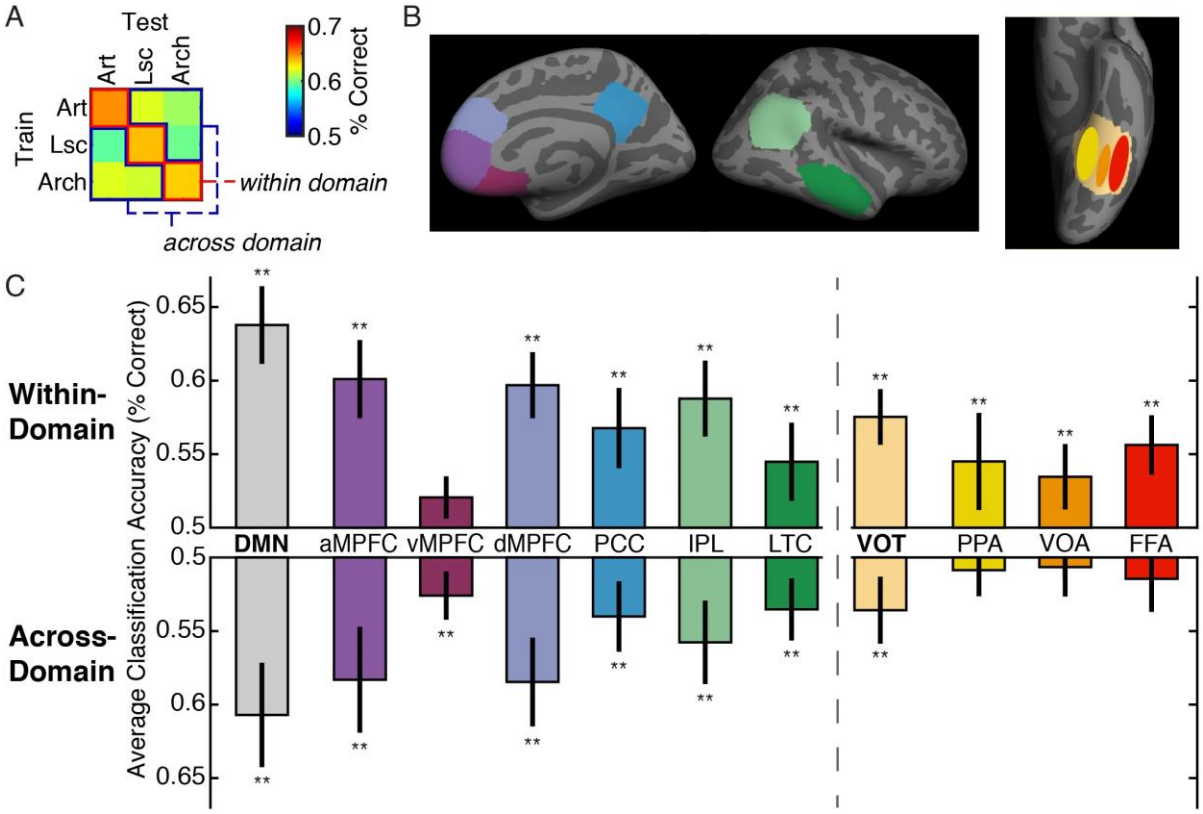
Support: NYU Research Challenge Fund to G.S.

Title: The default-mode network represents aesthetic appeal that generalizes across visual domains

Authors: ***E. A. VESSEL**[1], A. I. ISIK[1], A. M. BELFI[2], J. L. STAHL[3], G. G. STARR[4]; [1]Neurosci., Max Planck Inst. For Empirical Aesthetics, Frankfurt am MIn, Germany; [2]Missouri Univ. of Sci. and Technol., Rolla, MO; [3]Ohio State Univ., Columbus, OH; [4]Pomona Col., Claremont, CA

Abstract: Visual aesthetic evaluations, which impact decision-making and well-being, recruit the ventral visual pathway, subcortical reward circuitry, and parts of the medial prefrontal cortex overlapping with the default-mode network (DMN). However, it is unknown whether these networks represent aesthetic appeal in a domain-general fashion, independent of domain-specific representations of stimulus content (artworks versus architecture or natural landscapes). Using a classification approach, we tested whether the DMN or ventral occipitotemporal cortex (VOT) contains a domain-general representation of aesthetic appeal. Classifiers were trained on multivoxel fMRI response patterns collected while observers made aesthetic judgments about

images from one aesthetic domain. Classifier performance (high vs. low aesthetic appeal) was then tested on response patterns from held-out trials from the same domain to derive a measure of domain-specific coding, or from a different domain to derive a measure of domain-general coding. Activity patterns in category-selective VOT contained a degree of domain-specific information about aesthetic appeal, but did not generalize across domains. Activity patterns from the DMN, however, were predictive of aesthetic appeal across domains. Importantly, variation in classifier performance across observers reflected the distances (d') between each observers' behavioral ratings of images labeled as "high" or "low" appeal (R^2= 0.53). These findings support a model of aesthetic appreciation whereby domain-specific representations of the content of visual experiences in VOT feed in to a “core” domain-general representation of visual aesthetic appeal in the DMN. Whole-brain “searchlight” analyses identified additional prefrontal regions containing information relevant for appreciation of cultural artifacts (artwork and architecture) but not landscapes.



Disclosures: **E.A. Vessel:** None. **A.I. Isik:** None. **A.M. Belfi:** None. **J.L. Stahl:** None. **G.G. Starr:** None.

Poster

171. Human Social Cognition: Behavior, Mechanisms, and Disorders I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 171.01/AA22

Topic: H.02. Human Cognition and Behavior

Title: The influence of anti-social behavior in top-down modulation of motor resonance

Authors: *Y. SIONAKA, Y. MIYAWAKI, S. MORIOKA;
Kio Univ., Kitakatsuragi-gun, Japan

Abstract: Motor interference effects, which occur when observing the movements of others, are interpreted as a behavioral index of motor resonance that is based on the mirror neuron system comprising human premotor and parietal cortices. Some studies have suggested that motor resonance (i.e., mirror neuron system) does not occur when observing non-biological motions. However, we have previously shown that observing non-biological motions of a nonsense object could induce motor resonance through a positive attitude, such as the sense of affinity to the object. If the attitude toward the observed object has an impact on motor resonance, the effects of the positive attitude might be removed by an anti-social behavior of the object. The present study examined this hypothesis. Ten healthy participants performed horizontal arm movements while observing non-biological, incongruent (vertical) movements of a visual stimulus (nonsense object) in pre-test and post-test procedures. Participants played catch with the nonsense object by controlling a triangle object on a monitor between pre-test and post-test procedures, as in our previous study. In the middle of the catch, however, the nonsense object played catch only with a square object controlled by a computer, and thus, participants were left out of this catch by the nonsense object (i.e., anti-social behavior of the observed object). Our preliminary experiment confirmed that participants felt a significant sense of aversion to the nonsense object by being left out. Variance in the executed movements, which was measured as an index of motor interference effects, was compared between the pre-tests and post-tests. The results revealed no significant differences between the pre-tests and post-tests. Considering that our previous study showed that motor interference effects increased by the sense of affinity to the observed object by playing catch, the current result suggests that the anti-social behavior after playing catch inhibited motor resonance through the sense of aversion to the object. In conclusion, the present study supports the accounts of top-down modulation on motor resonance and mirror neuron system functioning.

Disclosures: **Y. Sionaka:** None. **Y. Miyawaki:** None. **S. Morioka:** None.

Poster

171. Human Social Cognition: Behavior, Mechanisms, and Disorders I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 171.02/AA23

Topic: H.02. Human Cognition and Behavior

Title: Top-down modulation of motor resonance through affective attitude toward a non-biological object

Authors: *Y. MIYAWAKI, Y. SHIONAKA, S. MORIOKA;
Kio Univ., Kitakatsuragi-gun, Japan

Abstract: Observing the movements of others can interfere with the execution of one's own movement. This motor interference is induced through motor resonance based on the mirror neuron system comprising human premotor and parietal cortices. Some studies have shown that motor resonance is insensitive to non-biological motions. However, other studies have shown that motor resonance is modulated by top-down processes associated with social cognition. The present study investigated whether observing non-biological motions can induce motor resonance through social interaction with a non-biological object. Twelve healthy participants performed horizontal arm movements while observing non-biological, incongruent (vertical) movements of a visual stimulus (nonsense object) in pre- and post-test procedures. Between these procedures, six participants in the interaction group played virtual catch with the nonsense object by controlling the triangle object on a monitor as the interaction experience. The six participants in the non-interaction group observed that the nonsense object was playing catch with a triangle object controlled by a computer. Variance in the executed movements was measured as an index of motor resonance. After the experiment, participants reported their subjective feeling toward the nonsense object by rating on a scale ranging from 1 (felt sense of aversion) to 5 (felt sense of affinity). The results showed that although there were no significant differences between the groups, the subjective feeling was significantly and positively correlated with increased variance in the post-test, suggesting that the sense of affinity induced motor resonance to non-biological motions. This study proposed that observing non-biological motions could enhance motor resonance (i.e., mirror neuron system functioning) through top-down processes, including affective attitudes. In this study, however, since some participants in the non-interaction group felt the sense of affinity even in just observing the catch, a continuous study of manipulating the subjective feeling toward the observed object should be conducted.

Disclosures: Y. Miyawaki: None. **Y. Shionaka:** None. **S. Morioka:** None.

Poster

171. Human Social Cognition: Behavior, Mechanisms, and Disorders I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 171.03/AA24

Topic: H.02. Human Cognition and Behavior

Support: Spectrum Health-MSU Alliance Corporation

Title: Investigating social motivation in young children with and without autism

Authors: *K. S. CARLSON, S. HULBERT GEORGE, B. L. THOMPSON;
Pediatrics and Human Develop., Michigan State Univ., Grand Rapids, MI

Abstract: There are significant gaps in our understanding of the neural mechanisms that underlie the heterogeneity of social-affective behaviors across human populations. In developing children, challenges arise when social-affective processing is disrupted, leading to difficulty in establishing relationships and effectively regulating emotions, which can ultimately influence quality of life. The factors that influence the heterogeneity of these social behaviors in both typically developing (TD) children and children with Autism Spectrum Disorder (ASD), for whom disruptions in social behaviors are among the core deficits, are currently unknown. To address this, we aimed to determine whether alterations in social behavior in children with ASD are driven by an aversion to, or lack of reward from, social interactions. We adapted our previously established associative learning paradigm of conditioned place preference (CPP) in young children for use with a social unconditioned stimulus (US). In this paradigm, during an initial preference test (IPT), TD children (30-60 months) and children with ASD (36-66 months) freely explored two neutral rooms in our child-friendly castle. Then, across training trials (TT), children alternated play between the two rooms while one of the rooms, the conditioned stimulus (CS), was paired with the US, a novel and interactive social experimenter. During the final preference test (FPT), children again freely explored both rooms, in absence of the US. Social CPP (sCPP) scores were calculated as the difference in time spent in the social-paired room during the IPT and FPT. Surprisingly, we found that both groups of children display robust sCPP ($p<0.05$) and that there were no significant differences between groups for sCPP. Interestingly, older chronological age was associated with stronger sCPP scores. This work provides a more comprehensive understanding of the mechanisms driving social motivation. It may also offer a unique opportunity to distinguish the endophenotypes of social behaviors within an ASD diagnosis, allowing for more precisely targeted behavioral interventions to improve social and affective behaviors.

Disclosures: K.S. Carlson: None. **S. Hulbert George:** None. **B.L. Thompson:** None.

Poster

171. Human Social Cognition: Behavior, Mechanisms, and Disorders I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 171.04/AA25

Topic: H.02. Human Cognition and Behavior

Support: MSU Faculty Startup Funds

Title: Predicting social motivation in children with and without autism using eye-tracking and machine learning

Authors: *S. HULBERT GEORGE, K. S. CARLSON, B. L. THOMPSON;
Pediatrics and Human Develop., Michigan State Univ., Grand Rapids, MI

Abstract: The quality of a child's social interactions plays an important role in their development and can act as a foundation for success in learning, relationships, and even employment. For individuals with neurodevelopmental disorders, such as Autism Spectrum Disorder (ASD), social deficits, including trouble anticipating a social routine or diminished/absent joint attention, often shape their daily interactions. Further complicating our understanding of the behavioral phenotype of ASD is not knowing whether altered social interactions arise as a result of *aversion* or a *lack of reward* to social stimuli. Therefore, the objectives of this study were to determine whether eye-tracking and machine learning techniques could elucidate an individual's social preference and potentially better describe the heterogeneity of social motivation.
Typically developing and children with ASD aged 2 ½ to 5 ½ years old were enrolled. Participants viewed a 6-minute video while gaze behavior was recorded at 600Hz using a Tobii Pro Spectrum eye-tracker and software. Video clips were separated into 6 blocks and pseudorandomized into 36 timelines, which were randomly assigned to each participant. The videos (n = 90) consisted of cartoons, natural scenes, geometric shapes, and people. Eye-tracking features including fixations, saccades, and several features derived from these foundational metrics were extracted offline and analyzed using SPSS and custom MATLAB scripts. Features were compared across all scenes as well as across different scene and area-of-interest subgroups. Then, these eye-tracking features were combined with social motivation behavior collected from these same children and a supervised machine learning algorithm, Enhanced Probabilistic Neural Network, to predict social preference. Preliminary results (n = 20) indicate that a subset of eye-tracking features can predict social motivation preference (aversion, non-reward, reward) with an accuracy of 70%.
This exploratory project seeks to better understand the neurobiological mechanisms of social motivation by combining eye-tracking biometrics and machine learning to more

comprehensively define the social motivation phenotype for each child. Ultimately, results from this work have the potential to help personalize interventions for children with social deficits.

Disclosures: S. Hulbert George: None. **K.S. Carlson:** None. **B.L. Thompson:** None.

Poster

171. Human Social Cognition: Behavior, Mechanisms, and Disorders I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 171.05/AA26

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01MH112517

Title: Examining the relationship between individual differences in spontaneous theory of mind and neural correlates of explicit theory of mind

Authors: *D. SHARIQ[1], D. ALKIRE[2], S. DZIURA[1], J. MERCHANT[2], A. RASHID[1], K. MCNAUGHTON[2], E. REDCAY[1];
[1]Psychology, [2]Neurosci. and Cognitive Sci., Univ. of Maryland, College Park, MD

Abstract: Theory of mind (TOM) is a fundamental ability that allows for making inferences about the mental states of others. In everyday social interactions, we often engage in spontaneous TOM (STOM), that is, the ability to infer the mental states of others without being prompted to do so. However, TOM is often researched with the use of explicit TOM (ETOM) tasks, which call upon the subject to deliberately and explicitly reason about another person's thoughts, feelings, and intentions. Recent studies have related performance on a STOM task to cortical thickness in brain regions functionally linked to TOM processing (Rice and Redcay, 2015). The aim of this ongoing study is to determine if there is a relationship between performance on a STOM task and brain activity in so-called TOM regions. Twenty subjects (age, 20.75 ± 2.2 yrs; 11 F) completed two TOM tasks on separate occasions. In the first session, subjects completed the Spontaneous Theory of Mind Protocol (STOMP) in which they watch two silent film clips portraying complicated social interactions and describe the scene. A STOMP index is calculated based on the ratio of internal clauses (e.g. intentions, emotions, and mental states) to the total number of clauses, including internal and external clauses (e.g. physical descriptions and inferences). In the second session, subjects undergo fMRI scanning and complete an ETOM localizer task. In this task, subjects read short stories and answer questions about the verity of an ensuing statement. Half of the statements require subjects to explicitly reason about the story's characters (false belief), while the other half require subjects to think about a physical representation (false photo). To examine whether performance on the STOMP had any relation with functional activation in ETOM regions of the brain, contrast values between false belief-false photos were extracted from seven regions of interest (dmPFC, vmPFC, precuneus, LTPJ,

RTPJ, LATL, and RATL) and used as independent variables in regression models with the STOMP index as a predictor variable. Analysis of these regions suggest that there is no relation between performance on the STOMP and functional activation in the TOM regions (ps>0.1). Contrary to our hypotheses, these findings demonstrate that neural sensitivity during ETOM is not related to individual differences in STOMP, suggesting potentially different mechanisms underlying explicit and spontaneous TOM. Further analysis including a larger sample size may help to elucidate the true nature of the relationship.

Disclosures: **D. Shariq:** None. **D. Alkire:** None. **S. Dziura:** None. **J. Merchant:** None. **A. Rashid:** None. **K. McNaughton:** None. **E. Redcay:** None.

Poster

171. Human Social Cognition: Behavior, Mechanisms, and Disorders I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 171.06/AA27

Topic: H.02. Human Cognition and Behavior

Support: R01MH112517-01

Title: Modulation of joint attention by naturalistic social and emotional stimuli in FMRI and eye tracking

Authors: *A. RASHID, D. SHARIQ, S. DZIURA, K. A. MCNAUGHTON, D. ALKIRE, J. MERCHANT, E. REDCAY;
Psychology, Univ. of Maryland, College Park, MD

Abstract: Joint attention is a key social-communicative skill that is important for interaction and social learning. Traditionally, static images have been used in studies of joint attention and social processing but the real world is dynamic and contains socio-emotional stimuli. Little is known about how joint attention behaviors are affected by emotion nor the extent to which joint attention behaviors during a naturalistic interaction are related to neural processing. This study used videos with emotional content to present naturalistic stimuli in joint (social) versus solo (non-social) settings to investigate how much attention is paid to a social partner. Simultaneous fMRI and eye-tracking(ET) data from 10 subjects (Male = 5, mean age = 20.2 years) in an ongoing study were analyzed. Subjects watched video clips that were 20 or 30 seconds long and were of three affect types (Positive, Neutral, Negative). A pseudo livestream of a peer's face in joint and solo was shown next to affect video. Peer's face was oriented toward the affect video in joint trial and oriented away during solo trial. The ET data was analyzed by calculating a "peer-ratio" (PR), defined as percent of total trial time subjects spent looking at the peer's pseudo live-stream divided by the percent of total trial time subjects spent looking at the affect video. We examined the effect of joint and emotion context on PR with a 2x3 repeated-

measures ANOVA. FMRI data were analyzed with standard preprocessing and with each video representing one event per condition in a blocked design with canonical hrf. The difference of PR between joint and solo conditions of the ET data was used as a whole-brain covariate to examine the relation between activation to joint vs. solo conditions in fMRI.
Behaviorally, the PR was significantly larger in joint contexts ($p<.0001$). PR was also affected by emotion context ($p<.044$), with positive being the largest, followed by negative, and neutral being the smallest. Interaction between joint and emotion contexts ($p<.02$) was such that the effect of joint context was largest in positive and negative videos. At the neural level, greater activation in the precuneus was related to greater PR ($p<.005$, uncorrected).
Joint attention is modulated by social and emotional conditions. Significantly larger PR for joint than solo suggests a modulation by social factor in allocation of joint attention to peer. In emotional condition, PR was largest for positive, followed by negative, and neutral being the smallest. Though the positive and negative videos were affect stimuli, the positive PR was larger than negative PR. This suggests a modulation of social condition by an emotional factor.

Disclosures: **A. Rashid:** None. **D. Shariq:** None. **S. Dziura:** None. **K.A. McNaughton:** None. **D. Alkire:** None. **J. Merchant:** None. **E. Redcay:** None.

Poster

171. Human Social Cognition: Behavior, Mechanisms, and Disorders I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 171.07/AA28

Topic: H.02. Human Cognition and Behavior

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Title: Co-localization of hemodynamic and theta band activity during interactive joint attention

Authors: *S. DRAVIDA[1], J. A. NOAH[2], X. ZHANG[2], J. HIRSCH[2,3,4,5];
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Abstract: Questions regarding the relationship between hemodynamic and electrocortical responses are long-standing in neuroscience and under-studied in live social interactions such as in joint attention. Joint attention is a form of communication during which one person ("the initiator") directs the attention of another person ("the responder") to an object. Using

simultaneous functional near-infrared spectroscopy (fNIRS) and electroencephalography (EEG), we examined the relationship between hemodynamic and electrocortical signals that occurs when people engage in live joint attention interactions. Based on prior studies of joint attention[1], we predicted increased hemodynamic responses in brain areas involved in social cognition and communication during interactive compared to non-interactive joint attention. Further, based on prior findings of theta band associations with attention[2] and our recent findings of co-localization of theta band sources with hemodynamic activity during face perception[3], we hypothesized that the source of theta oscillations during interactive joint attention would co-localize to these areas of increased hemodynamic activity. Twenty pairs of adults participated in the fNIRS experiment and of these, nine pairs also underwent simultaneous EEG. Each partner performed three conditions: one as initiator, one as responder, and one with a non-social cue. During the task, LEDs cued the initiator to direct their attention to one of three targets, and the initiator used only eye movements to cue the responder to the target (interactive runs), or the LED indicated to both participants which target was correct (non-interactive runs). When participants engaged in social interaction as either the initiator or responder, social cognition (right temporoparietal junction) and language areas (left superior temporal gyrus) were engaged more than during the non-interactive runs. As predicted, the theta band oscillations also localized to these areas during interactive runs, while alpha and beta band oscillations did not. The results suggest a possible role of theta band oscillations in relating electrocortical and hemodynamic signals during the dynamic interactions associated with joint attention. **References:** [1]Redcay, E. et al., Human brain mapping, 2013. [2]Başar, E. et al., International Journal of Psychophysiology, 2001. [3]Dravida, S. et al., Submitted.

Disclosures: **S. Dravida:** None. **J.A. Noah:** None. **X. Zhang:** None. **J. Hirsch:** None.

Poster

171. Human Social Cognition: Behavior, Mechanisms, and Disorders I

Location: Hall A

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Program #/Poster #: 171.08/AA29

Topic: H.02. Human Cognition and Behavior

Title: Effects of ketamine on the perception of animacy and metacognition

Authors: *S. WASSERTHAL[1], J. SCHULTZ[2], U. ETTINGER[3], M. LEHMANN[4], R. HURLEMANN[5];
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Abstract: Background: Psychosis is known to be associated with deficits in meta- and social cognition. The uncompetitive NMDA-receptor antagonist Ketamine has been identified to trigger psychotomimetic symptoms in healthy adults. We used Ketamine to investigate whether perception of animacy and metacognition during this perceptual task are impaired in participants in an experimentally-induced psychotomimetic state.
Methods: Fifty healthy participants (n = 23 Ketamine; n = 27 placebo: NaCL) performed a perceptual task in which visual stimuli displaying goal-directed motion evoke percepts of animacy (Schultz, Friston, O' Doherty, Wolpert, and Frith, 2005) during functional magnetic resonance imaging. In this signal-detection task, participants judged whether two moving dots were chasing each other. Task difficulty varied with the velocity and degree of chasing of the moving dots. Every fifth trial, participants had to report their confidence about the chasing decision (metacognitive decision). Ketamine (target plasma level = 100 ng/ml) or sodium chloride was administered intravenously throughout the scan using a computer-controlled infusion-pump. Psychotomimetic effects were assessed using an adjusted Positive and Negative Symptoms Scale (PANSS). Behavioral data were analyzed according to type 1 (d-prime) and type 2 (meta-d-prime) signal detection theory using a MATLAB toolbox by Maniscalco and Lau (Consciousness and Cognition 2012).
Results: As expected, participants under the influence of Ketamine showed significantly increased PANSS ratings. Both groups showed performance replicating earlier findings and task performance did not differ significantly between groups. In contrast, the Ketamine group's metacognitive efficiency was differently affected by the amount of goal-directed motion cues than the placebo group: Metacognitive efficiency was higher with stronger cues.
Conclusions: Our data show that doses of Ketamine leading to psychotomimetic effects do not affect animacy perception, a basic step in social cognition, but tend to influence metacognitive efficacy during animacy perception.

Disclosures: S. Wasserthal: None. **J. Schultz:** None. **U. Ettinger:** None. **M. Lehmann:** None. **R. Hurlemann:** None.

Poster

171. Human Social Cognition: Behavior, Mechanisms, and Disorders I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 171.09/AA30

Topic: H.02. Human Cognition and Behavior

Title: Unpacking the temporal self: Evidence that future and past selves are temporally compressed

Authors: *S. C. BRIETZKE[1], M. L. MEYER[2];
[2]Psychological and Brain Sci., [1]Dartmouth Col., Hanover, NH

Abstract: Although it is well-known in social psychology that people feel disconnected from their past and future selves, the underlying behavioral and neural mechanism supporting this phenomenon is underspecified. In visual perception, as objects become distant, they appear compressed, making them hard to distinguish. Here, we examined whether temporal self-perception abides by the same principle. In Study 1, participants (n=178) made traits ratings across 9 evenly dispersed time intervals in the past and future. A linear mixed model revealed a significant cubic effect of time, (B=-10.17, p=0.002) such that participants compressed their past and future selves relative to their present self. To examine whether such effects are preferential to the self, in Study 2, participants (n=174) rated themselves and another known-target (Angela Merkel) across the same set of time intervals. A linear mixed model revealed a significant cubic time by target interaction (B=-2.01, p=0.001), such that participants showed temporal compression for the self but not Angela Merkel. In Study 3, we have tested this phenomenon in 30 Dartmouth undergraduates, with a total goal of N=40 subjects. Thus far, we have employed multivariate whole brain representational similarity analysis (RSA) to identify the neural underpinnings of the self-compression effect. We have found that the self similarity structure across time fits an *a priori* theoretical logarithmic compression model in multiple brain regions. Specifically, this self-compression effect is preferential to the self (relative to Merkel) in bilateral medial frontal gyrus, bilateral cingulate gyrus, bilateral inferior frontal gyrus, bilateral middle frontal gyrus, bilateral middle temporal gyrus, and right precuneus (voxelwise primary threshold $p<0.005$). Moreover, no brain regions showed a compression effect for Merkel, further suggesting that self-representations are preferentially compressed across time. Taken together, these findings reveal a "self-compression" effect, with rapid changes in self-perception occurring closer to the present and tapering out with increased temporal distance both at the behavioral and neural level.

Disclosures: S.C. Brietzke: None. **M.L. Meyer:** None.

Poster

171. Human Social Cognition: Behavior, Mechanisms, and Disorders I

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant UH3 ODD023313
NIH Grant R01 MH087510

Title: Maternal education moderates infant and early childhood functional networks important for reading performance

Authors: *M. M. K. BRUCHHAGE[1,2], G. NGO[1], S. C. L. DEONI[1], V. D'SA[1,2];
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Abstract: Motivation:Maternal education (ME) is an important factor for school readiness and robustly predicts vocabulary and language skills in preschool children (Richels et al., 2013). Lower ME has been associated with reduced volume and functional activity in language areas of the brain (for a review, Farah, 2017). High phonological awareness (PA) links language development to reading ability and PA performance in pre-school children predicts later reading success (Ziegler and Goswami, 2005). In this study, we investigate the relationship between ME and PA on brain functional connectivity (fc) using functional MRI resting state data. Methods:89 typically developing participants (49 male, 40 female), ages 3-5 years.
ME: ME was coded from 1 (basic education) to 7 (graduate training; masters, PhD). PA: PA composite score of the CTOPP-2 (Herford, 2003), completed at the day or the following scanning appointment (within 6 months).MRI: Siemens 3T Trio (fMRI images of brain obtained using: TR/TE=2.5s/34ms, 80^0 flip angle, 80x80 acquisition matrix, 3.6mm slab thickness). The CONN fMRI toolbox (Whitfield-Gabrieli et al., 2012) was used toextract fc values and perform ROI-to-ROI network connectivity analyses to estimate the impact of high and low ME and PA individually, as well as their interactions on fc. Nuisance covariates and realignment parameter noises were reduced using CompCor (Behzadi et al., 2007), and fMRI data were band-pass filtered ($0.008 < f < 0.09$ HZ). All results were controlled for age, biological sex and FDR-corrected for multiple comparisons at $p \leq .05$. Results: Independent t-tests comparing low and high groups (lower and upper 25th percentile for PA; ME≥6, college graduate) revealed greater fc for both high ME (salience and sensorimotor network) and PA (language sensorimotor, posterior cerebellum). The combination of ME and PA groups revealed the following: low ME x low PA show the most negative fc (visual, default mode, language, sensorimotor, posterior cerebellum); low ME x high PA show negative sensorimotor and frontoparietal fc; high ME x low PA show negative and positive fc (visual, attention and default mode); and high ME x high PA only positive fc (visual, language). Conclusion: Increasing levels of maternal education and phonological awareness both increase functional brain connectivity independent of age. Importantly, independent of PA values, high ME consistently increased fc in networks important for reading ability, and low ME but not PA always resulted in reduced fc. These results could indicate a strong effect of ME on fc networks enabling early reading readiness and ability.

Disclosures: M.M.K. Bruchhage: None. **G. Ngo:** None. **S.C.L. Deoni:** None. **V. D'Sa:** None.

Poster

171. Human Social Cognition: Behavior, Mechanisms, and Disorders I

Location: Hall A

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Program #/Poster #: 171.11/AA32

Topic: H.02. Human Cognition and Behavior

Support: NRF-2017R1D1A1B032115

Title: The influence of choice attributes and memory on choice induced preference change

Authors: *Y. HWANG, S. CHO;
Psychology, Chung-Ang Univ., Seoul, Korea, Republic of

Abstract: Choice Induced Preference Change (CIPC) refers to the phenomenon in which self-reported preference increases or decreases after a decision is made for chosen vs. rejected options, respectively. The most widely accepted explanation for CIPC is based on the Cognitive Dissonance (CD) theory. The CD theory explains that people justify their decisions by adjusting subjective beliefs about their own preferences about chosen or rejected options. The present study aimed to investigate whether the degree of CIPC is modulated by different types of choice (i.e., preference-based vs. non-preference based) and memory of the options using a free choice paradigm with face stimuli.

Participants first gave ratings of their subjective preference for each face stimuli (R1 phase). In the choice phase (C), participants were presented with two faces to which they had given similar ratings at R1 phase. In the preference-based choice task, participants were forced to make a choice between the two faces based on subjective preference. In the non-preference-based choice task, participants were asked to choose a face for which they would recommend a certain pair of eye glasses. In the R2 phase, participants were asked to rate their subjective preference for each face for the second time. The experiment was conducted in two sequences; 1) R1-C-R2 (hereafter RCR) and 2) R1-R2-C (control procedure, hereafter RRC) to verify that the CIPC is due to decision making rather than an artifact of measuring preference twice using the free choice paradigm. Comparison with this control sequence establishes the degree of "true" CIPC that cannot be attributed to experimental artifact (Chen & Risen, 2010). At the end of either sequence, memory of all face stimuli was tested. The dependent variable was calculated by subtracting the degree of preference change for rejected faces from that of chosen faces.

Participants were 69 young adults, among which 33 performed the preference-based choice task, while the rest performed the non-preference-based choice task. A 2 by 2 mixed repeated measures ANOVA with type of Choice (preference-based, non-preference-based) as a between-subjects factor and Sequence (RCR, RRC) as a within-subject factor revealed a significant two-way interaction effect. There was a significantly greater preference change in the RCR compared to RRC sequence only for the preference-based choice task. There was also a significantly greater preference change in the RCR compared to RRC sequence only for remembered (but not forgotten) faces.

Our findings suggest that only choices that reflect subjective preference induce CIPC and that memory of the options may lead to stronger CIPC.

Disclosures: Y. Hwang: None. **S. Cho:** None.

Poster

171. Human Social Cognition: Behavior, Mechanisms, and Disorders I

Location: Hall A

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Program #/Poster #: 171.12/AA33

Topic: H.02. Human Cognition and Behavior

Support: DFG: KI 588/16-1
DFG: STR 1146/4-1

Title: Effects of group membership on neural correlates of mentalizing in the prisoner's dilemma game

Authors: *G. NEZIROGLU, M. STEINES, T. KIRCHER, B. STRAUBE;
Philipps Univ. of Marburg, Marburg, Germany

Abstract: The ability to infer mental states of others -defined as theory of mind (ToM) - is known to be influenced by group membership of the person we are interacting with. There is increasing neuroscientific evidence that people interact with their ingroup and outgroup members differently. Studies on ToM have so far focused either on pre-existing (i.e. sport fan) or experimentally induced (arbitrary) groups. However, the effects of such group defining factors on the neural correlates of mentalizing in an interactive task remain unknown. The aim of the present study was to investigate how neural correlates of social interactions were influenced by group membership defined by cues representing pre-existing and novel groups. The pre-existing social groups were based on migration background (game partners with and without migration background). The minimal group paradigm was used to determine arbitrary social groups whereby group membership was generated by a bogus problem-solving test (minimal in- vs. outgroup). During the acquisition of functional MRI, 46 German participants without migration background played a modified version of Prisoner’s Dilemma game with alleged members of their pre-existing and minimal in- and outgroups. The task was to gain as many points as possible and more than their counterparts. Although participants reported identifying more strongly with their ingroup game partners independent of the group defining factor (pre-existing or arbitrary group), they adopted a competitive strategy towards all players. fMRI results demonstrated that interacting with a game partner significantly activated the ToM network across all conditions. Interestingly, interacting with a game partner of the pre-existing outgroup was linked to significantly more activity in areas associated with mentalizing - including the middle cingulate gyrus, middle frontal gyrus, inferior frontal gyrus, superior parietal lobule and angular gyrus. These results indicate that interacting with pre-existing outgroup members requires more neural resources for mentalizing, probably to be able to anticipate their game strategies. Interaction with outgroup members in competitive contexts might increase consideration of

outgroup members' mental states and leads to greater activations in regions associated with mentalizing.

Disclosures: **G. Neziroglu:** None. **M. Steines:** None. **T. Kircher:** None. **B. Straube:** None.

Poster

171. Human Social Cognition: Behavior, Mechanisms, and Disorders I

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Program #/Poster #: 171.13/AA34

Topic: H.02. Human Cognition and Behavior

Support: MOST 107-2420-H-194-006

Title: Neural modeling and computational approaches to investigating the brain mechanisms underpinning emotional expression processing of East Asian faces

Authors: *G. C.-W. SHYI[1], S.-T. T. HUANG[1], J. O. GOH[2], C.-C. J. LEE[4], Y.-Y. CHEN[1], C.-H. CHEN[3], W.-T. HSIEH[4], F. F.-S. TSAI[4], C.-C. CHEN[2];
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Abstract: Neural modeling such as dynamic causal modeling (DCM) embodies a hypothesis-driven approach, whereas machine learning such as convolutional autoencoder (CAE) embraces a data-driven approach, to uncovering the meaningful patterns that may be hidden in the neuroimaging data. Here we adopted both the DCM and one-dimensional-CAE (1D-CAE) to investigate the brain mechanisms that may underlie the processing of emotional expression of East Asian (Taiwanese) faces. Facial expressions of different identities portraying the six basic emotions were contrasted with those portraying neutral expressions. At the beginning of each fMRI block, an affective label in Chinese was displayed and followed by eight sequentially presented faces from the same emotional category. Participants with differential thresholds in discriminating emotions were asked to judge the emotional intensity of each facial expression. DCM results indicated that (a) the fusiform gyrus (FFG) and posterior superior temporal sulcus (pSTS) in the two hemispheres were interconnected, and (b) the projection between bilateral FFG and amygdala were modulated by bilateral pSTS, suggesting a critical role for pSTS in emotional processing even in the processing of emotion conveyed via faces. On the other hand, the BOLD signals of 47,636 voxels based on the 90 ROIs specified according to the Automated Anatomical Labelling (AAL) were fed to the 1-D -CAE model with three encoding layers and three decoding layers. The results of reconstructed brain activities based on the learned voxel weights (the core features set) were then remapped onto the AAL-defined ROIs to locate brain regions that may contribute differentially to the emotional judgments of facial expression among

high- and low-thresholders. For low-thresholders who required smaller image differences to discriminate facial expressions, the results of the CAE suggest that brain regions that are involved in basic visual processing appear to play a sufficient role. In contrast, for high-thresholders, brain regions responsible for emotion processing and those that are involved in top-down emotional regulations appear to play a greater role. Taken together, the results of 1-D CAE complement those from the DCM model and suggest that the latter can be extended to include prefrontal regions that are related to emotion processing and regulation in order to better understand the brain mechanisms underpinning emotional processing of East Asian faces.

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Poster

171. Human Social Cognition: Behavior, Mechanisms, and Disorders I

Location: Hall A

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Program #/Poster #: 171.14/AA35

Topic: H.02. Human Cognition and Behavior

Title: Teaching machines to recognize neurodynamic correlates of team and team member uncertainty

Authors: *R. STEVENS[1], T. GALLOWAY[2];
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Abstract: We describe efforts to make humans more transparent to machines by focusing on uncertainty, a concept with roots in neuronal populations yet scales across social interactions. To be effective partners machines will need to learn why uncertainty happens, how it happens, how long it will last, and possible mitigations the machine can supply. EEG-derived measures of team neurodynamic organization were used to identify times of uncertainty in military, healthcare and high school problem-solving teams. A set of neurodynamic sequences was assembled that differed in the magnitudes and durations of uncertainty with the goal of training machines to detect the onset of prolonged periods of high level uncertainty, i.e. when a team might require support. Variations in uncertainty onset were identified by classifying the first 70s of the exemplars using self-organizing maps (SOM), a machine architecture that develops a topology during training that separates closely related from desperate data. Clusters developed during training that distinguished patterns of no uncertainty, low-level and quickly resolved uncertainty, and prolonged high level uncertainty, creating opportunities for neruodynamic-based systems that can interpret the ebbs and flows in team uncertainty and provide recommendations to the trainer or team in near real time when needed.

Disclosures: R. Stevens: None. **T. Galloway:** None.

Poster

171. Human Social Cognition: Behavior, Mechanisms, and Disorders I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 171.15/AA36

Topic: H.02. Human Cognition and Behavior

Title: Antisocial personality traits, voice modulation, and appraisal of music impact social discounting behavior

Authors: D. TOSSAVAINEN, S. E. MCCLELLAND, S. SPIVACK, L. E. CRANMER, G. IENNER, A. POINCOT, ***P. WALLISCH**;
New York Univ., New York, NY

Abstract: Social discounting - how prosocial behavior is modulated by social distance - is increasingly well studied. However, many aspects of this behavior remain unexplored. Here, we query the social discounting behavior of a large sample of participants in terms of both gains and losses in relation to social distance. In addition, participants listened to music prior to social discounting trials and we elicited voice samples as well as personality traits. We found a small, but consistent effect of music on social discounting - positive music appraisals foster prosocial behaviors, whereas negative appraisals hinder it. In addition, we found that prosocial behavior in our social discounting task was related to increased voice modulation and lack of antisocial personality traits. We conclude that social discounting is a valid behavioral assay to characterize prosocial behavior. This behavior can be modulated by extra-social factors, such as music, and predicted by a distinct prosody pattern as well as personality traits.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01MH110831
NIH Grant U01NS103792
NSF CAREER Grant

Title: Single-neuron representations in human orbitofrontal cortex and amygdala of internal emotional states evoked by contextual information

Authors: J. FELDMAN[1], D. BEAM[2], J. YIH[3], A. N. MAMELAK[1], J. PARVIZI[4], *U. RUTISHAUSER[1,5];
[1]Neurosurg., Cedars-Sinai Med. Ctr., Los Angeles, CA; [2]Neurol. and Neurolog. Sci., Stanford Univ., Palo Alto, CA; [4]Neurol. and Neurolog. Sci., [3]Stanford Univ., Stanford, CA; [5]Biol. and Biol. Engin., Caltech, Pasadena, CA

Abstract: The orbitofrontal cortex (OFC) and amygdala play a significant role in the processing of emotional stimuli. Both areas are critical for representations of one's own emotional state and such representations are thought to modulate ongoing processing in a top-down fashion. However, it remains largely unknown how one's own emotional state modulates the processing of sensory information. Here, we utilized single-neuron recordings in human epilepsy patients to study the representation of emotional faces and its modulation by context. We utilized two tasks: an emotional faces task, in which faces expressed an emotion (happy, angry, or neutral) and an appraisal task, in which faces with a neutral expression were presented preceded by a context-setting appraisal frame that was positive, negative, or neutral. In this pilot study, we recorded neurons from the OFC and amygdala of two epilepsy subjects that performed the two tasks (n=125 and 149 neurons in total, respectively). First, we categorized neurons according to their selectivity to specific facial expressions. As expected, we found neurons in the amygdala that modulated their firing as a function of the emotion expressed by the faces viewed by the subjects (10.3% of neurons). These neurons typically modulated firing from baseline within about 200 ms. Second, we determined whether neurons responded differentially to the neutral faces depending on the context (framing). This revealed neurons in the OFC that modulated their response to neutral faces based on the valence of the auditory frame (14.3% of neurons). These cells typically varied firing rate from baseline within 100 ms of face onset. Remarkably, there was little overlap between these two types of neurons. For example, cells that modulated firing rate for a happy face did not also show significant modulation for neutral faces that were presented following a happy frame. This indicates that the representations of registering someone else's emotional state (viewing faces) are likely different from those representing one's own emotional state. This pilot work thus indicates that this paradigm might be suitable to examine how internal representations of one's own emotional state colors processing of incoming sensory information.

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Poster

171. Human Social Cognition: Behavior, Mechanisms, and Disorders I

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Program #/Poster #: 171.17/AA38

Topic: H.02. Human Cognition and Behavior

Title: Real-time assessment of brain dynamics during embodiment: An IVR-TMS-EEG study

Authors: *E. P. CASULA[1], G. TIERI[2], M. MAIELLA[2], R. PEZZETTA[2], E. F. PAVONE[3], S. M. AGLIOTI[4], G. KOCH[5];
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Abstract: In daily life, we experience the world through our bodily actions without doubting about the integrity, continuity and sense of ownership of our body. This phenomenon is critical to establish a normal self-consciousness and can be investigated with immersive virtual reality (IVR) by inducing the illusory experience of embodiment over a virtual body. However, the neural mechanisms underlying the embodiment process of artificial limbs are mostly unknown. In the present project, we took advantage of the novel combined TMS-EEG (transcranial magnetic stimulation and electroencephalography) approach to *in vivo* investigate the real-time neural processes underlying the embodiment of a virtual arm.We tested 19 healthy volunteers in three IVR-TMS-EEG sessions during which they wore a head-mounted display through which they observed a virtual arm projected out of their right shoulder in a first-person perspective. During IVR, the participant was instructed to passively observe the virtual arm (overlapped to the real one) and to refer whether he/she feel it as a part of his body. In two IVR sessions, the participant observed a standard right full arm and received 160 TMS single-pulses over the left (full-lM1 condition) or over the right primary motor cortex (full-rM1 condition). In another IVR session, the participant observed an arm with detached hand due to a missing wrist (detached-lM1 condition). All the IVR sessions were preceded and followed by a TMS-EEG block of stimulation during which 120 TMS single-pulses were delivered over the left M1. Behavioral effects were investigated in terms of self-reported sense of ownership, whereas neurophysiological effects were investigated in terms of TMS-evoked cortical response. Behavioral results showed that the full-lM1 condition induced the highest embodiment feeling ($p<0.01$). In the same condition, neurophysiological results showed a strong reduction of TMS-evoked cortical activity ($p<0.001$). A trial-by-trial analysis conducted during IVR, showed that suppression of cortical activity started concurrently with the self-reported feeling of embodiment. These effects were focused over the left motor area, comprising M1 and the pre-motor areas. No effects were observed in the detached-lM1 condition ($p>0.05$). We provide the first evidence of the *in vivo* neural mechanisms underlying embodiment of a virtual arm. The embodiment

experience of a virtual arm was accompanied by a dramatic decrease in the contralateral motor area activity, compatible with a disembodiment feeling of the real hand. Interestingly, the timing of such neurophysiological effect was coincident with the self-reported embodiment feeling.

Disclosures: **E.P. Casula:** None. **G. Tieri:** None. **M. Maiella:** None. **R. Pezzetta:** None. **E.F. Pavone:** None. **S.M. Aglioti:** None. **G. Koch:** None.

Poster

171. Human Social Cognition: Behavior, Mechanisms, and Disorders I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 171.18/AA39

Topic: H.02. Human Cognition and Behavior

Support: DoD Grant N660011824050

Title: Association of arousal-related neural activation with disposition towards social issues: A pupillometric fMRI study

Authors: *D. P. CHRISTIANO[1], H. WANG[1], A. TAM[1], D. G. GHAHREMANI[1], E. D. LONDON[1,2,3];
[1]Psychiatry and Biobehavioral Sci., [2]Mol. and Med. Pharmacol., [3]Brain Res. Inst., UCLA, Los Angeles, CA

Abstract: Viewing media that depict social issues may engage neural mechanisms of arousal and activate dispositions. We tested this hypothesis by examining associations between arousal-related neural activation and self-reported dispositions to video clips that depict contemporary social issues. Prior to fMRI scanning, participants (ages 18-45) viewed 30 two-minute publicly available video clips on issues ranging from immigration to the environment while peripheral psychophysiological measurements (e.g., heart rate variability, HRV) were recorded. After viewing each video, participants rated the intensity of their affect during viewing, their agreement with the message of the video, and their willingness to share the video on social media. Of 30 videos evaluated by each individual, 16 were selected for presentation during fMRI based on highest and lowest agreement ratings and HRV. During fMRI, pupillary diameter was measured as a dynamic index of arousal, and participants provided self-report ratings of the videos. Ratings obtained before and during fMRI were highly consistent. The fMRI images were preprocessed (motion correction, denoising using independent component analysis) and then analyzed using the general linear model, in which separate regressors were used to model each video presentation. A regressor representing the interaction of video presentation and moment-to-moment pupillary diameter (convolved with the hemodynamic response function) was computed, providing an arousal-related activation map for each video. From these maps, we extracted values from the left and right amygdala and performed correlations with the

participant's rating of each video. Arousal-related amygdala activation was significantly correlated with willingness to share the videos (left: r=-0.42, P=0.005; right: r=-0.30, P=0.04). These preliminary results suggest that neural measures of dynamics of arousal may have predictive value in determining the decision to share content related to social issues.

Disclosures: D.P. Christiano: None. **H. Wang:** None. **A. Tam:** None. **D.G. Ghahremani:** None. **E.D. London:** None.

Poster

171. Human Social Cognition: Behavior, Mechanisms, and Disorders I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 171.19/AA40

Topic: H.02. Human Cognition and Behavior

Support: Scientific Research Network on Decision Neuroscience awarded to DVS [Subaward of NIH R24-AG054355 (PI Samanez-Larkin)]
College of Liberal Arts at Temple University (DVS)
NIH grant R21-MH116422 (DVS)

Title: Aging alters corticostriatal interactions during shared reward processing

Authors: *V. KELLY[1], K. HACKETT[1], N. M. HENNINGER[1], T. GIOVANNETTI[1], D. V. SMITH[1], D. S. FARERI[2];
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Abstract: Maintaining a network of close social relationships supports overall well-being throughout the lifespan, suggesting that close relationships are intrinsically rewarding. We have previously shown in healthy young adults that rewards shared with close friends (versus strangers) evoke stronger representations of value in the ventral striatum (Fareri et al., 2012). Because the nature of our relationships with close others may change with age, one outstanding question is how aging influences the way functional connectivity during shared reward processing is modulated by social closeness. To investigate this issue, we recruited 32 participants (20 younger adults, ages 20 - 35; and 12 older adults, ages 65 - 80). Each participant completed a block-design reward processing task in which they played a game requiring them to guess whether a hidden card is higher/lower than 5. Responses led to shared monetary gains (correct guess)/losses (incorrect guess) with a same-sex close friend, a stranger, and a computer while undergoing fMRI (adapted from Fareri et al., 2012). Participants received feedback on each trial as to whether they guessed correctly (gain of $10) or incorrectly (loss of $5). Overall, experiences shared with a close friend were rated as significantly more intense than those shared with a stranger or computer ($F_{(2,44)} = 11.88$, $p < 0.001$). We found that older adults rated

outcomes more positively than younger adults ($F_{(1,22)} = 8.15$, $p < 0.01$). Psychophysiological interaction analyses revealed that older adults relative to younger adults exhibited reduced connectivity between the ventral striatum and ventrolateral prefrontal cortex in during shared outcomes with a close friend relative to a stranger (cluster-defining threshold of Z > 3.1, FWE = 0.05). Taken together, the results suggest that integration of value with socially relevant information may take on less importance during later stages of life. Future analyses will examine changes in network connectivity during shared reward processing as a function of age (Utevsky et al., 2017).

Disclosures: V. Kelly: None. **K. Hackett:** None. **N.M. Henninger:** None. **T. Giovannetti:** None. **D.V. Smith:** None. **D.S. Fareri:** None.

Poster

171. Human Social Cognition: Behavior, Mechanisms, and Disorders I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 171.20/AA41

Topic: H.02. Human Cognition and Behavior

Title: Neural substrates of variations in momentary happiness during social decisions

Authors: *O. SALAH[1], M. GÄDEKE[2], T. WILLEMS[2], B. WEBER[1], R. HURLEMANN[2], J. SCHULTZ[1];
[1]Ctr. for Econ. and Neuroscience, Inst. of Exptl. Epileptology and Cognition Res., [2]Div. of Med. Psychology, Dept. of Psychiatry, Rheinische Friedrich-Wilhelms-Universität Bonn, Bonn, Germany

Abstract: In social contexts, people often need to take decisions for others, or to be submitted to a decision taken for them by other people. To investigate which neural structures underlie the differences in how participants experience the outcomes of these decisions, we modified a recently published social decision task (Rutledge et al, Nature Communications 2016) as follows. Participants lying in an fMRI scanner or a friendly social partner chose between a safe and a risky option in a gamble for money. Choosing the risky option lead to the gamble being played out independently for both players, such that both could either win or lose the gamble. We used bootstrapped backward stepwise regression to predict the variations of momentary happiness occurring during the experiment using BOLD signal from regions of interest specified a priori. We found higher prediction accuracy in all experimental conditions with models including brain regions associated with Theory-of-Mind (ToM) compared with models including only emotion processing and reward evaluation regions. In addition, we found that in the best predictive models, ToM related regions were predictive of momentary happiness only when the game included a social partner. Our findings highlight the relative contributions of different ToM-

associated regions to the changes in momentary happiness occurring during social decision-making

Disclosures: O. Salah: None. **M. Gädeke:** None. **T. Willems:** None. **B. Weber:** None. **R. Hurlemann:** None. **J. Schultz:** None.

Poster

171. Human Social Cognition: Behavior, Mechanisms, and Disorders I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 171.21/AA42

Topic: H.02. Human Cognition and Behavior

Title: Individual differences in social and non-social cognitive control

Authors: *K. M. DARDA, E. E. BUTLER, R. RAMSEY;
Bangor Univ., Bangor, United Kingdom

Abstract: A remarkable feature of the human cognitive system is its ability to adapt behaviour depending on current goals. This process is known as cognitive control, and has been the focus of growing research in cognitive psychology. However, the extent to which shared cognitive and brain systems underlie cognitive control in social and non-social contexts, as well as how these systems may vary across individuals, remains largely unexplored. To investigate this, we used a multimodal approach across three large-sample behavioural experiments (N=165, N=205, N=189), and a large-sample fMRI experiment (N=50). Our behavioural results consistently showed that cognitive control systems are largely invariant to stable aspects of personality, but exhibit a sex difference, such that females show greater interference than males. Further, we showed that the sex difference is unrelated to the sex of the interaction partner and does not reflect an in-group bias based on sex. The sex difference is also neither completely domain-general i.e. it does not generalise across all types of cognitive control, nor is it domain-specific i.e. it is not solely tied to social control. Instead, our findings suggest that a robust sex difference exists in the system or set of subsystems that operate in resolving a form of non-social spatial conflict. Our fMRI findings found that along with non-social control, social control also recruited areas of the domain-general multiple demand network, instead of a domain-specific brain network unique to social cognition. However, in contrast to the behavioural results, no sex differences were found on neural correlates of social or non-social spatial control. Functional connectivity profiles differed for social and non-social control, thus suggesting that domain-general brain networks that operate across a wide range of cognitive control tasks may be engaged differentially for different types of cognitive control. In addition, current models of social and non-social control need updating to account for the potential interplay between domain-general and domain-specific neural networks which might make a process distinctly social than non-social.

Disclosures: **K.M. Darda:** None. **E.E. Butler:** None. **R. Ramsey:** None.

Poster

171. Human Social Cognition: Behavior, Mechanisms, and Disorders I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 171.22/AA43

Topic: H.02. Human Cognition and Behavior

Title: Effects of transcranial direct current stimulation (tDCS) of the anterior prefrontal cortex on Theory of Mind tasks

Authors: *A. KARIM[1,2], Z. ZHENG[3], R. KHALIL[3], B. GODDE[3];
[1]Dept. of Psychology and Methods, Univ. of Tübingen, Tübingen, Germany; [2]Dept. of Neurorehabilitation and Hlth. Psychology, SRH Mobile Univ., Riedlingen, Germany; [3]Psychology & Methods, Jacobs Univ., Bremen, Germany

Abstract: Neuroimaging have shown that the anterior prefrontal cortex (aPFC; BA9/10) is involved in Theory of Mind (ToM) tasks such as the recognition of facial emotional expressions (for a review see Kripple & Karim, 2011; Khalil et al., 2018). However, since neuroimaging methods only allow correlative statements the causal contribution of the aPFC in ToM tasks remain elusive. In this study we investigated in N=37 students the effects of inhibiting the aPFC with cathodal transcranial direct current stimulation (tDCS) compared with sham tDCS as placebo control while subjects were required to recognize facial emotional expressions using the Amsterdam Dynamic Facial Expression Set (ADFES) (Schalk et al., 2011). Moreover, we investigated the interaction between inhibiting the aPFC and Machiavellian personality traits. Our data reveal that inhibition of the aPFC impairs recognition of facial emotional expressions only in subjects with low Machiavellian personality traits but not in subjects with high Machiavellian personality traits. These findings provide crucial information in understanding the pathophysiology of ToM deficits in personality disorders and my offer novel treatment options in the neurorehabilitation of ToM deficits using tDCS.
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Khalil R, Tindle R, Boraud T, Moustafa A, Karim AA. (2018). Social decision making in autism: On the impact of mirror neurons, motor control, and imitative behaviors. CNS neuroscience & therapeutics 24 (8), 669-676.
Krippl M & Karim AA, (2011). " Theory of mind" and its neuronal correlates in forensically relevant disorders. Nervenarzt. 82: 843-852.
Schalk JV, Hawk ST, Fischer AH, & Doosje B. (2011). Moving faces, looking places: validation of the Amsterdam Dynamic Facial Expression Set (ADFES). *Emotion, 11 4*, 907-20.

Disclosures: **A. Karim:** None. **Z. Zheng:** None. **R. Khalil:** None. **B. Godde:** None.

Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 172.01/AA44

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: R&D program of China 2018YFC2000100
CAS Strategic Priority Research Program XDB32060000

Title: Cytosine base editor generates substantial off-target single-nucleotide variants in mouse embryos

Authors: *H. YANG[1], E. ZUO[2], Y. SUN[3], T. YUAN[4];
[1]Inst. of Neurosci., Inst. of Neuroscience, Chinese Acad. of Scie, Shanghai, China; [2]Inst. of Neurosci., Shanghai, China; [3]CAS-MPG Partner Inst. for Computat. Biol., Shanghai, China; [4]Chinese Acad. of Agr. Sci., Shenzhen, China

Abstract: Genome editing holds promise for correcting pathogenic mutations. However, it is difficult to determine off-target effects of editing due to single-nucleotide polymorphism in individuals. Here we developed a method named GOTI (genome-wide off-target analysis by two-cell embryo injection) to detect off-target mutations by editing one blastomere of two-cell mouse embryos using either CRISPR-Cas9 or base editors. Comparison of the whole-genome sequences of progeny cells of edited and nonedited blastomeres at embryonic day 14.5 showed that off-target single-nucleotide variants (SNVs) were rare in embryos edited by CRISPR-Cas9 or adenine base editor, with a frequency close to the spontaneous mutation rate. By contrast, cytosine base editing induced SNVs at more than 20-fold higher frequencies, requiring a solution to address its fidelity.

Disclosures: H. Yang: None. **E. Zuo:** None. **Y. Sun:** None. **T. Yuan:** None.

Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 172.02/BB1

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: National Key R&D Program of China 2018YFA0108000

National Natural Science Foundation of China 31771196, 31471037
Shanghai Science and Technology Commission Innovation Fund 17JC1401500
Shanghai Commission of Health Youth Program 2017YQ016

Title: A single-allele conditional gene inactivation and *in situ* restoration strategy utilizing two recombinases

Authors: *X. LIU, H. LIU, L. MA, J. GAN, T. ZHANG, Y. XU, L. GONG, P. MU, M. HE;
Inst. of Brain Science, Fudan Univ., Shanghai, China

Abstract: Studying neurodevelopment and modeling neurological diseases rely on genetic methods such as conditional gene regulation. We have designed a novel strategy that conditionally inactivates and restores gene function through reversible inversion of a critical part of its genomic sequence by Cre- and Flp-mediated recombinations. As a proof of principle, we applied this strategy to *Mecp2*, an X-linked dosage sensitive gene whose mutations cause Rett syndrome. Combining with multiple Cre and Flp mouse driver lines and viral tools, we demonstrated the efficiency, specificity and flexibility of this method by inactivating and restoring *Mecp2* in germline and in several neuron types. Some phenotypes caused by cell type specific *Mecp2* inactivation were prevented or rescued by in situ *Mecp2* restoration. This versatile method allows reversible gene regulation in specific cell types and will facilitate studies of gene function in development and disease. The mouse lines generated in this study will be valuable for Rett syndrome research.

Disclosures: X. Liu: None. **H. Liu:** None. **L. Ma:** None. **J. Gan:** None. **T. Zhang:** None. **Y. Xu:** None. **L. Gong:** None. **P. Mu:** None. **M. He:** None.

Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 172.03/BB2

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: Whitehall Foundation 2017-05-20

Title: Developing adeno-associated virus reporters for intersectional cell-type targeting

Authors: *Y. ZHU[1], **J. XU**[2];
[1]Dept. of Ophthalmology and Physiology/ Feinberg Sch. of Med., [2]Dept. of Physiology/ Feinberg Sch. of Med., Northwestern Univ., Chicago, IL

Abstract: The low toxicity and efficient transduction of adeno-associated virus (*AAV*) made it the most commonly used viral vector in neuroscience. When combined with site-specific

recombinase such as Cre or Flp, expression of transgene can be controlled in cell-type specific manner. Furthermore, expression of transgenes can be controlled by two recombinases simultaneously, allowing viral vectors to target intersections of two distinct neuronal populations. To gain control of the first recombinase, one can use DIO/FLEX genetic switch. This strategy provides a tight control because of the initial inverted orientation of transgene. To gain control of the second recombinase, one can place certain stop cassette, flanked by recombinase recognition sites (LoxP, FRT, etc), in front of transgene. These stop cassettes often comprise one or multiple polyA signals (polyA-STOPs) to force transcription into termination. However, noticeable levels of leaky expression in the absence of recombinase are often encountered with this strategy. To reduce "read-through" leaky expression, we designed a series of STOPs based on Kozac sequences. The Kozac sequences are recognized by the ribosome as the translational start site. Our Kozac-STOPs contain consensus Kozac sequence (ccAccAUGG) placed in front of translation termination codon (TAG, TAA or TGA). These Kozac-STOPs are very short (< 100 base pairs). When inserted between promoter and transgene, they significantly reduced expression of transgenes. However, we found that leaky expressions were still quite strong. We then made new STOPs by combining Kozac-STOP with polyA-STOP. We noticed lowest level of the leaky expression, suggesting that the new STOPs can be used to generate AAV intersectional reporters. Experiments are being conducted to examine the utility of the new AAV vectors for intersectional cell-type targeting in mammalian retina.

Disclosures: **Y. Zhu:** None. **J. Xu:** None.

Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 172.04/BB3

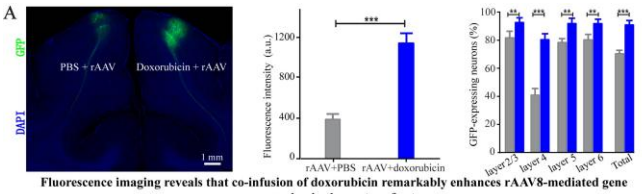
Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: the Strategic Priority Research Program of Chinese Academy of Sciences, Grant No. XDB32060200
the Shanghai Municipal Science and Technology Major Project, Grant No. 2018SHZDZX05

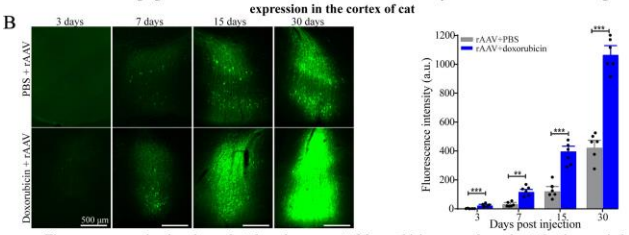
Title: Doxorubicin accelerates and enhances rAAV-mediated gene expression in the cortex of mouse, cat and monkey

Authors: *H. GONG[1,2], C. TANG[1,2], L. QIAN[1], Y. LU[1], I. ANDOLINA[1], S. ZHANG[3], H. YANG[1,2], J. WU[3], W. WANG[1,2];
[1]Inst. of Neuroscience, Chinese Acad. of Sci., Shanghai, China; [2]Univ. of Chinese Acad. of Sci., Beijing, China; [3]Dept. of Ophthalmology, Eye and ENT Hosp. of Fudan Univ., Shanghai, China

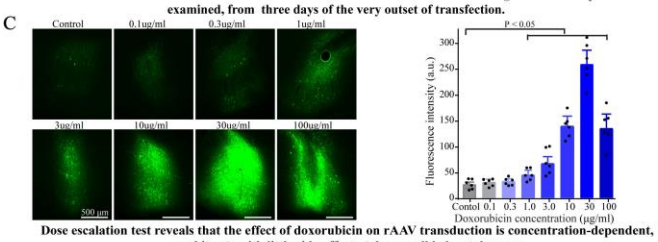
Abstract: Recombinant adono-associated viruses (rAAVs) are widely used as vehicles for gene transfer to mammalian cortices of central nervous system (CNS). However, the time-delay in transgene expression together with insufficient transduction has limited rAAVs' full application, and through vectoring approaches alone is not enough to meet some specific requirements in research and therapy. Here, by exploitation of the clinical pharmacological agent doxorubicin as an agonist, we have described a practical strategy for rapid and efficient rAAV-mediated transgene expression in the mammalian cortices across rAAV serotypes and species (mouse, cat and non-human primate). Firstly, we found that the co-infusion of doxorubicin remarkably enhanced rAAV8-mediated gene expression throughout the time-period examined from three days of the very outset of transfection. The following *in vivo* dose-finding and cytotoxic studies revealed that the action of doxorubicin could take prominent effects at doses without cytotoxicity. Finally, the agonist effect of doxorubicin on rAAV8 could be reproduced with other rAAV serotypes, suggesting a universal effect of doxorubicin upon *in vivo* rAAV transduction. In short, this study provides a practical and effective strategy that can be synergistic with vector amelioration for further improvement on *in vivo* rAAV transduction, so as to obtain rapid and efficient gene transduction with lower doses of viral vector.



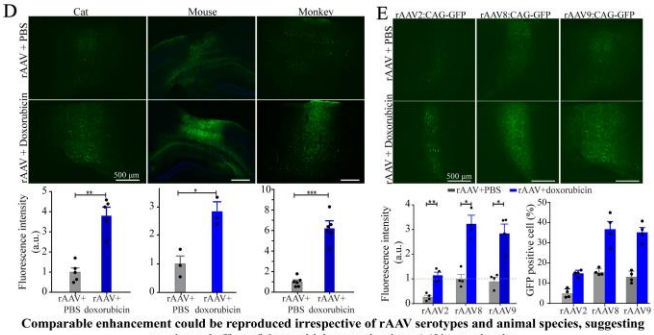
Fluorescence imaging reveals that co-infusion of doxorubicin remarkably enhances rAAV8-mediated gene expression in the cortex of cat



Time course examination shows that the enhancement of doxorubicin occurs throughout the time-period examined, from three days of the very outset of transfection.



Dose escalation test reveals that the effect of doxorubicin on rAAV transduction is concentration-dependent, and it acts with little side-effect at doses well below tolerance



Comparable enhancement could be reproduced irrespective of rAAV serotypes and animal species, suggesting a universal effect of doxorubicin upon in vivo rAAV transduction

Disclosures: **H. Gong:** None. **C. Tang:** None. **L. Qian:** None. **Y. Lu:** None. **I. Andolina:** None. **S. Zhang:** None. **H. Yang:** None. **J. Wu:** None. **W. Wang:** None.

Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 172.05/BB4

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: U01MH10913301
RF01MH117070-01
R21HG009750
T32GM008151-32
T32GM007200
T32HG000045
F30HG009986

Title: Transposon mediated, cell type specific transcription factor recording in the mouse brain

Authors: *A. J. CAMMACK[1], A. MOUDGIL[2], T. LAGUNAS, JR[2], M. J. VASEK[2], M. SHABSOVICH[2], J. HE[2], X. CHEN[2], M. HOODA[2], M. N. WILKINSON[2], T. M. MILLER[3], R. D. MITRA[2], J. D. DOUGHERTY[4];
[1]Washington Univ. In St. Louis, St. Louis, MO; [2]Washington Univ. In St. Louis, Saint Louis, MO; [3]Dept Neurol, Washington Univ, Sch. Med., Saint Louis, MO; [4]Genet. and Psychiatry, Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Transcription factors (TFs) play a central role in the regulation of gene expression, controlling everything from cell fate decisions to activity dependent transcription. However, widely-used methods for TF profiling *in vivo* (e.g. ChIP-seq) yield only an averaged picture of TF binding across all cell types present within the harvested tissue; thus, it is challenging or impossible to determine how the same TF might bind different portions of the genome in different cell types, or even to identify its binding events at all in rare cell types in a complex tissue such as the brain. Here we present a versatile methodology, FLEX calling cards, for the mapping of TF occupancy in specific cell types from heterogenous tissues. In this method, the TF of interest is fused to a hyperactive *piggyBac* transposase (hypPB), and this bipartite gene is delivered, along with donor transposons, to mouse tissue via a Cre-dependent adeno-associated virus (AAV). The fusion protein is expressed in Cre-expressing cells where it inserts transposon "calling cards" near to TF binding sites. These transposons permanently mark TF binding events and can be mapped using high-throughput sequencing. Alternatively, unfused hypPB interacts with and records the binding of the super enhancer (SE)-associated bromodomain protein, Brd4. To demonstrate the FLEX calling card method, we first show that donor transposon and

transposase constructs can be efficiently delivered to the mouse brain with AAV and that insertion profiles report TF occupancy. Then, using a Cre-dependent hypPB virus, we show utility of this tool in defining cell type-specific TF profiles in multiple cell types of the brain. Finally, we demonstrate utility of FLEX calling cards in longitudinal, integrative recording of the promoter-associated TF, SP1, providing a historical record of SP1 occupancy across time. This approach will enable important cell type-specific studies of TF-mediated gene regulation in the brain and could provide valuable insights into brain development, homeostasis, and disease.

Disclosures: **A.J. Cammack:** None. **A. Moudgil:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent filed on SRT technology. **T. Lagunas:** None. **M.J. Vasek:** None. **M. Shabsovich:** None. **J. He:** None. **X. Chen:** None. **M. Hooda:** None. **M.N. Wilkinson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent filed on SRT technology. **T.M. Miller:** None. **R.D. Mitra:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent filed on SRT technology. **J.D. Dougherty:** None.

Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 172.06/BB5

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Title: Development of novel IDLV lentiviral vector platform as an efficient vehicle for CRISPR/dCas9-DNMT3A gene therapy: The new generation drug for precised modulation of SNCA gene expression

Authors: O. CHIBA-FALEK[1], L. TAGLIAFIERRO[1], E. ILICH[2], J. GU[1], Z. Y. HUANG[3], S. MURPHY[3], ***B. KANTOR**[2];
[1]Neurol., [2]Neurobio., [3]Obstetrics and Gynecology, Duke Univ., Durham, NC

Abstract: Here we describe the development of novel integrase-deficient *all-in-one* lentiviral vectors (*IDLVs*) for delivery of dCas9/CRISPR system into dopaminergic progenitor neurons derived from a patient with *SNCA* triplication (*SNCA*-Tri) and *in vivo*. The system carried dCas9-DNA methyltransferase 3A (*DNMT3A*) designed to enrich DNA methylation within *CpGs* island at the *SNCA* intron 1. We demonstrate that IDLV-gRNA/dCas9-DNMT3A system is capable to downregulate *SNCA* overexpression in efficient and precise manner. Importantly, we demonstrate the rescue of Parkinson's disease (PD)-related phenotypes in the neuroprogenitor cells (NPCs) derived from the PD patient. These proof-of-concept experiments provide a strong foundation for further development of the approach for gene therapy use.

Disclosures: **O. Chiba-Falek:** None. **L. Tagliafierro:** None. **E. Ilich:** None. **J. Gu:** None. **Z.Y. Huang:** None. **S. Murphy:** None. **B. Kantor:** None.

Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 172.07/BB6

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: HHMI

Title: A CRISPR/Cas9 based recombination system to facilitate the design of complex transgenic animals for neuroscience experimentation

Authors: ***J. LUO**[1], C. HUANG[2], J. LI[2], M. J. SCHNITZER[1,2];
[1]HHMI, Stanford, CA; [2]Stanford Univ., Stanford, CA

Abstract: Transgenic reporters and effectors are important experimental tools for imaging and manipulating neural activity. To avoid influencing essential genes, researchers generally integrate transgenic elements at a limited number of well-characterized genetic loci. However, to achieve highly specific expression patterns, or to combine the use of multiple reporters and effectors, it is often desirable to create transgenic animals that contain multiple transgenic elements. Yet, due to the constraints imposed by genetic linkage and crossing-over, the probability of recombining multiple transgenic elements at the same docking site is near zero. Hence, traditional approaches to recombination are poorly suited to designing animals with a greater number of transgenic elements than the number of available docking sites. To overcome this limitation, we created and tested a set of genetic tools in *Drosophila melanogaster* based on the CRISPR/Cas9 genome editing system. For a given pair of transgenic elements to be combined, we first add an adaptor sequence upstream or downstream of each element. These adaptors then facilitate integration of the two original transgenes into a single, large transgene. The efficiency of our recombination system is dramatically higher than natural recombination. To illustrate, we used our approach to concatenate multiple copies of the gene encoding a fluorescent optical indicator of neural transmembrane potential, thereby enabling substantial increases in the indicator's expression level and optical signal-to-noise ratio during *in vivo* imaging experiments. Overall, our approach represents a new strategy for designing transgenic animals that have multiple transgenic elements and that are in increasing demand for state-of-the-art behavioral, imaging and optogenetic experiments in neuroscience.

Disclosures: **J. Luo:** None. **C. Huang:** None. **J. Li:** None. **M.J. Schnitzer:** None.

Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 172.08/BB7

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: NIH/NCI T32CA154274-02

Title: Combinatorial optimization of CRISPR/Cas9-mediated knockin yields high-performance editing *in vivo*

Authors: *R. RICHARDSON, M. STEYERT, B. ALTAS, A. ROMANOWSKI, A. POULOPOULOS;
Pharmacol., Univ. of Maryland Baltimore Sch. of Med., Baltimore, MD

Abstract: Gene knockin enables tagging and experimental manipulation of endogenous proteins while retaining native regulation of spatial and temporal expression patterns. While genome editing using CRISPR/Cas9 has emerged as an effective strategy for producing such knockins, the high incidence of on-target genome damage by undesired nucleotide insertion or deletion (NHEJ-mediated indels) causes an intrinsic bias towards knockout rather than knockin. In culture, strategies can be employed to select for cells with precise knockin, thereby obviating the impact of undesirable editing outcomes. While CRISPR/Cas9-mediated knockin can similarly be performed directly in the brain, its utility is hampered by this inherently low efficiency and fidelity, coupled with the inability to apply selection *in vivo.* In this study, to enable high-performance *in vivo* knockin directly in the brain, we combine existing variants of Cas9 and donor DNA and quantify their cumulative effect on knockin *in vitro* and *in vivo*. Our findings show that fusing Cas9 to Ctip, a critical component of the endogenous homology-directed repair pathway, in combination with long, in-situ linearized dsDNA donor templates, yields greater than 20-fold increases in knockin efficiency and editing specificity. Additionally, these optimizations result in a 40% increase in the occurrence of biallelic knockin, and, combined with *in utero* electroporation, provide an effective platform for *in vivo* genome editing in the developing brain. Applying this approach with high-fidelity Cas9 variants increases on-target stringency with minor sacrifice to overall efficiency. This high-performance combinatorial *in vivo* knockin strategy allows rapid and targeted experimental knockin into wild-type animals, and lays the groundwork for potential *in situ* use of CRISPR/Cas9 in future gene therapy approaches in patients.

Disclosures: R. Richardson: None. **M. Steyert:** None. **B. Altas:** None. **A. Romanowski:** None. **A. Poulopoulos:** None.

Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 172.09/BB8

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: R01MH103374
R01NS102456
Duke Bryan Scholar Award

Title: Plug and play protein modification using homology-independent universal genome engineering

Authors: *Y. GAO, E. HISEY, T. W. BRADSHAW, E. ERATA, W. E. BROWN, J. L. COURTLAND, A. UEZU, Y. XIANG, Y. DIAO, S. H. SODERLING;
Duke Univ., Durham, NC

Abstract: Analysis of endogenous protein localization, function, and dynamics is fundamental to the study of cellular neurobiology. However, current approaches are often low-throughput and resource-intensive. Here we describe a novel CRISPR/Cas9-based method for endogenous protein modification that is straightforward, scalable, and highly flexible in terms of genomic target and utility, employing AAV vectors to allow efficient application *in vitro* as well as *in vivo*. We demonstrate rapid alterations of proteins for labeling and dynamic visualization, neural circuit-specific protein modification, subcellular rerouting and sequestration, and truncation-based structure-function analysis. We also report successful detection of various novel proteomic candidates of the inhibitory synapses and axonal initial segments of neurons. Together, the “plug and play” nature of the system enables high-throughput and modular analysis of mechanisms driving protein functions in cellular neurobiology.

Disclosures: **Y. Gao:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent pending. **E. Hisey:** None. **T.W. Bradshaw:** None. **E. Erata:** None. **W.E. Brown:** None. **J.L. Courtland:** None. **A. Uezu:** None. **Y. Xiang:** None. **Y. Diao:** None. **S.H. Soderling:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent pending.

Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 172.10/BB9

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: Medical Research Council MC_UP_1201/2
ERC Starting Grant STG-677029
ERANET-NEURON Micronet

Title: Circuit-based genome editing using self-inactivating rabies virus

Authors: E. CIABATTI[1], H. LEE[2,1], M. TRIPODI[1];
[1]MRC Lab. of Mol. Biol., Cambridge, United Kingdom; [2]Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Neural networks are emerging as the fundamental computational unit of the brain. Network dysfunction is also the culprit of many psychiatric and neurodegenerative disorders, such as schizophrenia and Alzheimer disease. However, our ability to precisely and safely manipulate neural networks and deliver network-specific therapeutic treatments remains limited. To overcome this, we recently engineered a monosynaptically restricted Self-inactivating Rabies virus (SiR, Ciabatti et al 2017) that provides permanent life-long genetic access to neural circuits without cytotoxicity or detrimental effects on circuit physiology and function. All RNA viruses are known to be subject to high rate of mutations. Such mutations could target key regulatory elements of the SiR, potentially leading to the loss of self-inactivating behaviour and hence to the emergence of wild type-like revertant mutants. To appreciate the likelihood of these events, we systematically investigated the genomic stability of SiR. We found that the occurrence of revertant mutations is rare and that they do not accumulate when the virus is produced in appropriate conditions (as described in Ciabatti et al, 2017). To expand the scope of SiR as a neural tracer and potential therapeutic vector we further developed the technology in two directions: incorporating genome-editing machinery in the SiR genome and modifying the parental viral strain to reduce its immunogenicity. We screened multiple designs to generate functional SiR viruses that genomically integrate the two components of the CRISPR technology (the CAS9 protein and a guide RNA) and successfully edit genomic loci *in vitro* and *in vivo*. To reduce the SiR-induced immune response that could be elicited shortly after infection and before viral silencing, we applied the SiR technology to a more neurotropic Rabies strain (N2c), known to be less immunogenic than the B19 strain used in the first generation SiR. This new-generation of SiR virus (SiR2.0) retains the ability to permanently label neural networks before self-inactivation, but displays improved neurotropism, more efficient transsynaptic transfer and markedly reduced immunogenicity during the virus' early transcriptionally active phase. These

new advancements in the SiR technology expands the scope of SiR viruses to probe the relationship between genes and networks in brain function and pave the way to its potential use to deliver therapeutic treatments to dysfunctional neural networks.

Disclosures: E. Ciabatti: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); attend application WO2018203049A1. **H. Lee:** None. **M. Tripodi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); attend application WO2018203049A1.

Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 172.11/BB10

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: JST ERATO (JPMJER1801)

Title: Auxin inducible rapid degradation in hippocampal neurons

Authors: *R. NAKANO[1], N. IHARA[1], A. NAKASHIMA[1], S. MORIKAWA[1], M. KANEMAKI[2], Y. IKEGAYA[1], H. TAKEUCHI[1];
[1]Grad. Sch. of Pharmaceut. Sci., The Univ. of Tokyo, Tokyo, Japan; [2]Natl. Inst. of Genet., Osaka, Japan

Abstract: Genetic manipulation of protein levels is a promising approach to identify the function of a specific protein in living organisms. Previous studies demonstrated that the auxin-inducible degron (AID) strategy provides rapid and reversible degradation of various proteins in fungi and mammalian mitotic cells. In this study, we employed this technology to postmitoticneurons to address whether the AID system could be applied to the nervous system. Using adeno-associated viruses, we simultaneously introduced EGFP fused with an AID tag, and an F-boxfamily protein, TIR1 from *Oryza sativa*(OsTIR1) into hippocampal neurons. In dissociated hippocampal neurons, EGFP fluorescence signals rapidly decreased when adding a plant hormone, auxin. Further, auxin-induced EGFP degradation was also observed in hippocampal acute slices. Taken together, these results open the door for neuroscientists to manipulate protein expression levels by the AID-system in a temporally-controlled manner.

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Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 172.12/BB11

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: NIH R01 MH087463
Carver Chair in Neuroscience
Nellie Ball Trust

Title: Single cell RNA sequencing reveals regulation of protein folding gene expression by the nuclear receptor Nr4A in hippocampal CA1 neurons after learning

Authors: *S. CHATTERJEE[1], E. BAHL[2], E. WALSH[1], J. D. LEDERMAN[1], J. MICHAELSON[2], S. SAFE[3], T. ABEL[1];
[1]Dept. of Mol. Physiol. and Biophysics, Iowa Neurosci. Inst., [2]Dept. of Psychiatry, The Univ. of Iowa, Iowa City, IA; [3]Dept. of Vet. Physiol. and Pharmacol., Texas A&M Univ., College Station, TX

Abstract: New experiences are initially encoded as labile short-term memories and then converted into stable long-term memory by transcription- and translation-dependent processes. Gene expression after learning involves a transient wave of transcription that is critical for memory consolidation. One group of transcription factors induced during the first wave of transcriptional events are the Nr4A subfamily of nuclear receptors. In this study, we show that transgenic mice expressing a dominant-negative form of Nr4A (Nr4ADN) in forebrain excitatory neurons have impaired long-term spatial memory. Total RNA and single neuronal nuclear RNA sequencing (sn-nuc RNA seq) following a spatial object recognition task revealed that genes related to endoplasmic reticulum (ER) protein chaperones are downregulated in Nr4ADN mice especially in dorsal CA1 excitatory neurons. The RNA sequencing results were validated at the single-cell level using RNA scope flourescent *in situ* hybridization techniques. We also found that several ER protein processing genes regulated by Nr4ADN are also induced by learning in wild-type mice. AAV-mediated overexpression of Nr4ADN exclusively in CA1 excitatory neurons resulted in impaired long-term spatial memory suggesting Nr4A regulates CA1 gene expression to form long-term memory. To study if the downregulated genes in Nr4ADN mice are directly regulated by Nr4A, we generated a Nr4A1-Tavi knock-in mice using CRISPR technology. Nr4A1-Tavi mice were infused with biotin ligase enzyme (BirA) in the dorsal hippocampus to biotinylate Tavi sequence fused with Nr4A1. Chromatin pulldown using streptavidin beads showed enriched occupancy of Nr4A1 on ER chaperone gene promoters following learning. As learning leads to protein translation, the newly synthesized proteins in naïve form require folding by ER chaperones into their correct conformation to be transported to

destinations. Therefore, our study reveals a critical mechanism by which Nr4A regulates learning induced expression of ER protein processing genes which ultimately fold the newly synthesized proteins required for memory.

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Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 172.13/BB12

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Title: Novel AAV mediated genetic sensor of somatic mosaicism in postmitotic neurons- implications in environmental toxicant elicited neurotoxicity and sporadic Parkinson's disease

Authors: *M. ELSAADI, X. TIAN, X.-H. LU;
Pharmacology, Toxicology & Neurosci., Lsuhsc-Shreveport, Shreveport, LA

Abstract: The majority of all Parkinson’s disease (PD) cases are sporadic (sPD). Epidemiologic studies report an increased risk of sPD associated with environmental toxicant exposure, and many toxicants cause genomic instability (i.e., increase in mutational rate). Recent single-neuron sequencing studies demonstrate an age-dependent increase in somatic brain mutations, suggesting adult terminally differentiated neurons can acquire somatic mutations, possibly precipitated by environmental factors- these observations offer a new perspective on neurodegenerative pathogenesis. sPD linked toxicants, like Paraquat (PQ), can drive DNA damage. However, the significance of neuronal genomic instability in neurodegenerative pathogenesis remains unknown. A main hurdle in the field is the lack of in-vivo tools with sufficient spatiotemporal resolution and sensitivity to identify single-neurons with DNA-instability, and to subsequently measure pathological changes. To fill this gap we engineered an Adeno-associated virus (AAV) mediated genetic sensor of genomic instability in terminally differentiated neurons. Our method exploits rAAV single strand DNA replication in postmitotic neurons, genetic-instability of microsatellite repeats and utilizes conditional genetic reporter systems resulting in sparse neuronal labeling and detailed 3D-reconstruction. Our stratergy significantly reduces cell labeling frequency both in-vitro and in-vivo; a known frameshift mutagen (9-AA) stimulates labeling of adult neurons in-vivo, which suggests labeling is depent on microsatellite instability. As proof of concept, we tested ability of PQ to elicit DNA-instability in adult neurons following systemic sub-chronic exposure in age/sex/litter-matched mice. Low dose PQ elicited a 10-fold increase of genetically labeled neurons compared to saline-treated mice. We then reconstructed unobstructed dorsal striatal medium spiny neurons in 3D. Sholl analysis revealed dose-dependent neuronal atrophy. Data was supported by IF/IHC staining

of γH2AX, a well-characterized substrate central to DNA damage response. Our work establishes a novel genetic approach to evaluate the pathogenic role of brain DNA-instability. By employing a novel "seek and destroy" strategy, we can genetically modify mosaic neurons with DNA-instability to identify relevant substrates, explore therapeutic targets and test causative links between toxicants and sporadic CNS disease. Our easy-to-use genetic sensor may transform our current understanding of toxicant exposure in neurotoxicity and sPD pathogenesis, and offers translational value in aging and neurodegeneration research.

Disclosures: **M. Elsaadi:** None. **X. Tian:** None. **X. Lu:** None.

Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

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Program #/Poster #: 172.14/BB13

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: VA CDA IK2RX002013

Title: Utilizing a CTIP2 promoter-driven trans-synaptic tracing system to interrogate the development of cortical circuitry

Authors: ***J. T. LIM**[1], D. JGAMADZE[1], T. DUONG[2], J. A. WOLF[1,3], J. A. MILLS[2], H. I. CHEN[1];
[1]Dept. of Neurosurg., [2]Dept. of Opthalmology, Univ. of Pennsylvania, Philadelphia, PA; [3]Corporal Michael J. Crescenz Veterans Affairs Med. Ctr., Philadelphia, PA

Abstract: Introduction: Trans-synaptic retrograde neuronal tracing has become a valuable method for studying cortical circuitry and function in the last decade. Replication-deficient rabies (RABVdG) can be used for monosynaptic tracing, and can be utilized in tandem with genetic techniques to examine specific neural circuits. In mature cerebral cortex, layer V neurons receive vertical connections from upper cortical layers and horizontal connections within layer V. However, less is known about how this canonical circuitry arises during development. Here, we use a CTIP2 promoter-driven RABVdG system to begin examining factors contributing to the development of the afferent connectivity of layer V cortex. **Methods:** We constructed a polycistronic plasmid expressing the CTIP2 promoter sequence followed by a fluorescent marker (DsRedExpress2), the Rabies virus glycoprotein (G), and an avian leukosis virus receptor (TVA). Lentiviral vectors were generated from this plasmid. This construct was then transduced into dissociated rat embryonic day 18 cortical neurons, and promoter specificity was validated with flow cytometry using a CTIP2 antibody. Transduced cultures were then infected with RABVdG-GFP to confirm the viability of this tracing system. CTIP2 promoter-driven retrograde tracing was performed in embryonic day 18 rat cortical neurons, human induced pluripotent stem

cell-derived cortical neurons (hiPSC), and hiPSC-derived cortical organoids to study the upstream cortical connectivity of layer V. **Results:** In rat cortical neurons transduced with lenti-CTIP2-DsRedExp2-G-TVA, we found that 94.7 +/- 1.7% of the DsRedExp2+ cells were also CTIP2+ by flow cytometry analysis. DsRedExp2+ rat cortical neurons (starter cells) were subsequently transduced with RABVdG-GFP, resulting in the identification of GFP+ cells sending afferent inputs into these starter cells. Immunocytochemistry and immunohistochemistry was performed to evaluate the cortical layer phenotypes of the upstream partners of CTIP2 starter cells in 2D and 3D neuronal cultures, respectively. **Conclusion:** Our results demonstrate the feasibility of driving trans-synaptic tracing using a cortical layer-specific promoter. Other layer-specific promoters could be utilized to generate a suite of viral vectors that enable more thorough analysis of *in vitro* and *in vivo* cortical connectivity. In particular, these constructs could help illuminate the formation of cortical circuitry during fetal development.

Disclosures: J.T. Lim: None. **D. Jgamadze:** None. **T. Duong:** None. **J.A. Wolf:** None. **J.A. Mills:** None. **H.I. Chen:** None.

Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 172.15/BB14

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Title: A viral intersectional approach for cortex-wide projection-specific gene expression

Authors: *X. R. SUN[1], S. MUSALL[1], A. KHANAL[1], S.-J. LI[1], A. KEPECS[1], V. GRADINARU[2], A. K. CHURCHLAND[1];
[1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]Biol. and Biol. Engin., CALTECH, Pasadena, CA

Abstract: Genetically accessing projection pathways using viral approaches facilitates circuit-specific understanding of neural connectivity and behavior and carries clinical and therapeutic implications in many neuropsychiatric and movement disorders. While significant advances have been made in viral vector engineering, targeting of broadly-distributed projection pathways remains an ongoing challenge. The importance of cortex-wide expression is demonstrated in recent work showing complex behaviors, like decision-making, can recruit spatially-diverse cortical structures (Musall et al. 2019). Using a combination of retrograde and systemic viral vectors, we implemented an intersectional viral strategy to express transgenes efficiently in the corticostriatal and corticothalamic circuits, where cell bodies are distributed across all regions of the cortex.We performed stereotactic delivery of retrograde vectors (AAV-retro (Tervo et al. 2016) or CAV-2 (Soudais et al. 2004)) expressing Cre recombinase to activate and amplify Cre-dependent transgenes transduced by the intravenously-administered AAV-9 capsid variant AAV-

PHP.eB (Chan et al. 2017). This strategy was applied by targeting the striatum or the thalamus to express the genetically-encodable calcium indicator GCaMP7s (Dana et al. 2018). Brain-wide gene expression level and distribution was evaluated histologically using immunofluorescence. Calcium imaging in skull-cleared awake behaving mice was performed using wide-field epifluorescence microscopy. Both AAV-retro and CAV-2 drive GCaMP7s expression in corticostriatal neurons across the cortex. Corticostriatal expression levels and labeled neuron density are higher in animals injected with CAV-2, which also demonstrates reduced anterograde uptake in the striatum. With thalamic targeting, AAV-retro drives robust expression in both cortex and thalamus. Lastly, this approach enabled us to track neural activity through GCaMP7s using wide-field calcium imaging to generate visually-evoked cortical maps.This work demonstrates a dual-virus strategy for cortex-wide transgene expression specified by target selection. We are testing CAV-2 to unidirectionally label corticothalamic projections, which is part of a bidirectional pathway. To optimize CAV-2 retrograde uptake and tropism, we are focusing on co-expressing the coxsackie and adenovirus receptor (CAR) to complement CAV-2 internalization (Li et al. 2018). Furthermore, we are using this approach in combination with calcium imaging to investigate the corticostriatal and corticothalamic representations of sensory decision-making in mice.

Disclosures: X.R. Sun: None. **S. Musall:** None. **A. Khanal:** None. **S. Li:** None. **A. Kepecs:** None. **V. Gradinaru:** None. **A.K. Churchland:** None.

Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 172.16/BB15

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: University of Michigan

Title: Drug- and calcium-gated transcription factor for mapping functional neuronal circuits

Authors: *J. SHEN, W. WANG;
Univ. of Michigan, Ann Arbor, MI

Abstract: It remains a major challenge to identify and characterize the neuronal ensembles underlying specific behaviors. Various methods have been developed to label the activated neurons in a designated time window, including immediate early gene (IEG) based tools, CaMPARI, FLARE and Cal-Light. IEG based tools are the most widely applied so far and have resulted in many insightful findings. IEG based tools usually use drug as the temporal gate, allowing labeling of activated neurons in the whole brain region. However, IEG based tools have different activation patterns in different neuron types due to different IEG responses to stimuli.

CaMPARI, FLARE and Cal-Light use calcium, a more universal neuronal activity indicator, as the neuronal activity gating. They also use light as the temporal gate, giving seconds to minutes temporal resolution. However, light has poor penetration in brain tissues, restricting the labeling radius and depth of these methods. We are interested in designing a new category of tools that are double gated by drug for whole brain labeling and calcium, the universal neuronal activity indicator. We will show the design and optimization of the drug gated protein switch and its application in the drug- and calcium-gated transcription factor for mapping functional neuronal circuits.

Disclosures: J. Shen: None. **W. Wang:** None.

Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 172.17/BB16

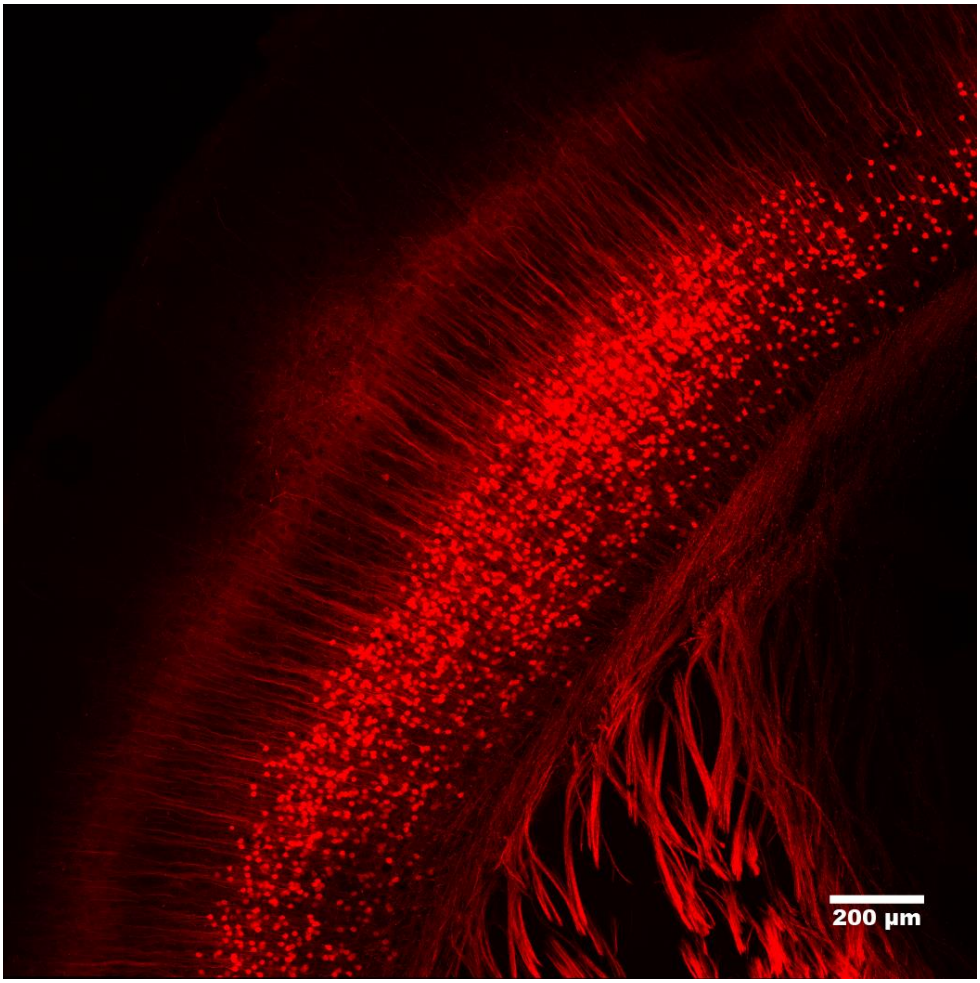
Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: NIH grant U01MH106018

Title: Third-generation, nontoxic, single-deletion-mutant rabies viral vectors

Authors: L. JIN, M. MATSUYAMA, T. K. LAVIN, H. A. SULLIVAN, N. E. LEA, ***I. R. WICKERSHAM**;
MIT, Cambridge, MA

Abstract: Rabies viral vectors have become important components of the systems neuroscience toolkit, allowing both direct retrograde targeting of projection neurons and monosynaptic tracing of inputs to defined postsynaptic populations. We recently introduced "second-generation", double-deletion-mutant vectors that cause no detectable toxicity and showed that they are efficient means of retrograde transduction of projection neurons for expression of recombinases; even more recently, we have shown that they can be used for monosynaptic tracing as well, albeit with lower efficiency than the first-generation system. Here we introduce "third-generation" rabies viral vectors which appear to be as nontoxic as second-generation vectors but which, like their first-generation counterparts, have only a single gene deleted from their genomes. Using longitudinal two-photon structural and functional imaging in mouse visual cortex over four months, we show that labeled neurons do not die, and maintain stable visual response properties, for as long as we follow them. In addition to now representing the state of the art in direct retrograde targeting of projection neurons, this new class of vectors may serve as the foundation of a simplified nontoxic monosynaptic tracing system.



Disclosures: **L. Jin:** None. **M. Matsuyama:** None. **T.K. Lavin:** None. **H.A. Sullivan:** None. **I.R. Wickersham:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Monosynaptix, LLC. **N.E. Lea:** None.

Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

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Program #/Poster #: 172.18/BB17

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: BRAIN Initiative U01MH117079

Title: Detailed morphological imaging of sparsely labeled and genetically-defined neuronal populations in Cre-dependent MORF mice

Authors: *C. S. PARK[1], M. B. VELDMAN[1], C. M. EYERMANN[1], M. ZHU[2], K. MARRETT[1], E. ZUNIGA-SANCHEZ[1], A. A. HIRANO[1], N. C. BRECHA[1], S. L. ZIPURSKY[1], J. CONG[1], H. DONG[2], X. W. YANG[1];
[1]UCLA, Los Angeles, CA; [2]USC, Los Angeles, CA

Abstract: In advancing one of the principle visions of the BRAIN Initiative to generate an unbiased cell census of neuronal cell types in the mammalian brain, we aim to construct a comprehensive profile of the dendritic morphologies (i.e. dendritome) of genetically-defined neuronal populations, including the striatal medium spiny neurons (MSNs) and layer V cortical pyramidal neurons (CPNs). To this end, we have developed a series of novel mouse tools (collectively called the MORF mice) that confer Cre-dependent, sparse (1-5%) and stochastic labeling of genetically-defined neuronal populations. To achieve comprehensive, brain-wide imaging of all the MORF-labeled neurons in the mouse brain, we implemented a pipeline that includes serial thick sectioning (up to 600 μm), optimized iDISCO$^+$ immunostaining and clearing, and high-resolution, high-speed imaging. We will present data on the imaging and reconstruction of several neuronal cell populations, including layer V CPNs, striatal MSNs, and retinal horizontal cells. We are in the process of developing a MORF neuronal image processing pipeline that includes G-Cut, a new software for neuronal reconstruction/segmentation. Moreover, we plan to apply machine learning and hardware-based acceleration (FPGA) to speed up the analyses of our MORF-based dendritome datasets.

Disclosures: C.S. Park: None. **M.B. Veldman:** None. **C.M. Eyermann:** None. **M. Zhu:** None. **K. Marrett:** None. **E. Zuniga-Sanchez:** None. **A.A. Hirano:** None. **N.C. Brecha:** None. **S.L. Zipursky:** None. **J. Cong:** None. **H. Dong:** None. **X.W. Yang:** None.

Poster

172. Genetic and Genome Engineering Techniques

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Program #/Poster #: 172.19/BB18

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: NIH Grant 1R01DA036909
NIH Grant U19MH114830

Title: TIGRE3.0 and VIEDL: Transgenic and viral tools for combinatorial labeling and perturbation of cell types in the mouse brain

Authors: *L. T. GRAYBUCK, M. WALKER, G. LENZ, L. SIVERTS, E. SZELENYI, A. SEDENO-CORTES, T. NGUYEN, B. KALMBACH, E. GARREN, J. MICH, S. YAO, M. MORTRUD, B. LEVI, J. TING, E. LEIN, A. CETIN, H. ZENG, T. DAIGLE, B. TASIC;
Allen Inst. For Brain Sci., Seattle, WA

Abstract: Labeling and perturbation of specific cell types in the brain has transformed our ability to understand them. To enable access to specific neural populations in the mouse brain, we have developed a new transgenic platform in the TIGRE locus using a new landing pad mouse ES cell line for Bxb1-integrase-mediated cassette exchange. This platform allows the insertion of new constructs that integrate inputs from multiple recombinases (e.g. Cre and Flp) to gate expression based on AND/OR logic provided by combinations of recombinase drivers. We call this new combinatorial reporter platform TIGRE3.0.
TIGRE3.0 reporters are compatible with traditional, genomically encoded drivers (e.g. Penk-IRES2-Cre), as well as with virally-delivered drivers. Along with TIGRE3.0, we present new FlpO driver lines designed with intersectional cell targeting strategies in mind. In addition, we have developed a set of Viruses for Intersectional Enhancer Driven Labeling (VIEDL) that can be used separately or in combination with genomic drivers to enable intersectional labeling and perturbation of cell types in the mouse brain. These viruses were generated by mining scATAC-seq and scRNA-seq data for cell subclass- or type-specific enhancer elements, which were cloned into viral genomes to drive recombinase expression, and were tested by retro-orbital injections.
Finally, we present a TIGRE3.0 triple reporter with 3 cassettes separately driven by three recombinases: Cre, Flp, and Nigri to enable the labeling of multiple cell types in the same mouse brain. This new generation of TIGRE3.0 reporter mouse lines, driver lines, and viruses will enable experiments with unprecedented cell type specificity and flexibility in the mouse brain.

Disclosures: L.T. Graybuck: None. **M. Walker:** None. **G. Lenz:** None. **L. Siverts:** None. **E. Szelenyi:** None. **A. Sedeno-Cortes:** None. **T. Nguyen:** None. **B. Kalmbach:** None. **E. Garren:** None. **J. Mich:** None. **S. Yao:** None. **M. Mortrud:** None. **B. Levi:** None. **J. Ting:** None. **E. Lein:** None. **A. Cetin:** None. **H. Zeng:** None. **T. Daigle:** None. **B. Tasic:** None.

Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 172.20/BB19

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: NIMH RF1MH114106
NIDA R01DA036909
DARPA

Title: Photoactivable recombinases for targeted single-cell genomic modification

Authors: *P. BALARAM[1], S. YAO[1], P. YUAN[2], S. CHATTERJEE[1], B. OUELLETTE[1], T. ZHOU[1], M. MORTRUD[1], T. DAIGLE[1], B. TASIC[1], R. CHRAPKIEWICZ[2], M. SCHNITZER[2,3],

H. ZENG[1], **A. CETIN**[1];
[1]Allen Inst. for Brain Sci., Seattle, WA; [2]Biol., [3]Applied Physics, Stanford Univ., Palo Alto, CA

Abstract: Photoactivable recombinases enable spatiotemporally targeted genetic modification without relying on transgenic or promoter-driven expression strategies, making them a powerful genetic tool for neural circuit dissection. We developed a suite of light-inducible recombinases, collectively termed the RecV system, in which split-Cre or -Flp constructs fused to a photoactivable protein, VIVID, are recombined and activated when exposed to light. The RecV system exhibits increased spatial specificity and minimal leak compared to other light-inducible recombinase systems. Intersectional constructs that drive Cre- or Flp-dependent RecV expression allow cell type-specific targeting, with no crossreactivity between traditional and photoactivable recombinases at floxed sites and no background expression in the absence of light. In awake, head-fixed mice, RecV-dependent recombination can be spatially restricted to a single cortical layer or individual cortical neurons via laser-mediated induction of target regions during two photon imaging sessions. Thus, the RecV system expands the range of available genetic tools for neural circuit dissection in the mouse brain, and provides significant advantages in targeting specificity over currently available photoactivable recombinase systems.

Disclosures: P. Balaram: None. **S. Yao:** None. **P. Yuan:** None. **S. Chatterjee:** None. **B. Ouellette:** None. **T. Zhou:** None. **M. Mortrud:** None. **T. Daigle:** None. **B. Tasic:** None. **R. Chrapkiewicz:** None. **M. Schnitzer:** None. **H. Zeng:** None. **A. Cetin:** None.

Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 172.21/BB20

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: University of Illinois

Title: Repurposing protein degradation for optogenetic modulation of protein activities

Authors: *N. HAACK, P. MONDAL, V. V. KRISHNAMURTHY, S. R. SHARUM, K. ZHANG;
Dept. of Biochem., Univ. of Illinois, Urbana-Champaign, Urbana-Champaign, IL

Abstract: Signaling pathways extensively crosstalk among each other and result in different cellular phenotypes depending on the dynamic profile of protein activity. Conventional genetic and pharmacological approaches such as gene overexpression, use of growth factors or inhibitors have helped us delineate interaction maps of signaling components, however these techniques provide limited means to determine contribution of a target protein for specific cellular

phenotype. Therefore, to find out the molecular mechanism for a cellular outcome, there is an urgent need for a tool that can specifically activate or inactivate a protein of interest and study it's role towards a specific cell fate. Optogenetic techniques utilize light to control protein functions with high spatial and temporal resolution. Here we introduce a generalizable light modulated protein stabilization system (GLIMPSe) that enables fine spatiotemporal control of specific signaling pathways. This optogenetic system has been used to re-purpose the protein degradation machinery by combining a small peptide sequence called degron, an evolved LOV (eLOV) protein and a light-inducible nuclear export system (LEXY). We applied GLIMPSe to control two distinct classes of proteins: mitogen-activated protein kinase phosphatase 3 (MKP3), a negative regulator of the extracellular signal-regulated kinase (ERK) pathway, as well as a constitutively active form of MEK (CA MEK), a positive regulator of the same pathway. Kinetics study showed that light-induced protein stabilization could be achieved within 1 minute of blue light stimulation. GLIMPSe enables target-independent optogenetic control of protein activities and therefore minimizes the systematic variation embedded within different photoactivatable proteins. Overall, GLIMPSe promises to achieve light-mediated post-translational stabilization of a wide array of target proteins in live cells.

Disclosures: **N. Haack:** None. **P. Mondal:** None. **V.V. Krishnamurthy:** None. **S.R. Sharum:** None. **K. Zhang:** None.

Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 172.22/BB21

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: University of Michigan

Title: Optimization of a light-gated transcriptional system for tracking protein-protein interactions in the brain

Authors: *R. M. MILLER, W. WANG;
Univ. of Michigan, Ann Arbor, MI

Abstract: A central aim of neuroscience is to understand the link between neuronal activity and physiological response. Optogenetic tools are a novel method that can be used to provide evidence of this connection.[1] Converting this general concept to a format useful for tracking neuronal signaling requires harnessing the molecular level effects of a neuron firing an action potential, such as rises in concentration of cytosolic calcium. FLARE (**F**ast **L**ight- and **A**ctivity-**R**egulated **E**xpression) was designed to convert transient neuronal activity to a permanent genetic marker in functionally relevant neurons in response to the presence of both high

concentrations of calcium and light stimulation. A light-induced conformational change is coupled to a calcium concentration-induced protein-protein interaction (PPI) to affect the transcription of a fluorescent reporter gene.[2] However, there is a significant limitation to this method *in vivo*. Calcium concentrations fluctuate on the order of milliseconds as signals are passed from one neuron to the next.[1] Therefore, the challenge of using FLARE successfully *in vivo* is that the high calcium concentration required to trigger the system is not sustained long enough to promote the necessary PPI. Here we demonstrate methods for optimization of FLARE, including the use of different protein binding partners to elongate the effects of a high calcium state.
References: **[1]** Wang, W.; et al. Nat. Chem. Biol. 2019, 15, 101-110. **[2]** Wang, W.; et al. Nat. Biotechnol. 2017, 35, 864-871.

Disclosures: R.M. Miller: None. **W. Wang:** None.

Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 172.23/BB22

Topic: I.04. Physiological Methods

Support: University of Utah startup fund
RO1 NS102444

Title: Development of a novel live-cell reporter for identification of neuronal activity modulators

Authors: *A. SANTOS, G. YANG, A. SHCHEGLOVITOV, S. PARK;
Neurobio. & Anat., Univ. of Utah Sch. of Med., Salt Lake City, UT

Abstract: The ability to measure changes in neuronal activity in a quantifiable and precise manner is of fundamental importance to the study of neurons and neuronal circuits. However, monitoring neuronal activity of a population of neurons over several days is challenging and, typically, a low-throughput process. Here, we describe a biochemical reporter assay that allows for repeated measurements of neuronal activity, in a cell-type specific manner and applicable to pharmacological treatments and high-throughput screens. We coupled an activity-dependent driver to a secreted luciferase reporter in order to quantify neuronal activity without sacrificing the neurons. We have thus termed the assay SNAR, for Secreted Neuronal Activity Reporter. By repeatedly measuring the accumulation kinetics of the reporter in culture, we quantify changes in neuronal activity during neuronal development in different culture conditions. This assay is a useful tool to identify modulators of neuronal activity as validated by using known anti-seizure drugs and protein factors. The assay enables the direct comparison of changes in neuronal activity of the same population of neurons upon pharmacological manipulations, which

significantly improves its consistency and statistical power. Importantly, the assay allows for kinetic analysis of drug effects over days, thus distinguishing acute from long-term responses. Furthermore, conditional expression of a floxed reporter by using Cre recombinase allows for selective monitoring of neuronal activity only in a sub-population of neurons within heterogeneous cultures. This simple, quantitative, cost-effective, automatable, and cell-type specific activity reporter will be a valuable tool to study the development of neuronal activity in normal and disease-model conditions, and to identify small molecules and protein factors that selectively modulate the activity of a specific population of neurons.

Disclosures: A. Santos: None. **S. Park:** None.

Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 172.24/BB23

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Title: Genetically modified rat toolbox: Characterization and application for neuron-type specific activity modulation

Authors: *Z. LIU, G. ZHAO, A. BROWN, K. FORBES;
Horizon Discovery, Saint Louis, MO

Abstract: Rats are preferred animal models for neuroscience research over mice thanks to the rat's higher intelligence and richer behaviors. Taking advantage of zinc finger nuclease and CRISPR/Cas9 based genome editing technology, we created an optogenetic toolbox utilizing the rat, consisting of about a dozen of neuron-type-specific Cre driver lines (such as CamKII-cre, VGAT-Cre and Parvalbumin-cre), Cre-activity-dependent excitatory and inhibitory opsin expression lines as well as a fluorescent protein-based Cre-activity reporter lines. Here we report our new Cre and Optogenetic lines with our continued efforts to characterize this toolbox and showcase how it is being used for groundbreaking neuroscience-based research and drug development.

Disclosures: Z. Liu: A. Employment/Salary (full or part-time):; Horizon Discovery. **G. Zhao:** A. Employment/Salary (full or part-time):; Horizon Discovery. **A. Brown:** A. Employment/Salary (full or part-time):; Horizon Discovery. **K. Forbes:** A. Employment/Salary (full or part-time):; Horizon Discovery.

Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 172.25/BB24

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: 2018YFC1003002

Title: Establishment of CaMKIIα-ChrimsonR-GFP transgenic macaque monkey model

Authors: *S.-T. HE, Z. LIU;
Inst. of Neurosci., Shanghai City, China

Abstract: Though rodents serve as important animal models in neuroscience, they do differ in important ways from humans, such as brain circuitry, cognitive capacities, and behavioral repertoires. The nonhuman primate thus has a unique role to play as a model for studying the human brain. In addition, cell type-specific expression of optogenetic molecules allows temporally precise manipulation of targeted neuronal activity. Here, we use lentivirus-based transgenic cynomolgus monkeys (Macaca fascicularis) to establish optogenetic model of non-human primates. We used excitatory neuron-specific promotor CaMKIIα to drive expression of ChrimsonR-GFP in lentivirus vector. We activated cortical pyramidal neurons expressed CaMKIIα-ChrimsonR-GFP *in vitro* upon light stimulation, which demonstrating light sensitivity of virally transduced neurons.
After evaluating the efficacy of the virus and investigating the neurophysiology of ChrimsonR expression in excitatory neurons of mice, we will inject lentivirus of CaMKIIα-ChrimsonR-GFP into one-cell embryos of macaque monkeys to generate transgenic monkey models processing robust and consistent expression of ChrimsonR in cortical excitatory neurons. This optogenetic model of non-human primate will certainly provide critical information for the advancement of optogenetic primate research models and for initiating the development of optogenetically based cell-specific therapies with which to treat neurological diseases in humans.

Disclosures: S. He: None. **Z. Liu:** None.

Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 172.26/BB25

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: NIH/NIMH Intramural support
NIH Grant RO1MH117069
NIH Grant DP2NS087949
NIH Grant DP1OD025535
DARPA Grant W911NF-17-2-0036

Title: Transgenic expression directed to the CNS of non-human primates

Authors: *J. PICKEL[1], A. C. CUMMINS[2], N. FLYTZANIS[3], N. GOEDEN[4], V. GRADINARU[5];
[1]Natl. Inst. of Mental Hlth., NIH, Bethesda, MD; [2]LN NIMH NIH, Bethesda, MD; [3]Biol. and Biol. Engin., [4]Div. of Biol., Caltech, Pasadena, CA; [5]Biol. and Biol. Engin., CALTECH, Pasadena, CA

Abstract: Genetic manipulation of the primate CNS is essential to advance both research and therapies. We have developed unique viral vectors that cross the blood brain barrier from the systemic circulation and deliver a transgene to CNS neurons of non-human primates. The protein product of the transgene is expressed throughout the brain. With the development of standard techniques to produce transgenic lines of primates it has become possible to alter their genomes for a range of uses. Transgenes can be used as tools to mark specific cell types, indicate neuronal activity or model aspects of human disease. In particular, marmoset monkeys (a new world primate), have a shorter life cycle than other primates and have been used extensively to generate lines of germline transgenics. Even so, their gestation period of five months and an eighteen month delay to sexual maturity restrict their use in biomedical research. In comparison, old world monkeys have even longer life cycles. While genetic engineering techniques decrease the variability of transgene insertion and expression, many experiments rely on invasive techniques, such as intracranial injections and chemical or physical ablation of cells or circuits. As an alternative to germline transmission of transgenes that are expressed in the CNS we have developed AAV9 capsid variants that cross the blood brain barrier efficiently. Using a modification of the CREATE system a library of AAV9 capsid gene variants has been produced. Variants were first extensively screened in mice. Those that crossed the blood brain barrier, and had reduced transduction of liver were further refined. Next, after several rounds of selection several promising variants were delivered through the peripheral circulatory system into marmoset monkeys. Variant viruses were infused into the femoral vein and analyzed after 6 weeks. Expression from the transgene (a synapsin transcriptional promoter driving the expression of an HA-tagged protein) was seen throughout the cortex and in other brain nuclei. The protein level was robust, while there was little expression in the liver. This method expands the potential for neuroscience research by manipulating CNS neurons without invasive techniques or the delay necessary for germline transmission.

Disclosures: J. Pickel: None. **A.C. Cummins:** None. **N. Flytzanis:** None. **N. Goeden:** None. **V. Gradinaru:** None.

Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 172.27/BB26

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: NIH 1R21GM114852
NIH 5R01MH110932

Title: Lineage Tracker Bitbow2: A convenient and versatile spectral barcoding tool for neuronal lineage studies in the *Drosophila* brain

Authors: *Y. LI[1], Y. ZHAO[1], E. EDWARDS[1], T. CHEN[1], M. GHAZZI[1], D. ROOSSIEN[1], D. CAI[1,2,3];
[1]Cell and Developmental Biol., [2]Biophysics, LS&A, [3]Neurosci. Grad. Program, Univ. of Michigan, Ann Arbor, MI

Abstract: The complex functions of the nervous system are built upon the precise development of its computational units, neurons. As distinct neural stem cells, neuroblasts (NBs) in the Drosophila brain give rise to neurons that are functional heterogenous, it is critical to unveil the lineage composition of neurons when studying the molecular mechanisms that govern the neurogenesis and differentiation process. Genetic tools such as MARCM [Lee and Luo, Neuron 1999] and Flip-Out based systems (including MultiColor FlipOut, MCFO [Nern et al., PNAS 2015]) have been broadly used in lineage tracing. These methods rely on sparse clonal labeling of few NBs and their progenies by heat-shock induced fluorescent protein expression, so that their spatially separated patterns help verify individual lineages. The stochastic nature of heat-shock requires many trial-and-errors to acquire enough samples with proper labeling density. To increase sampling efficiency, MCFO generates ~10 unique colors to differentiate neighboring clones. However, MCFO is not reliable to solely use color to determine whether adult neurons are lineage-related. This is because the limited colors that MCFO provides result in a high chance of labeling more than one lineages in the same color. Once the adult neurons migrate away from their clones there is no more spatial information to help determine their lineage compositions. To overcome the color limitation, we developed Lineage Tracker Bitbow (LT-Bitbow), a digital format of Brainbow to provide ~30 unique spectral lineage barcodes upon heat-shock using a single recombination cassette [Veiling, Li, Veiling, et. al, bioRxiv 2019]. Here, we report the development of LT-Bitbow2, which provides up to ~1000 unique spectral-spatial lineage barcodes without the need of heat-shock. With a simple one-step cross, LT-Bitbow2 enables studying different aspects of the neurogenesis process in a high-throughput manner. For instance, it can trace all the progenies generated by NBs that expressed a particular

transcription factor during development. In another example, lineage composition of neurons belonging to a functional subtype can be easily mapped in an adult brain.

Disclosures: Y. Li: None. **Y. Zhao:** None. **E. Edwards:** None. **T. Chen:** None. **M. Ghazzi:** None. **D. Roossien:** None. **D. Cai:** None.

Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 172.28/BB27

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Title: Identifying efficient chemical-based nucleic acid transfection compound for primary neurons and neuronal cell lines

Authors: *V. T. TOUSSAINT, F. PREMARTIN, T. BENCHIMOL, M. DENU, M. DUMONT, G. FREUND, P. FRIEDEL, Y. PHILIPSON, M. HELLAL, P. ERBACHER;
Res. Develop., POLYPLUS-TRANSFECTION, ILLKIRCH GRAFFENSTADEN, France

Abstract: Efficient gene expression in neurons is indispensable for the study of neuronal cell biology, such as investigating gene and protein function, cell behavior and or cell morphology. The need for more physiologically relevant cellular models has become a requirement to further validate studies performed in neuron cell lines that are easier to transfect compared to primary neurons. Primary neurons are fragile and difficult to transfect, and with currently available transfection method either results in low transfection efficiency or low cell viability. Currently, the most efficient methods for exogenous gene delivery into slow to non-dividing neurons are electroporation or viral-based transduction methods. These methods are often associated with side-effects on cellular viability and morphology. Here we describe the screening of a new library of chemical compounds to identify candidates as potent nucleic acid transfection reagents in different primary neurons (such as primary hippocampal or cortical neurons from rat) and neuronal cell lines. Following the optimization of hits-to-lead, we selected the best candidate based on its high transfection efficiency and its ability to maintain excellent cell viability and morphology.

Disclosures: V.T. Toussaint: None. **F. Premartin:** None. **T. Benchimol:** None. **M. Denu:** None. **M. Dumont:** None. **G. Freund:** None. **P. Friedel:** None. **Y. Philipson:** None. **M. Hellal:** None. **P. Erbacher:** None.

Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 172.29/BB28

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: ICT (2017M3A9G8084463)
the Ministry of Trade, industry & Energy (10062378)

Title: Development of OR-expressing stable cell lines using lentiviral genomic integration and those uses for biomimetic noses

Authors: *J. CHOE, C. MOON;
DGIST, Dept. of Brain & Cognitive Sci., Daegu, Korea, Republic of

Abstract: Olfaction is one of five senses. The olfactory signalling begins with binding of a specific chemical to a particular odorant receptor (OR) localized at the cilia of the olfactory sensory neurons (OSNs), and the OSN sends its olfactory signals to the brain for the olfactory perception. There are about 400 kinds of ORs in human, and people have been tried to mimic the olfactory organs using ORs. However ORs are not easily expressed in the heterologous system. Thus most of researchers have used Hana3A cell line based on HEK293 to express ORs in vitro. Since the OR-based biomimicking system is designed to realize the responsiveness to odorants similarly in the OSN, cell lines expressing ORs functionally may be necessary to develop a biomimicking chemical sensor. To this end, we made several OR-expressing stable cell lines using lentiviruses. We established two models. First, OR inducible expression model using tet-on system. Second one is a permanently OR expressing model. We verified quantification of OR in the OR-expressing stable cell lines. We will apply 2X2 OR array system which contains 4 distinct OR-expressing cell lines at each well. Ultimately we will measure the reactivity to a specific odorant or odorant mixtures using this newly designed OR-based electrochemical or optical sensors. Both sensors may be faster than traditional molecular and cellular assay since those measure the electrical property changes in this cell lines. Taken together, our OR-based bioelectronics nose may help us not only to understand the signal transduction in the peripheral olfactory system but also to develop a biomimetic electronic nose.
Keywords: smell, odorant, odorant receptor, electric nose
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Disclosures: J. Choe: None. **C. Moon:** None.

Poster

172. Genetic and Genome Engineering Techniques

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 172.30/BB29

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: NIH/NIDA Intramural funds.

Title: Non-invasive and longitudinal opsin monitoring for research and potential clinical optogenetics applications

Authors: *J. BONAVENTURA, S. LAM, J. GOMEZ, C. RICHIE, M. MICHAELIDES; NIDA/NIH, Baltimore, MD

Abstract: With the advance of genetic strategies to correct brain dysfunction (i.e. optogenetics and chemogenetics) and the development of non-invasive viral vectors and delivery systems, a critical hurdle to the clinical implementation of such technologies is an effective, reproducible, and non-invasive reporter system that allows for longitudinal monitoring of the expression of the transduced therapeutic protein in a sensitive and quantitative manner. Positron Emission Tomography (PET) is a clinical diagnostic tool that offers unique possibilities to remotely track ligand binding to a given target and the Ligand Binding Domain of the Estrogen Receptor alpha (ERa-LBD) offers a small, inactive, and non-immunogenic protein moiety that can be potentially attached to any effector protein and used as a dock for the PET ligand. With this strategy, a universal reporter could be used to monitor the expression and analyze the neuroanatomical distribution of therapeutic transgenes, such as the several opsins used in optogenetics that lack a known ligand binding domain.We fused the ERa-LBD to commonly used opsins in several positions to ensure their functionality. In most cases, the opsin moieties were active and the ligand binding properties of estradiol were conserved. A small molecule radiolabeled with short-lived isotopes is needed for PET applications. The ideal isotope for PET applications is fluorine-18, with a 110 min half-life, that allows synthesis, shipping and application within a few hours. We used (18)F-fluorestradiol (a radioligand which is currently used clinically) to effectively localize ChannelRhodopsin 2 in living rats. With the combination of the ERa-LBD reporter and a robust PET ligand we now have a unique PET-based gene therapy reporter system (not limited to optogenetics) that can non-invasively asses the correct expression of transgenes after viral transduction in the desired target areas and, maybe more importantly, evaluate the long-term progression of this expression, and hence adjust and optimize the therapeutic outcomes of the intervention.

Disclosures: J. Bonaventura: None. **S. Lam:** None. **J. Gomez:** None. **C. Richie:** None. **M. Michaelides:** None.

Poster

173. Anatomic Methods: Image Acquisition I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 173.01/BB30

Topic: I.03. Anatomical Methods

Support: Project 973 2015CB755602
Science Fund for Creative Research Group of China (Grant No.61721092)
National Natural Science Foundation of China (Grant No. 91749209)

Title: Brain region segmentation at single cell scale based on Markov random field

Authors: X. XU, ***Y. GUAN**, H. GONG, Z. FENG, W. SHI, A. LI, M. REN, J. YUAN, Q. LUO;
Huazhong Univ. of Sci. and Technol., Wuhan, China

Abstract: The precise delineation of brain region boundaries is important for brain region identification, atlas illustration and a structured view of neuro-information processing. In this paper we propose a hierarchical model based on Markov random field for the brain region segmentation, where a MRF is applied to the downsampled images and the result is used to initialize another MRF for the original high-resolution images. A fractional differential feature and gray level co-occurrence matrix are extracted as the visual vector for the MRF and the hidden vector is the brain region label. A new potential energy function, which can capture the spatial characteristic of brain regions, is proposed as well for the MRF learning. A fuzzy entropy criterion is used to fine-tune the boundary from the hierarchical MRF model. We test the model both on synthetic images and real histological mouse brain images. The result suggests that the model can accurately identify target regions and even the whole mouse brain outline as a special case. An interesting observation is that the model cannot only segment regions with different cell density but also can segment regions with similar cell density but different cell morphology texture. Thus this model shows great potential for building the high-resolution 3D brain atlas.

Disclosures: **X. Xu:** None. **Y. Guan:** None. **H. Gong:** None. **Z. Feng:** None. **W. Shi:** None. **A. Li:** None. **M. Ren:** None. **J. Yuan:** None. **Q. Luo:** None.

Poster

173. Anatomic Methods: Image Acquisition I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 173.02/BB31

Topic: I.03. Anatomical Methods

Support: NSFC projects Grant 61721092
NSFC projects Grant 91749209
NSFC projects Grant 31871088

Title: Whole brain mapping of long-range direct input to glutamatergic and GABAergic neurons in motor cortex

Authors: *P. LUO[1], J. TIAN[1], A. LI[2], H. GONG[3], X. LI[3];
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Abstract: Determining long-range inputs of motor cortex (MC) is important for understanding circuit mechanisms involved in regulating movement. Here, we used monosynaptic rabies tracing technique combined with fluorescent micro-optical sectional tomography to characterize the input atlas of MC. The two main cell types - glutamatergic neurons and GABAergic neurons in the motor cortex receive inputs from similar brain regions with quantitative difference. While the primary and secondary motor cortex (MOp and MOs) receive inputs from spatially separated populations in isocortex, thalamus, claustrum and amygdala. Based on these results, we analyzed the morphology and projection properties of these separated populations. And we performed the TRIO (for tracing the relationship between input and output) in these parallel motor subcircuits. These results would lay the anatomical foundation for understanding the organized pattern of motor circuits and the functional differences between MOp and MOs.

Disclosures: P. Luo: None. **J. Tian:** None. **A. Li:** None. **H. Gong:** None. **X. Li:** None.

Poster

173. Anatomic Methods: Image Acquisition I

Location: Hall A

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Program #/Poster #: 173.03/BB32

Topic: I.03. Anatomical Methods

Title: The whole brain afferent to cortical corticotropin-releasing hormone (CRH) neurons in mice

Authors: *P. ZHAO[1], H. WANG[1], J. TIAN[1], A. LI[1,2], H. GONG[1,2], X. LI[1,2];
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Abstract: Cortical CRH is an important neurotransmitter that involves in awake, study, anxiety, motor and spatial memory. Although the cortical CRH neurons were certified as the interneurons that mainly project in local area, their input neurons distributed in many brain regions. Here, we combined the fluorescence Micro-optical Sectioning Tomography(fMOST) with a genetically modified neurophagic virus tracer system, investigated the long-range input neurons to these neurons in different cortical areas in whole brain. The majority of input to cortical CRH neurons were found in cortex, thalamic nucleus and basal forebrain, and a small of which in hypothalamus, midbrain and pons. In the cortex,the input neurons mainly located in adjacent areas and some in the contralateral homonymous cortex. What's more, the ipsilateral orbitofrontal cortex(OFC) and olfactory-related cortex (OLF) contact different cortical CRH neurons stably. For the thalamus, the anterior part tended to project to the CRH neurons in the rostral cortex, while the posterior thalamus preferred to the caudal. In the basal forebrain(BF), steadily affernet neurons were found in differernt subregions. And immunological results showed that cholinergic positive input neurons clustered in SI and LGP while HDB to mPFC and V1. In addition, we found that the CRH neurons in mPFC received strong monosynaptic afferent from zona incerta (ZI) in hypothalamus and ventral hippocampus(vHIP).

Disclosures: P. Zhao: None. **H. Wang:** None. **J. Tian:** None. **A. Li:** None. **H. Gong:** None. **X. Li:** None.

Poster

173. Anatomic Methods: Image Acquisition I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 173.04/BB33

Topic: I.03. Anatomical Methods

Support: NSFC 81827901
MOST 2017YFA0700402

Title: A high efficient whole brain optical imaging platform

Authors: *J. YUAN, Q. ZHONG, A. LI, R. JIN, X. YANG, G. LIU, Z. DING, J. CHEN, X. LI, H. GONG, Q. LUO;
Huazhong Univ. of Sci. and Tenchnology, Wuhan, China

Abstract: Whole-brain optical imaging opens a door to decipher brain structure with single-neuron resolution. However, data size of TBs leads long acquisition and post-processing time and large cost of data saving. Here, we propose a high-definition fluorescence micro-optical sectioning tomography (HD-fMOST) to improve high throughput and accelerate data acquisition of whole-brain optical imaging. This technology is capable of high-resolution and high-quality imaging and on-line processing and analyzing. Benefiting from great imaging quality and high signal-to-background ratio (SBR), HD-fMOST enables to acquire very sharp whole-brain images and achieve on-line data compression rate of 1%. Our method potentially becomes a routine tool for anatomical data acquisition of neuroscience.

Disclosures: **J. Yuan:** None. **Q. Zhong:** None. **A. Li:** None. **R. Jin:** None. **X. Yang:** None. **G. Liu:** None. **Z. Ding:** None. **J. Chen:** None. **X. Li:** None. **H. Gong:** None. **Q. Luo:** None.

Poster

173. Anatomic Methods: Image Acquisition I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 173.05/BB34

Topic: I.03. Anatomical Methods

Support: Grant No. 81827901
2017YFA0700402

Title: Design of a precision tape-driven platform for thin tissue sections cutting, collecting and imaging

Authors: *L. DENG, J. CHEN, H. GONG, Q. LUO, J. YUAN;
Huazhong Univ. Of Sci. and Technol., Wuhan, China

Abstract: To avoid the absorption and scattering effect, when image large bio-sample via optical microscopy, one possible manner is to cut thick tissue into thin slices, collect and image them one by one. Conventionally, this is a manual process, which is labor-intensive and possible to damage the thin slice. In this work, we present a novel tape-driven system integrated with microtome and microscopy that can cut, collect and image thin tissue sections automatically. In the cutting part, a custom-designed sliding microtome is used to cut the embedded tissue into thin slices. In the collecting part, a biocompatible tape guided by a roll-to-roll system is used to collect these thin slices. Among these rollers, a pressing roller is used to drive the tape to make contact with the separated slice. To make this process with precision, two ends of the pressing roller are mounted on a pair of custom-designed nano-positioning stages so that the contact displacement between the tape and the slice can be precisely controlled. In the imaging part, wild-field microscopy is placed over the tape to image the slices as they pass by. To demonstrate the capacity of the novel platform, we have performed some experiments: (1) minimize the

collecting defects by eliminating pressing roller's motion errors; (2) optimize the collecting performance via adjusting the contact displacement; (3) collect and image the cryo-embedded and paraffin-embedded sections. The results indicate that such a platform can be used for three-dimensional reconstruction of large bio-sample information from the continual collected thin slices.

Keywords: Neural circuits, Whole brain optical imaging, Micro-optical sectioning tomography (MOST)

Acknowledgements: This work was supported by the National Key Scientific Instrument and Equipment Development Program of China (Grant No. 81827901), the National Key Research and Development Program of China (2017YFA0700402).

Disclosures: **L. Deng:** None. **J. Chen:** None. **H. Gong:** None. **Q. Luo:** None. **J. Yuan:** None.

Poster

173. Anatomic Methods: Image Acquisition I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 173.06/BB35

Topic: I.03. Anatomical Methods

Support: Grant No. 81827901
2017YFA0700402

Title: Real-time optical-sectioning method based on deep learning

Authors: *K. NING, X. ZHANG, A. LI, H. GONG, Q. LUO, J. YUAN;
Wuhan Natl. Lab. For Optoelectronics, Wuhan, China

Abstract: Optical-sectioning techniques are capable of producing clear focal-plane images from thick samples. Current optical-sectioning methods require complex hardware or considerable processing time to obtain high quality imaging data. Here, we developed a deep learning algorithms to remove the out-of-focus light in wide-field (WF) images and achieved real-time reconstruction. We imaged three different biological samples including PI-counterstained cytoarchitecture, tdTomato-labeled heart tissue and Golgi-stained brain tissue to train convolutional neural networks (CNN). We used the normalized root mean square error, structural similarity index and correlation coefficient to quantify the close resemblance of network outputs to the ground truth images. The average results were 0.0181, 0.9169 and 0.8803, respectively. To further quantify the transformation reliability, we imaged 100 nm FluoSpheres and measured 3D point-spread function distributions by structured-illumination microscopy (SIM) and CNN reconstructions. The lateral and axial full-width-at-half-maximum of the CNN reconstructions were similar with the one of SIM reconstruction. Moreover, benefited from the light-weighted network structure, we can reconstruct an image of 1024*1024 pixels in less than 0.07s, reaching

the level of video rate imaging. In summary, we proposed a high-throughput fast optical sectioning method based on deep learning. Quantitative and qualitative results demonstrate that this data-driven approach potentially facilitates the data acquisition of whole brain.

Disclosures: **K. Ning:** None. **X. Zhang:** None. **A. Li:** None. **H. Gong:** None. **Q. Luo:** None. **J. Yuan:** None.

Poster

173. Anatomic Methods: Image Acquisition I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 173.07/BB36

Topic: I.03. Anatomical Methods

Support: NSFC Grant 61721092
NSFC Grant 81671374

Title: Processing and analysis pipeline for mesoscopic brain-wide big data in Brainsmatics studies

Authors: ***A. LI**, Y. GUAN, Z. FENG, T. QUAN, S. ZENG, H. GONG, Q. LUO;
Wuhan Natl. Lab. For Optoelectronics, Huazhong Univ. of Sci. and Technol., Wuhan, China

Abstract: The spatial brain structure can cross several magnitudes, from synapse, single neuron, and neural circuit to brain nucleus, brain region and organ. Structures at mesoscopic scale include soma, neurite, and neural circuits, and the neural circuits also have macroscopic spatial distribution characteristics. In the past decade, we developed MOST/fMOST series to obtained the dataset of whole mouse brain at single-neuron resolution which directly demonstrates the brain-wide structural connectivity. Similar techniques are also included STP tomography and Light-sheet tomography. However, mesoscopic scale imaging on rodent's brain will generate a 10 TB scale dataset. As the advance of the image techniques, higher resolution, more detect channels, wider dynamic range will generate even more data. Therefore, biology labs need a mature and comprehensive solution for mesoscopic whole brain images when studying neuron types and circuits connections. In this presentation, we give a brief introduction to our pipeline for processing and analyzing the big data obtained by MOST/fMOST. From the raw images to the brain science knowledge, the pipeline includes image processing, image registration, information extraction, quantitative analysis, and visualization. The big data also brings a full range of challenges to data processing, storage, analysis, management, and sharing, to address the issues we also have some preliminary solutions.

Disclosures: **A. Li:** None. **Y. Guan:** None. **Z. Feng:** None. **T. Quan:** None. **S. Zeng:** None. **H. Gong:** None. **Q. Luo:** None.

Poster

173. Anatomic Methods: Image Acquisition I

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Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 173.08/BB37

Topic: I.03. Anatomical Methods

Support: 973 project Grant 2015CB755602

Title: Projection pattern defines anatomical subtypes of pyramidal neurons in layer 5 of the motor cortex

Authors: *S. JIANG, Y. GUAN, A. LI, Q. LUO, H. GONG;
Wuhan Natl. Lab. for Optoelectronics, Huazhong Univ. of Sci. and Technol., Hubei, China

Abstract: Pyramidal neuron is an important cell type in the mouse motor cortex. Different projection patterns of pyramidal neurons can reveal different properties about their circuit connectivity. Therefore, the anatomical subtypes of pyramidal neurons can be defined by their projection patterns. However, it is not easy to acquire neuron anatomy at single axon resolution with elaborate cytoarchitecture information, particularly for long projection neurons in the mouse motor cortex. As a result, the anatomical subtypes of pyramidal neurons have yet to be studied at single axon resolution. In this research, we use a fluorescence micro-optical sectioning tomography system to image a Thy1 H-line mouse brain. This image dataset is at single axon resolution with cytoarchitecture information, which is elaborate enough for us to reconstruct long projection neuron morphologies. There are 42 pyramidal neurons in layer 5 of the motor cortex that have been reconstructed in this image dataset. Based on the reconstructed neuron anatomies, we analyze the neuron arborization patterns and their soma spatial distributions. We summarize the projection patterns which reflect the brain circuit connectivity and suggest five input-output function of single neurons.

Disclosures: S. Jiang: None. **Y. Guan:** None. **A. Li:** None. **Q. Luo:** None. **H. Gong:** None.

Poster

173. Anatomic Methods: Image Acquisition I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 173.09/BB38

Topic: I.03. Anatomical Methods

Title: A registration pipeline for mapping histological brain slices to standard stereotaxic coordinate system

Authors: *Z. ZHANG, Z. FENG, H. GONG, A. LI;
Wuhan Natl. Lab. for Optoelectronics, Huazhong Univ. of Sci. and Technol., Hubei, China

Abstract: The positional information of neuron circuits and brain spatial localization is crucial for analyzing the structure and function of neural connectivity and brain mapping. Histological brain slices are widely used in neuroscience to study anatomical structure of neural circuit. However, because most laboratory histological images produced by conventional microtome only provides highly resolved tissue properties on a cellular level in 2D, it is difficult to achieve precise spatial localization of neural circuit and brain structure through traditional registration methods or manual correction. Here, we proposed a reconstruction-based slices-to-volume registration pipeline which mapping 2D histological slices to 3D reference atlas and then enable the precise positioning of neural circuits. Compared with traditional methods, our pipeline introduced coarse registration of brain region's shape in 2D slice images for image reconstruction and fine registration between 3D reconstruction result and 3D reference atlas to achieve robust and accurate. In summary, our slice-to-volume registration pipeline provided an effective solution for spatial localization of 2D histological slices studies such as neuronal projection, cell distribution and gene expression.

Disclosures: Z. Zhang: None. **Z. Feng:** None. **H. Gong:** None. **A. Li:** None.

Poster

173. Anatomic Methods: Image Acquisition I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 173.10/BB39

Topic: I.03. Anatomical Methods

Title: Functional projection at single-neuron level in the visual cortex in mice

Authors: *S. KE, J. CHANG, R. MENG, W. LI, A. LI, W. ZHOU, H. GONG;
Britton Chance Ctr. for Biomed. Photonics, Wuhan Natl. Lab. for Optoelectronics-Huazhong Univ. of Sci. and Technol., Hubei, China

Abstract: The functional connectivity of neural circuit is the basis of revealing the information processing mechanism of brain neural network,which aims at the projection characteristics of neurons in the circuit and their associated functional features. However, due to the current platform system for exploring neural function is incompatible with that for studying neural structure, the research on function and structure is still relatively independent, and cannot provide direct evidence for the functional connection in the neural network. Here, we combined

two-photon calcium imaging with fluorescent Micro-Optical Sectioning Tomography (fMOST) technique, both based on single-neuron level, to study functional projection of neurons in primary visual cortex (V1). In order to obtain the fine structure of neural circuit of calcium-labeled brain, we developed a new set of resin embedding method, which solved the problem that the signal-to-noise ratio of calcium-labeled samples for whole-brain structure imaging in previous was too poor to track the single-neuron projection. After registration and tracing of the function-annotated neurons in brain, we obtained the functional projection of V1 at the single-neuron level of the whole brain. This approach fills the gap between the functional and structural research of neural circuit, and will uncover more details for the logic of functional connection in brain.

Disclosures: **S. Ke:** None. **J. Chang:** None. **R. Meng:** None. **W. Li:** None. **A. Li:** None. **W. Zhou:** None. **H. Gong:** None.

Poster

173. Anatomic Methods: Image Acquisition I

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 173.11/BB40

Topic: I.03. Anatomical Methods

Support: the Science Fund for Creative Research Group of China (61421064)

Title: A new method for ventricle expansion correction to assist the precise location of neuron projection pathway

Authors: *Z. FENG, C. TAN, H. NI, H. GONG, Q. LUO;
Wuhan Natl. Lab. For Optoelectronics, Huazhong Univ. of Sci. and Technol., Wuhan, China

Abstract: The emerging micro-optical imaging techniques allow scientists to depict the intact projection pathway of neurons in μm-scale spatial resolution throughout the whole brain, which provides essential knowledge for the study of brain functions and diseases in mesoscopic scale. The key step for elucidating the precise projection pathways of neurons acquired by micro-optical imaging is to accurately locate the position of neuron fibers via image registration techniques. However, many tissue preparing methods that are used to label the projection pathways would cause severe brain ventricle expansions, which are rather difficult to recover with commonly used image registration methods. This would prevent us from correctly obtaining the neurons' projection targets and the brain regions passing by. Here, we propose a new method that can effectively correct the expansion of brain ventricles with precision of μm-scale spatial resolution, as well as avoiding the unreal stretching of brain tissue around the ventricles due to the non-rigid image registration. The proposed method first reconstructs the surfaces of the tissues around brain ventricles with triangular meshes, and then applies the non-rigid iterative

closest point algorithm to construct a controlling point pair between the image datasets of the brain sample and a standard reference brain. Finally, a global non-rigid warping is established from brain sample to the standard reference brain with thin-plate spline model. Compared to methods based on Demons algorithm or B-spline model, the proposed method can eliminate the overstretching phenomenon of brain tissues around the ventricles during image registration, which can simulate the inverse process of real brain expansion occurred in brain tissue preparing more effectively, and can help to precisely locate the neuron projection pathway.

Disclosures: **Z. Feng:** None. **C. Tan:** None. **H. Ni:** None. **H. Gong:** None. **Q. Luo:** None.

Poster

174. Anatomic Methods: Image Acquisition II

Location: Hall A

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Program #/Poster #: 174.01/BB41

Topic: I.03. Anatomical Methods

Support: NIH R25MH060482
Jane Coffin Childs Memorial Fund
UCSF Physician Scientist Scholar Program
Simons Foundation Autism Research Initiative
HHMI

Title: Scalable and cost-effective processing for Micro-CT imaging of postmortem brain structure

Authors: *D. B. KASTNER[1], V. KHARAZIA[2], Z. YANG[1], S. JANA[2], J. SANCHEZ[2], D. Y. PARKINSON[3], L. M. FRANK[2];
[1]Dept. of Psychiatry, [2]Dept. of Physiol., UCSF, San Francisco, CA; [3]Advanced Light Source Div., Lawrence Berkeley Natl. Lab., Berkeley, CA

Abstract: Postmortem evaluation of the brain is a critical validation step for much of correlative and causal systems neuroscience. Often, the 3-dimensional structure of the brain is sacrificed during histologic processing and staining to enable relatively easy, but time consuming, analyses. More specialized processing exists, such as tissue clearing methods, to maintain the 3-dimensional structure of the brain while still gaining histological access, but these techniques can be labor intensive and quite expensive. Furthermore, such techniques are not well suited to evaluate lesions or electrode placements. Micro-CT provides an excellent intermediate option for these purposes, and a protocol has recently been developed to provide the necessary contrast for whole brain imaging using osmium as the contrast agent (Masis et al. *Sci Rep*, 2018). Drawing upon the recent use of eosin to provide contrast in postmortem liver and kidney (Busse et al. *PNAS*, 2018, Müller et al. *Sci Rep*, 2018), we developed a simplified protocol that uses eosin to

provide a high resolution 3-dimensional view of brain structure while also maintaining compatibility with subsequent traditional brain-slice histology. Unlike the osmium-based technique, there is minimal additional histochemical processing beyond the initial fixation of the brain tissue. We utilized the tomography beamline 8.3.2 at the Advanced Light Source at Lawrence Berkeley National Labs and the monochromatic and phase contrast imaging that it provides. The protocol provides a scalable, and cost-effective processing pipeline to provide up to 6 micrometer resolution of structures and lesions in the rat brain.

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Poster

174. Anatomic Methods: Image Acquisition II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 174.02/BB42

Topic: I.03. Anatomical Methods

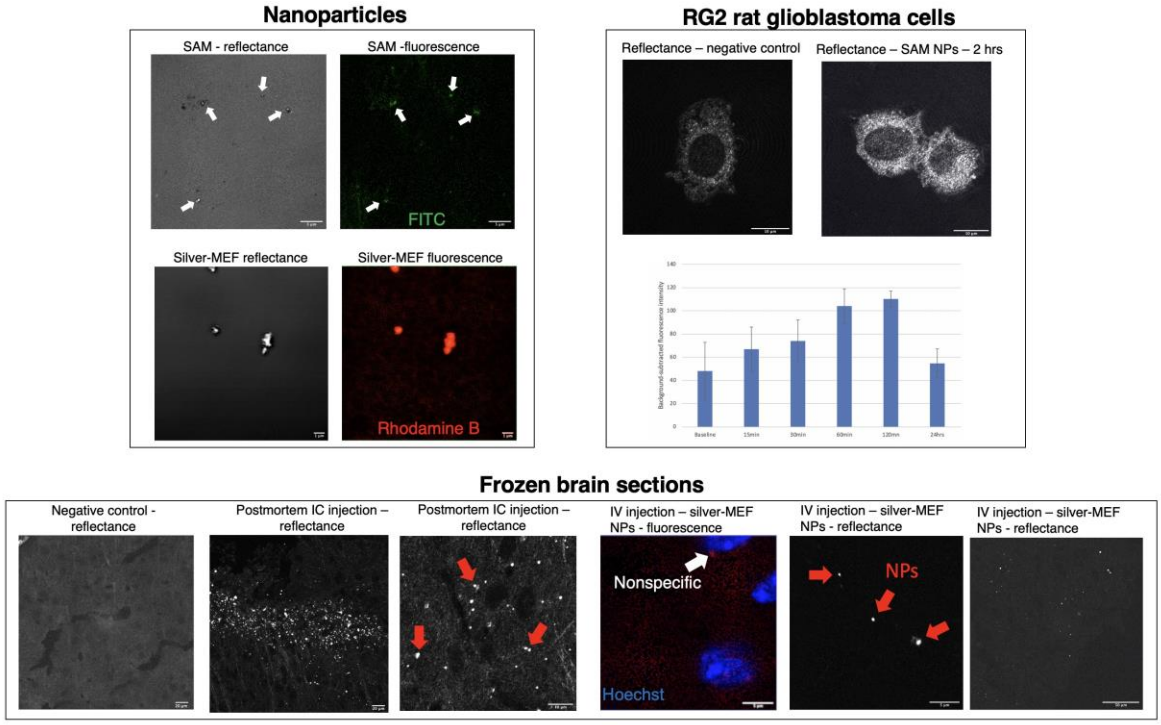
Support: Hacettepe University Research Projects Coordination Unit (010 A 105 001-106)
The Science Academy, Turkey

Title: Confocal reflectance microscopy for metal and lipid nanoparticle visualization in the brain

Authors: C. CAKIR AKTAS, ***S. ERDENER**, B. TEKE, S. PEHLIVAN, Z. KAYA, A. TASKIRAN SAG, T. DALKARA, M. MUT;
Hacettepe Univ., Ankara, Turkey

Abstract: An essential element of nanoparticle (NP) research is their visualization within cells and tissues. Technical challenges of electron microscopy and the low yield of fluorescent tagging of NPs is a bottleneck for this goal. Here, we utilized confocal reflectance microscopy (CRM) for imaging of silver and lipid-core NP uptake in vitro and in vivo, after intracerebral (IC) and intravenous (IV) administrations. A Leica SP8 confocal microscope with a 64x (NA:1.4) objective was used for imaging at 488 nm laser illumination. Custom-made FITC-conjugated 70-nm stearylamine (SAM) NPs and commercial 120-nm RhodamineB-conjugated silver NPs were visualized on glass slides as clear bright spots in CRM overlapping with their fluorescence, based on their distinguishing refractive indices. For in vitro confirmation of CRM to visualize NP uptake, RG2 rat glioblastoma cells were exposed to varying concentrations and durations of SAM NPs. Background-adjusted cytoplasmic reflectance intensity showed a dose and exposure-dependent increase compared to negative controls, peaking at 2 hours of incubation. For in vivo experiments, Swiss-Albino mice were used. SAM NPs IC injected (305 µg in 1µL) into a postmortem brain to minimize spread of NPs with cerebrospinal fluid flow, for better

localization. When 20 µm frozen sections were examined, NPs were detected along the injection tract. Then, SAM NPs were IC injected to an anesthetized mouse and as early as 15 min after injection, diffuse bilateral spread of NP aggregates were detected, indicating their high mobility and penetration. Likewise, parenchymal penetration of silver NPs were demonstrated after their IV injection with CRM. Nonspecific particulate fluorescence signals were present even in negative controls, while CRM reliably excluded these. Our findings demonstrate the value of CRM, which can be performed with a standard confocal microscope, for cell-level or tissue-level imaging of different types of NPs in central nervous system.



Disclosures: **C. Cakir Aktas:** None. **S. Erdener:** None. **B. Teke:** None. **S. Pehlivan:** None. **Z. Kaya:** None. **A. Taskiran Sag:** None. **T. Dalkara:** None. **M. Mut:** None.

Poster

174. Anatomic Methods: Image Acquisition II

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ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: I.03. Anatomical Methods

Support: Strategic Priority Research Program of the Chinese Academy of Sciences XDB32030200
Scientific Instrument Developing Project of the Chinese Academy of Sciences YZ201668

Title: Whole-brain connectomic mapping of rhesus monkey at micron-resolution

Authors: F. XU[1,2], ***Y. SHEN**[1], H. TAN[3], C. YANG[1], L. DING[1], H. WANG[1], Q. ZHU[1], X. HU[3], P.-M. LAU[1], G.-Q. BI[1];
[1]Univ. of Sci. and Technol. of China, Hefei, China; [2]Shenzhen Inst. of Advanced Technology, Chinese Acad. of Sci., Shenzhen, China; [3]Kunming Inst. of Zoology, Kunming, China

Abstract: Whole-brain mapping of neural structure, connectivity and activity traces is important for the study of brain functions and diseases. In the past few years, automated high-resolution fluorescence imaging techniques have been successfully used in systematic mapping of rodent brains. For non-human primates such as rhesus macaques, the very large size of the monkey brain poses significant challenges to the current 3D imaging approaches. Here, we report a new method and pipeline for high-throughput mapping of the whole rhesus macaque brain based on our recently developed VISoR technique that enables ultrafast volumetric imaging of cleared tissue slices. The pipeline combines a primate-optimized clearing and matching method (uClear), a VISoR imaging system optimized for large scale samples, and automated reconstruction and analysis software for mapping monkey brains labeled with tracing viruses and fluorescent dyes. The time for single-channel imaging of an entire rhesus monkey brain with 1 μm * 1 μm * 2.5 μm voxel size (total ~250TB of raw image data) was less than 100 hours. Using this system, we demonstrate high-resolution neuronal structures of the monkey brain, as well as whole brain projections traced by AAV virus originating from superior colliculus (SC).

Disclosures: F. Xu: None. **Y. Shen:** None. **H. Tan:** None. **C. Yang:** None. **L. Ding:** None. **Q. Zhu:** None. **X. Hu:** None. **P. Lau:** None. **G. Bi:** None. **H. Wang:** None.

Poster

174. Anatomic Methods: Image Acquisition II

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Program #/Poster #: 174.04/BB44

Topic: I.03. Anatomical Methods

Support: Swiss National Science Foundation 310030B_170269, F.H.
ERC Advanced Grant BRAINCOMPATH, project 670757; F.H.

Title: Whole-body imaging of vDISCO-cleared mice with the mesoSPIM light-sheet microscope

Authors: *F. F. VOIGT[1], S. ZHAO[2], R. CAI[3], M. I. TODOROV[4], A. ERTURK[5], F. HELMCHEN[6];
[1]Brain Res. Inst., Zurich, Switzerland; [2]Inst. For Stroke and Dementia Res. (ISD), Muenchen, Germany; [3]Inst. for Stroke and Dementia Res., Klinikum Der Univ. Muenchen, Muenchen, Germany; [4]Inst. for Stroke and Dementia Res., Klinikum der Univ. München, Munich, Germany; [5]Inst. for Stroke and Dementia, Munich, Germany; [6]Brain Res. Inst. / Univ. of Zurich, Zurich, Switzerland

Abstract: Recently, we launched the mesoscale selective plane illumination microscopy (mesoSPIM) initative, an open-source project aimed at providing the neuroscience community with instructions and software to set up versatile light-sheet microscopes for cleared tissue (mesospim.org). A mesoSPIM is capable of quickly exploring large (cm-sized) cleared samples at isotropic resolution. Typical single-channel datasets of whole mouse brains at 6.55-µm sampling are generated within 8 minutes. Uniform axial resolution is achieved by axially scanned light-sheet microscopy (ASLM), a technique which translates the beam waist region of the light-sheet through the sample in synchrony with the rolling shutter readout of the camera. In this approach, only the most axially confined region of the illumination beam is used to generate an image which enables uniform 6.5-µm axial resolution across a FOV of 13.3 mm. Currently, 7 mesoSPIM instruments are in operation at various institutions throughout Europe. While clearing techniques such as uDISCO and vDISCO render whole adult mice transparent, currently available commercial light-sheet instruments lack the capability to image such large samples without remounting and cutting. Typically, the major limitations are the insufficient size of the immersion chambers and inadequate sample travel ranges. In addition, commercial instruments for cm-sized samples often lack a rotation stage as most instruments feature a vertical detection path. Especially in large samples, however, a microscope capable of sample rotation is highly beneficial as users can select the optimal imaging direction for regions of interest and perform multi-view imaging. With its travel range of 44x44x100 mm (X/Y/Z) and integrated rotation stage, the mesoSPIM supports a larger imaging volume than other available microscopes. We therefore upgraded a mesoSPIM with a larger immersion chamber and improved sample holders which allowed us to image a whole cleared mouse including the nervous system. Beyond neuroscience, the combination of whole-body clearing and mesoSPIM imaging will streamline studies aimed at better understanding cancer metastasis and promoting treatments by visualizing the distribution of tumor-targeting therapeutic antibodies.

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Poster

174. Anatomic Methods: Image Acquisition II

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Program #/Poster #: 174.05/BB45

Topic: I.03. Anatomical Methods

Support: European Research Council Grant 268970
Fight for Sight

Title: 3D-MANC a computational platform for extracting and reconstructing single neurons in 3D from multi-colour labelled populations

Authors: *N. MILOSAVLJEVIC[1], C. A. PROCYK[2], E. ZINDY[1], R. J. LUCAS[1];
[1]The Univ. of Manchester, Manchester, United Kingdom; [2]UCL, London, United Kingdom

Abstract: The structure-function relationship of neurones underpins their classification and provides significant insights into how individual neurones and their processes integrate to form functional circuits. A powerful method by which to investigate these circuits is by using the multi-reporter labelling technique "Brainbow" and the evolving repertoire of related multi-reporter labelling technologies. Brainbow relies on the stochastic labelling of neurons in a targeted population such that individual cells can be visualized by their unique combination of reporter protein expression, usually represented in terms of a particular 'colour' in pseudocoloured images. These have been applied to a variety of neuronal and non-neuronal tissues in diverse model species to answer a wide range of scientific questions from cell lineage tracking to neuronal architecture and connectivity. However, a significant limitation to these multi-reporter technologies is the absence of a reliable and objective analysis tool that is not only capable of producing 3D images of individual cells from the labelled population but which can also produce quantitative data about them. Here, we address this challenge by developing a versatile analytical toolbox (3D-MANC) which allows for the reconstruction of multiple individually isolated single cells from Brainbow labelled tissue in 3D. The toolbox consists of a range of steps including filtering, processing and clustering of voxals and finally the spatial reconstruction of the individual cells in 3D that allow for quantitative morphological analysis. We demonstrate the utility of the 3D-MANC platform by using it to reconstruct images of individual intrinsically photosensitive retinal ganglion cells (ipRGCs) from the retina of *rd/rd* $Opn4^{Cre/+}$ mice transfected with a floxed Brainbow virus. Following reconstruction of their 3D morphology we are able to quantitatively compare the soma sizes and the extent, complexity and location within the inner-plexiform layer of dendritic fields with previous descriptions of ipRGC anatomy in the wildtype retina. We find ipRGCs whose characteristics match those reported for all 5 subtypes of ipRGC in the wildtype retina, indicating for the first time that all five subtypes of ipRGCs survive retinal degeneration.

Disclosures: **N. Milosavljevic:** None. **C.A. Procyk:** None. **E. Zindy:** None. **R.J. Lucas:** None.

Poster

174. Anatomic Methods: Image Acquisition II

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Program #/Poster #: 174.06/BB46

Topic: I.03. Anatomical Methods

Support: NHMRC 1140295

Title: The endorestiform nucleus of the human brainstem

Authors: *M. S. KASSEM, G. PAXINOS;
Neurosci. Res. Australia, Randwick, Australia

Abstract: Paxinos, Furlong and Watson (2019) identified a hitherto unknown structure within the inferior cerebellar peduncle (restiform body) of the human brainstem, via labelling with acetylcholinesterase (AChE) and Nissl, which they named the endorestiform nucleus. This nucleus can be seen most prominently via AChE, where positive cells can be easily seen against a light stained background. It is located dorsal-laterally within brainstem 6 to 9mm rostral to the obex. The endorestiform nucleus was outlined (but not identified) by Paxinos et al (1990) in a diagram plotting the ascending spinal fibre projections and connections. Microscopically charted Nauta-method selective silver impregnation of degenerating fibers of passage and preterminal connection sites were depicted at the level of the rostral part of the hypoglossal nucleus, what is now identified as the endorestiform nucleus. The present study attempts to find homologies of the entorestiform nucleus within other species. We initially thought the entorestiform nucleus might be a feature of only the human brain because we had not identified it our atlases of other primates and rodents. We now report it presence in the chimpanzee brain. Further investigation lead to the use of higher-power images of the rhesus monkey, suggesting that it is present there as well. We cannot find the endorestiform nucleus in the rat. The nucleus is in a privileged position to receive projections from the spinal cord related to sensory information.

Disclosures: M.S. Kassem: None. **G. Paxinos:** None.

Poster

174. Anatomic Methods: Image Acquisition II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 174.07/BB47

Topic: I.03. Anatomical Methods

Support: NIH DC003180
NIH DC014503

Title: Quantitative delineation of sub-regions of marmoset auditory cortex using ultra-high field MRI

Authors: *Y. ZHANG[1], X. WANG[2];
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Abstract: Marmoset is a highly vocal and social New-World monkey of growing interest in neuroscience. It has unique advantages as a non-human primate model for studying neural mechanisms underlying vocal communication and brain functions for social interactions. Understanding anatomical and functional organizations of marmoset auditory cortex is crucial to unveil the mechanisms of auditory perception. Auditory cortex of non-human primates has been divided into core, belt, and parabelt sub-regions. However, the borders between sub-regions defined by histology are usually drawn without quantification. In this study, we developed a method to quantitively define sub-regions of marmoset auditory cortex using ex-vivo multi-modal magnetic resonance imaging (MRI) based on T2-weighted and multi-shell diffusion images. We combined multiple MRI contrasts (orientation dispersion index, fractional anisotropy, principle direction and T2 signals) to provide a multi-dimensional feature space for delineation and validation of sub-regions in auditory cortex. Comparing to previous ex-vivo MRI studies that typically need several days of scan, we were able to obtain high resolution data (0.15mm isotropic) with a small surface coil covering the superior temporal gyrus in about 3 hours on a 11.7T Bruker MRI scanner. A unique feature of our method is that it does not require averaging data from multiple brains and thus can be applied to an individual brain. The method we have developed in this study has the potential to be applied to in-vivo studies in marmosets and can be applied to other cortical areas or other animal species.

Disclosures: Y. Zhang: None. **X. Wang:** None.

Poster

174. Anatomic Methods: Image Acquisition II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 174.08/BB48

Topic: I.03. Anatomical Methods

Support: FWF grant P23102-N22
FWF grant P31263-B26

Title: Light sheet microscopy of human brain tissue and tumors

Authors: *H.-U. DODT[1,2], S. SAGHAFI[1], I. SABDYUSHEVA-LITSCHAUER[1], J. GAUGELER[1], K. BECKER[1], M. FOROUGHIPOUR[1];
[1]Tech. Univ. Vienna, Vienna, Austria; [2]Med. Univ. Vienna, Vienna, Austria

Abstract: Light sheet microscopy has been applied very successfully to the investigation of chemically cleared animal brains since the first description of this approach[1]. However its application to human brain tissue has been hampered by difficulties to obtain specific neuronal staining. The use of endogenous fluorescent markers is not possible in humans and staining with antibodies is cumbersome and expensive due to diffusion problems in large tissue pieces. Also nuclear staining does not give much more information than the number of cells in a certain brain volume.
We therefore looked for alternative staining possibilities and found a way to strongly boost the autofluorescence of brain tissue. This allowed us to visualize the vascularization in brain tumors which is important in the context of neovascularization of malignancies. Cells in other tumors could be directly visualized by autofluorescence boosting.
A prerequisite for fast imaging of cm sized brain tissue blocks with cellular resolution is the generation of a light sheet with a thickness in the 1 µm range and a very large Rayleigh length. As this is not possible with any existing optics we constructed a new light sheet generator providing a new extremely flat and thin light sheet and applied it to human tumor samples. In addition we developed a new kind of clearing that makes cm large issue blocks completely transparent within one day.
We expect that this combination of fast clearing, autofluorescence boosting and high resolution light sheet microscopy will in future play an important role in the investigation and clinical diagnostics of human tissue.
[1]Dodt et al., Ultramicroscopy: three-dimensional visualization of neuronal networks in the whole mouse brain, *Nat. Methods* **4**: 331-36, 2007

Disclosures: H. Dodt: None. **S. Saghafi:** None. **I. Sabdyusheva-Litschauer:** None. **J. Gaugeler:** None. **K. Becker:** None. **M. Foroughipour:** None.

Poster

174. Anatomic Methods: Image Acquisition II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 174.09/BB49

Topic: I.03. Anatomical Methods

Support: P41EB027061
P30NS076408
R01DA038615
R01DA037229

U01EB025144

Title: Awake rhesus macaque imaging at ultra high field strength (10.5T)

Authors: *S. JUNGST[1], R. LAGORE[2], P. MEHTA[4], M. D. GRIER[3], S. R. HEILBRONNER[5], G. ADRIANY[2], B. Y. HAYDEN[6], J. ZIMMERMANN[2];
[1]Univ. of Minnesota Twin Cities Campus, Minneapolis, MN; [3]Neurosci., [2]Univ. of Minnesota Twin Cities, Minneapolis, MN; [4]Univ. of Minnesota, Minneapolis, MN; [5]Neurosci., Univ. of Minnesota, New Brighton, MN; [6]Univ. of Minnesota, Saint Paul, MN

Abstract: Functional magnetic resonance imaging in macaques offers the opportunity to assess whole-brain activity in a species that is amenable to recording and neuronal manipulation. We developed a novel tool set for imaging awake rhesus macaques at resolutions below 500 microns (isotropic, whole brain) with fidelities tuned towards microvasculature oxygenation changes. This resolution is made possible through three developments. First, the ultra high field strength of 10.5 Tesla with a large magnet bore diameter (88 cm) allows for the use of an SC 72 Siemens body gradient (60 cm bore liner diameter) which in turn provides a comfortable environment for monkeys to perform tasks despite the high field strength. Second, the use of custom designed shimming and calibration routines as well as sequence designs allows for heretofore unavailable signal to noise ratios. Third, we developed a custom coil. Our coil has 16 receive and 4 transmit channels with embedded electronics and customized pre-amplifiers to minimize cable losses while being easy to work with on a daily basis. The coil provides homogenous excitation as well as direct skin contact with head fixed non human primates. It also provides a wide view which is critical for stimulus presentation and eye tracking.The coil is constructed as two physically independent mirrored halves. Each half of the coil conforms to the side of the head and contains 8 receive loops and 2 transmit loops which also conform to the same curvature. The receive channels have on-coil tune and match circuits for operation at 447 MHz (proton imaging at 10.5T) and are both preamplifier decoupled and overlap decoupled with their nearest neighbors. The loops range in size from 30 mm to 40 mm and have up to four distributed capacitors to achieve resonance. Taken together, we demonstrate the first proof of principle in hemodynamic imaging of non human primates at ultra high field of 10.5T. We hope that our approach opens a new era of functional MRI in non human primates facilitating direct translation to human results.

Disclosures: S. Jungst: None. **R. Lagore:** None. **P. Mehta:** None. **M.D. Grier:** None. **S.R. Heilbronner:** None. **G. Adriany:** None. **B.Y. Hayden:** None. **J. Zimmermann:** None.

Poster

174. Anatomic Methods: Image Acquisition II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 174.10/BB50

Topic: I.03. Anatomical Methods

Support: NIH
CSCTR
Brain Research Foundation
The Hope Center for Neurological Disorders

Title: Airysynapse—A simple, robust super-resolution imaging technique to quantify synaptic loci in mammalian neuropil

Authors: *A. D. SAUERBECK[1], M. GANGOLLI[2,3], T. KUMMER[1];
[1]Washington Univ. in St. Louis, St. Louis, MO; [2]The Henry M. Jackson Fndn., Bethesda, MD; [3]Ctr. for Neurosci. and Regenerative Med., Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD

Abstract: Synapse loss is a classic early hallmark of Alzheimer disease (AD) and numerous other neurodegenerative conditions. Existing methods to accurately quantify synaptic loci in mammalian neuropil, such as electron microscopy and array tomography, are highly laborious and technically challenging, presenting a high barrier to widespread adoption. A straightforward method to perform accurate, high-throughput quantification of synaptic density could open the door to routine synaptic connectivity analysis, shedding light on critical questions related to acute and chronic brain injury, as well as normal circuit function. We report a novel, rapid, and easily adopted method for synapse quantification—AirySynapse—that combines the ease of confocal microscopy with the detection efficiency of super-resolution (S-R) imaging. AirySynapse leverages a widely-available pinhole plane scanning microscope to break the diffraction limit without the concomitant loss of sensitivity inherent in most optical implementations of S-R imaging. Custom localization analysis is applied to resulting datasets to identify pairs of pre- and postsynaptic structures at ultrastructurally-informed separation distances. AirySynapse was designed to maximize accessibility to the scientific community, providing a broadly useful tool for routine probing of gray matter microconnectivity. We validated AirySynapse for the measurement of plaque-associated and diffuse excitatory synapse loss in a pre-clinical AD model, confirming its sensitivity to pathological synaptic injury. Traumatic brain injury (TBI) is the best-established environmental risk factor for AD, but its structural-mechanistic connections to neurodegeneration are not understood. We therefore used AirySynapse to evaluate potential synaptic injury after TBI induced with a diffuse, closed-head injury model called modCHIMERA. AirySynapse revealed a delayed loss of cortical synapses following TBI, validated with electron microscopy, that may predispose to future AD-related cognitive decline. Ongoing work is focused on mesoscale imaging of synaptic networks to quantify microconnectivity in entire brain structures (*e.g.*, murine hippocampi or human cortical columns), which will shed new light on the spatial patterning of synaptic alterations. AirySynapse is a powerful, accessible platform for the characterization of synaptic connectivity that will provide new insights into synaptic dynamism in health and disease.

Disclosures: **A.D. Sauerbeck:** None. **M. Gangolli:** None. **T. Kummer:** None.

Poster

174. Anatomic Methods: Image Acquisition II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

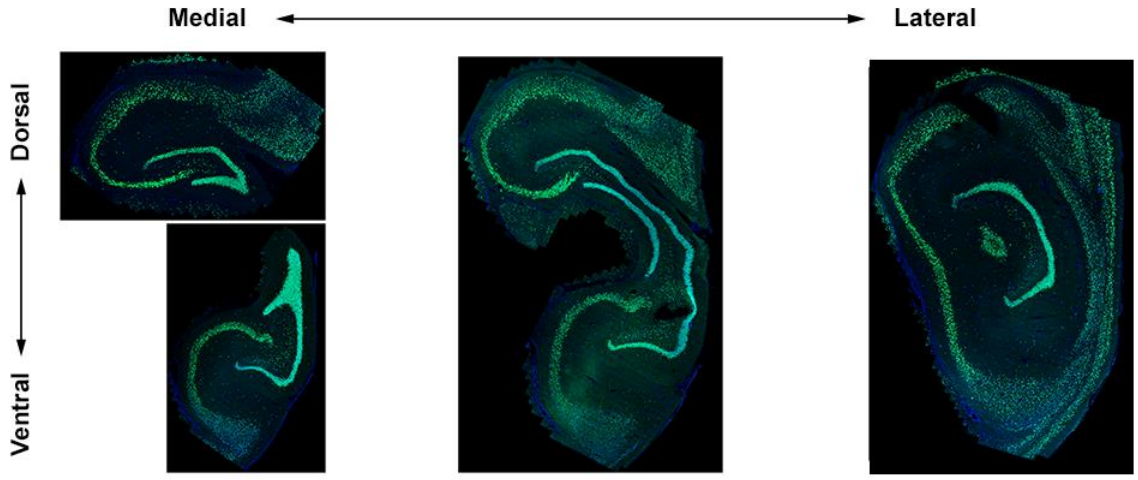
Program #/Poster #: 174.11/BB51

Topic: I.03. Anatomical Methods

Title: Hippocampal formation in Carollia perspicillata

Authors: *T. D. MORELLO[1], R. KOLLMAR[2], *M. G. STEWART[1];
[1]Physiol. & Pharmacol., [2]Dept. of Cell Biol., SUNY Downstate Med. Ctr., Brooklyn, NY

Abstract: With the advancement of optical sectioning techniques, image processing and segmentation, and 3 dimensional (3D) reconstructive techniques, there is an increasing call for 3D representations of histologic data. Here we show a workflow for the 3D reconstruction of NeuN stained hippocampus of Carollia perspicilatta, also known as Seba's short-tailed fruit bat, and discuss future uses for these techniques. As flying animals, memory of and navigation through space is especially critical in bats. The hippocampus holds many of the primary elements that allow this to take place. In the current study, we compare histologic observations in three areas of the hippocampus (CA3, CA1, and subiculum) between dorsal and ventral regions, and between medial to lateral sections. 3D reconstruction involves steps to optimize tissue section alignment, to create consistency in contrast, and to correct for histologic artifacts that result from fixation and mounting. The bat brain has a width of 1.4 mm meaning approximately 150 sagital slices for one half of the brain. Thus, automation is necessary for efficient processing and reconstruction of whole brain from serial sections. Furthermore, segmenting, or labeling images by features such as cell body, cell process, blood vessel, and so on, has relied on edge detection filters that usually specialize for one shape or another (e.g. Frangi vesselness filter, roundness filters, blobness filters). However, the use of convolutional neural networks, a form of machine learning, is becoming more prevalent in microscopy image processing. Our data shown here represent a stepping stone in the process of 3D reconstruction from serial tissue sections.



Disclosures: **T.D. Morello:** None. **R. Kollmar:** None. **M.G. Stewart:** None.

Poster

174. Anatomic Methods: Image Acquisition II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

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Topic: I.03. Anatomical Methods

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Jacobs Foundation grant 2016121703
Child Neurology Foundation
McDonnell Center for Systems Neuroscience
Mallinckrodt Institute of Radiology grant 14-011
Hope Center for Neurological Disorders

Title: Benchmarking diffusion tensor calculation for reliable individual analysis

Authors: *N. A. SEIDER[1], D. J. NEWBOLD[5], T. O. LAUMANN[2], J. RUTLIN[6], R. MILLER[1], B. ADEYEMO[3], A. Z. SNYDER[7], J. SHIMONY[4], N. DOSENBACH[8];
[1]Washington Univ. Sch. of Med., St Louis, MO; [2]Neurol., [3]Dept. of Neurol., [4]Washington Univ. Sch. of Med., Saint Louis, MO; [5]Washington Univ. in St Louis, Saint Louis, MO; [6]Washginton Univ. Sch. of Med., St Louis, MO; [7]Radiol Dept, Washington Univ. Sch. Med., Saint Louis, MO; [8]Neurol., Washington Univ. in St. Louis, Saint Louis, MO

Abstract: Diffusion Tensor Imaging is used to characterize white matter tracts. Currently DTI measures are averaged across a group and within a white matter tract to optimize the power of the study: localized and individual effects can be lost when taking this approach. Three individuals were scanned every day for over two weeks: each diffusion weighted scan consisted of 96 unique B-vectors measured at 5 different B values (shells). Eddy current correction and top-up processing was applied on each DWI and all images were registered. In total, a single subject had 74 B0 images and 1158 B-weighted images. Fifteen regions of interest (ROI) were chosen, to create reliability curves in large white matter tracts (e.g. corpus callosum), smaller contentious white matter tracts (e.g. fornix), and gray matter areas. A subset of *N* directions were pseudo-randomly chosen from the available 1158, where *N* was equal to 10 to 1100 in steps of 10. Directions were subdivided into 16 groups on the surface of a sphere, and directions were chosen to sample all 16 groups equally. For the set of *N* directions, the diffusion tensor was calculated using a non-negative least square method (Linear), a priors-constrained method (Bayes), and FSL's Ball-and-Stick model with 1-3 sticks. We calculated the fractional anisotropy (FA), radial diffusion (RD), axial diffusion (AD), mean diffusion (MD), and tensor angles theta and phi for each direction. The mean and standard deviation of each parameter of interest was calculated over 1000 permutations and plotted against the number of directions in that set. FA is underestimated at low numbers of B-vectors, while MD and RD are both overestimated at low numbers of B-vectors. AD is over- or underestimated depending on which computation method was used. Comparing the non-negative least-squares computation (Linear) to a Bayes model with biological priors (e.g. eliminating negative eigenvalues), resulted in different RD, AD, and MD values at 1100 directions: the difference can be up to 0.02 $\mu m/ms^2$. Additionally, the B-vector reliability threshold varies slightly in different ROIs given the cohesive organization of that ROI. Regardless of method, one needs a about 100 unique B-vector directions for a coefficient of variance less than 5% in the corticospinal tract. Following the example set in resting-state functional connectivity studies, we propose looking at the white matter microstructure at an individual and local level. In order to apply such principles, we must use highly oversampled and high-quality data sets from which to acquire reliable local DTI measures. Our results suggest we need a greater number of B-vector directions than what is currently accepted in the field.

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Poster

174. Anatomic Methods: Image Acquisition II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 174.13/BB53

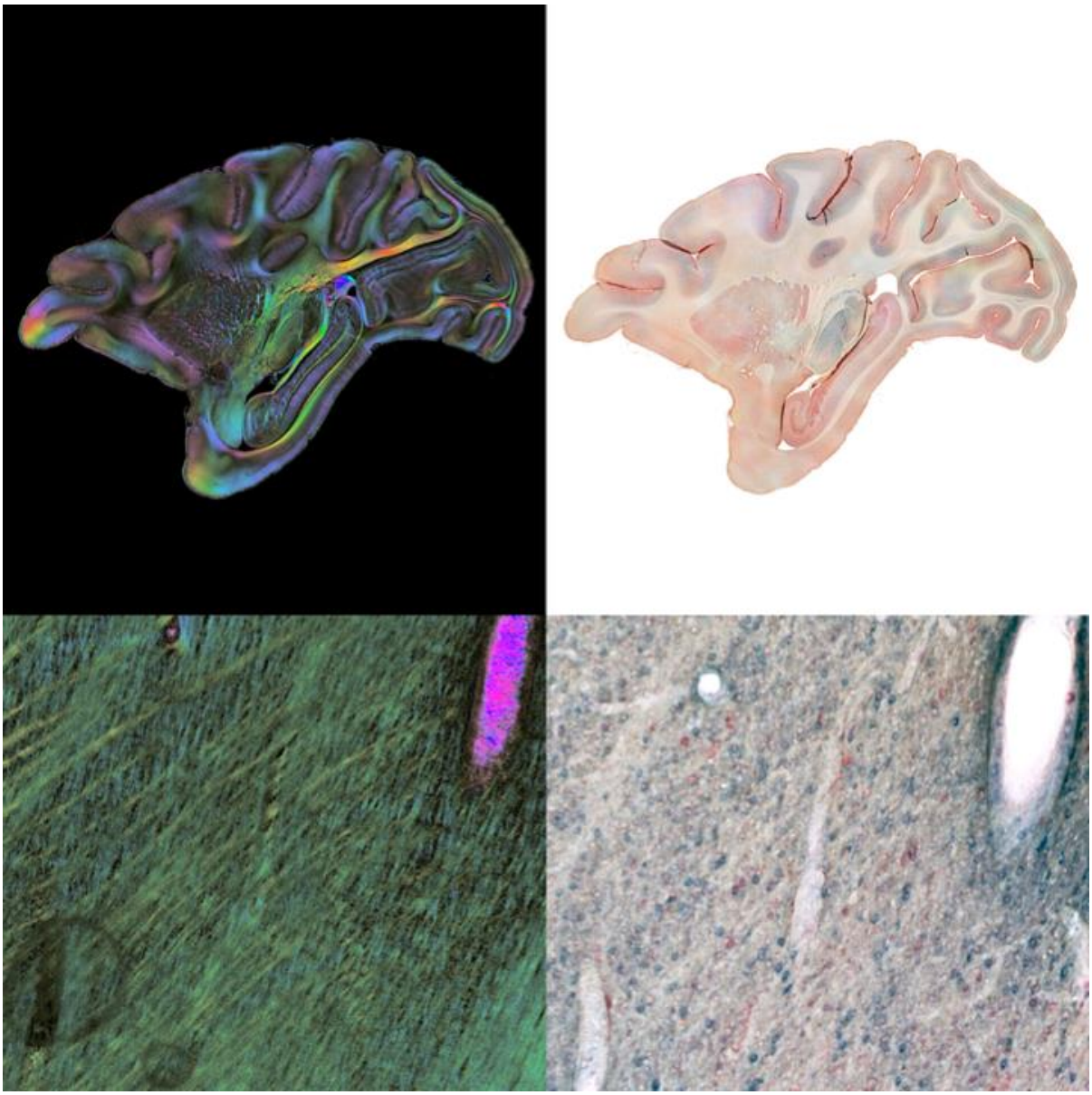
Topic: I.03. Anatomical Methods

Support: Human Brain Project SGA2 785907

Title: Multimodal cell and fiber mapping in full vervet brain sections

Authors: *R. N. KOOIJMANS[1,2], M. AXER[2], M. SCHOBER[2], D. GRÄßEL[2], P. SCHLÖMER[2], K. ZILLES[2], P. R. ROELFSEMA[1], K. M. AMUNTS[2];
[1]Vision & Cognition, Netherlands Inst. for Neurosci., Amsterdam, Netherlands; [2]Inst. of Neurosci. and Med., Res. Ctr. Jülich, Jülich, Germany

Abstract: Detailed multi-modal architecture information is the basis for understanding function, dysfunction, and potential treatment of the brain. There are multiple efforts to generate complete and consistent maps for various species, but none addresses (quantitative) protein expression in combination with direct imaging of fiber distribution patterns. We have refined a method to integrate multi-channel, cell-type specific immunohistochemistry with polarized light imaging (3D-PLI), for revealing protein expression, as well as fiber architecture in 3D-space, in the same, full, primate brain sections (figure). We image fiber tracts in 60µm-thick, unstained, vervet brain sections at 1.3 µm pixel size in-plane, using polarization microscopy (Axer et al.). Based on these measurements, regional fiber orientation maps are determined by means of big data analysis utilizing high-performance computing (JURECA supercomputer, JSC, Forschungszentrum Jülich), color-coded and visualized (figure). Subsequently, we unmount the tissue, and use immunohistochemistry to specifically label cells expressing calcium binding proteins parvalbumin, calbindin and calretinin, in the same section. We remount the sections, and acquire high-resolution, full color images (figure) with fast brightfield scanning. We segment cell bodies in these images using machine learning, and split the data into multiple channels based on color. This allows for acquiring more data at the same time, as well as relational data between targeted proteins. Finally, the two data sets are aligned section-wise, using non-linear registration tools.This approach enables the visualization, segmentation, classification, and quantification of distinct cell populations, as defined by protein expression, in the context of the local and global fiber architecture, within the same section. Ultimately, we aim to obtain a complete, consistent, and multi-modal map of the brain, to be integrated into the atlas of the Human Brain Project, and made available to the international research community.



Disclosures: **R.N. Kooijmans:** None. **M. Axer:** None. **M. Schober:** None. **D. Gräßel:** None. **P. Schlömer:** None. **K. Zilles:** None. **P.R. Roelfsema:** None. **K.M. Amunts:** None.

Poster

174. Anatomic Methods: Image Acquisition II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 174.14/BB54

Topic: I.03. Anatomical Methods

Title: Bessel beam tomography for fast volume imaging

Authors: *A. FLORES VALLE, J. SEELIG;
Caesar Fndn., Bonn, Germany

Abstract: For investigating the dynamics of neural circuits in the brain of behaving animals the activity of populations of neurons distributed over extended volumes needs to be recorded. To monitor an entire volume at high temporal resolution, we developed a tomography approach for scanning fluorescence microscopy which allows imaging a volume with a single frame scan. This is achieved by simultaneously recording four independent projections at different angles using temporally multiplexed, tilted Bessel beams. From the resulting projections, volumes are reconstructed using inverse Radon transforms combined with three dimensional convolutional neural networks (U-net). This tomography approach is suitable for experiments requiring fast volume imaging of sparsely labeled samples. We are planning to use this method for recording neural activity in behaving fruit flies and develop theoretical models to describe circuit dynamics underlying behavior.

Disclosures: A. Flores Valle: None. **J. Seelig:** None.

Poster

174. Anatomic Methods: Image Acquisition II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

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Topic: I.03. Anatomical Methods

Support: National Key Research and Development Program of China No. 2016YFC0103803, 2017YFA0205200, 2017YFA0700401
National Natural Science Foundation of China under Grant No. 81527805, 81671851, 81227901
CAS Grant XDBS01030200, GJJSTD20170004, YJKYYQ20170075

Title: Multiscale imaging of brain tumor using confocal laser endomicroscopy, light-sheet fluorescence microscopy and magnetic resonance imaging in small animal

Authors: *H. HUI[1], X. YANG[2], J. TIAN[3];
[2]Key Lab. of Mol. Imaging, CAS, [1]Inst. of Automation, Chinese Acad. of Scienc, Beijing, China;
[3]Key Lab. of Mol. Imaging, CAS, Beijing City, China

Abstract: In this work, we have developed a multimodality approach by combining magnetic resonance imaging (MRI) and optical imaging methods to assess brain tumor both at macro- and microscopic level for small animals. Macroscopic imaging technique, like magnetic resonance imaging (MRI), is widely used to evaluate the alternations of brain tumor. Compared with

computed tomography (CT) and positron emission tomography (PET), MRI has the advantage of no radiation. However, conventional MRI suffers from low resolution to detect brain tumor changes at cellular level. A newly developed imaging method, confocal laser endomicroscopy (CLE), provides over 10-fold higher resolution than MRI for *in vivo* tracking of fluorescently labeled tumor cells and microvessels of the tumor. In a CLE system, thousands of thin fibers are incorporated in a probe in which each fiber works as a point scanner and pinhole used in conventional confocal microscope. In general, with CLE for mouse applications, the lateral resolution can reach up to 1.4μm with 10μm optical sectioning and the field of view (FOV) has a diameter from 240μm to 600μm. Although CLE allows accurate assessment of brain tumor, a whole tumor imaging in three-dimensional is still challenging due to its small FOV and lack of 3D imaging ability. As a supplement for CLE, light-sheet fluorescence microscopy (LSFM) also known as ultramicroscopy is commonly used for *in-toto* imaging of the fluorescent labeling transparent sample at a focus plane. The aim of this work is to compare and correlate MRI, CLE and LSFM with respect to their potential as multimodality tools to assess brain tumor both at macro- and microscopic level.

Disclosures: **H. Hui:** None. **X. Yang:** None. **J. Tian:** None.

Poster

174. Anatomic Methods: Image Acquisition II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 174.16/BB56

Topic: I.03. Anatomical Methods

Title: Validation of experimental pipelines for mouse brain imaging with functional ultrasound (fUS) to quantitatively measure sensory responses and intrinsic functional connectivity patterns

Authors: J.-C. MARIANI[1], ***R. SANTOS**[2], L. BEYNAC[2], L. BOURGEAIS[2], T. DEFFIEUX[3], A. JOUTEL[2], M. TANTER[3], Z. LENKEI[2];
[1]Inst. of Psychiatry and Neurosci. of Paris, Inserm U1266- Univ. Paris Descartes, Paris, France; [2]Inst. of Psychiatry and Neurosci. of Paris, Paris, France; [3]Physics for Med. Paris, Inserm, ESPCI Paris, CNRS, PSL Res. Univ., Paris, France

Abstract: Functional imaging of the brain using fast ultrasound (fUS) has been validated in different models and paradigms in recent years. While these pioneering works obtained interesting data on neurovascular coupling, they were mainly limited to proof-of-concept studies. In our study, we focused on systematic testing of quantitative aspects of intrinsic functional connectivity (FC) during resting state (RS) and during hemodynamic response to sensory input (visual, whisker and tactile stimulation). We observed brain areas related to all three pathways under various preparations and experimental protocols (different anesthesia protocols and levels from deeply anesthetized to lightly sedated and awake, different degree of invasiveness from

simple shaving to craniotomy). We also studied the robustness of the method with chronic measurement following the same animal for weeks. These measurements allowed us to model to which extent these parameters are influencing the recordings. We show that non-invasive and chronic recording of hemodynamics is possible both in awake and sedated mice, and propose optimized protocols for these techniques.

Disclosures: J. Mariani: None. **R. Santos:** None. **L. Beynac:** None. **L. Bourgeais:** None. **T. Deffieux:** None. **A. Joutel:** None. **M. Tanter:** None. **Z. Lenkei:** None.

Poster

174. Anatomic Methods: Image Acquisition II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 174.17/BB57

Topic: I.03. Anatomical Methods

Title: A meso- and nano-scale pipeline for detailing butterfly brain evolution

Authors: G. WILDENBERG[1], ***K. NORWOOD**[2], M. KRONFORST[3], N. B. KASTHURI[4]; [1]Univ. of Chicago/Argonne Natl. Lab., Chicago, IL; [3]Ecology and Evolution, [4]Neurobio., [2]Univ. of Chicago, Chicago, IL

Abstract: Understanding how brains, and specifically neural circuits, evolve requires a deep appreciation of the multiscale nature of brains, from brain regions to individual neuronal connections. Brain evolution likely occurs at all these scales. There is currently few imaging pipelines that can traverse these scales in a single animal and, even more problematic, many of the species historically studied to understand evolution (e.g. butterflies) are not accessible by conventional imaging technologies due to a lack of antibody or genetic labeling tools. We describe here a method using synchrotron source micro-x-ray computed tomography (syn-µXCT) and automated serial electron microscopy for high-throughput imaging of Lepidoptera (i.e. butterfly and moth) brains. We utilize staining methods developed for electron microscopy to provide contrast for syn-µXCT that can be widely applied to any species. Our methodology allows for imaging at rates of ~ 15 min/mm3 at 600nm3 resolution and the resulting data-sets are amenable to automatic segmentation of brain structures (e.g. Flood Fill Networks, FFN) and downstream analyses for quantitatively comparing how the visual system varies across evolution. We used this approach to study the retinas of nocturnal and diurnal Lepidoptera and find both large and fine scale changes.

Disclosures: G. Wildenberg: None. **K. Norwood:** None. **M. Kronforst:** None. **N.B. Kasthuri:** None.

Poster

174. Anatomic Methods: Image Acquisition II

Location: Hall A

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Program #/Poster #: 174.18/BB58

Topic: I.03. Anatomical Methods

Support: NIH R01 NS045193
NIH R01 MH115750
New Jersey Brain Injury Research Fellowship
Veni ZonMW Fellowship
Erasmus MC Fellowship

Title: Crystal episkull: Chronic imaging of neocortical dendritic spines in mice using three-photon microscopy

Authors: *H.-J. BOELE, J. L. VERPEUT, D. PACUKU, S. Y. THIBERGE, S. S. H. WANG; Princeton Neurosciene Inst., Princeton, NJ

Abstract: In postmortem tissue we have found alterations in neocortical spine density in mice with Purkinje-cell specific deficiency in *Tsc1 e*xpression, as well as in wild-type mice with postnatal lesions of cerebellar crus I. These findings suggest that cerebellar activity is necessary for the normal maturation of neocortical circuitry. However, neocortical dendritic spine dynamics over a period of weeks are uncharacterized. We developed an approach to weeks-long monitoring using a new skull clearing technique that leads to a 'crystal episkull.' This preparation is amenable to three-photon fluorescence microscopy and allows imaging across a field of view spanning the dorsal surface of the neocortex. The preparation overcomes problems of *in vivo* two-photon imaging through chronic cranial window or thinned skull, which can cause substantial damage to the underlying tissue, have small fields of view, and can require repeated clearance of regrowing bone. We now seek to perturb cerebellar activity optogenetically, pharmacologically and/or chemogenetically in juvenile mice to test our *developmental diaschisis* hypothesis, which states that cerebellar activity in postnatal life helps refine circuitry in distal neocortical regions that are essential for normal cognitive and social function.

Disclosures: H. Boele: None. **J.L. Verpeut:** None. **D. Pacuku:** None. **S.Y. Thiberge:** None. **S.S.H. Wang:** None.

Poster

174. Anatomic Methods: Image Acquisition II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 174.19/BB59

Topic: I.03. Anatomical Methods

Support: NIH Grant MH64168
Hope for Depression Research Foundation

Title: Two-photon excitation enhances resolution of metal-stained tissue by scanning microscopy

Authors: M. S. SONDERS[1], G. ROSOKLIJA[1], C. LANGRECK[1], T. P. SCHNIEDER[4,1], C. HIKITA-DWORK[4], *A. J. DWORK[4,2], J. A. JAVITCH[3];
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Abstract: Various sophisticated techniques are available for microscopic examination of individual neurons in experimental animals. For human specimens, however, the options are much more limited. In particular, there are fluorescence methods for visualizing processes of individual neurons in laboratory animals, while in humans, attempts at such methods have met with limited success. Thus, the field has continued to rely upon Golgi methods, which extensively stain individual neurons, though sparsely and apparently at random (Ramon y Cajal, 1894). Golgi stains have always been viewed by bright field transmission microscopy, usually in thick sections (100-200 microns). Analysis of such material is complicated by the superimposition of signal from out-of-focus planes. Here we report that superior images of metal stained cells can be obtained with the use of a pulsed infrared (multi-photon; 2P) laser. Unexpectedly, several metal stains luminesce in visible wavelengths under low power 2P excitation. The luminescence shows a quadratic dependence on excitation power, effectively limiting signals to the plane of focus, which results in excellent axial resolution (~1 micron). Resolution is further improved by deconvolution based on an empirically determined point spread function. Striking improvement over bright field imaging was obtained with mercury-based (Golgi Cox) and silver-based (Golgi-Kopsch) methods for visualizing dendrites and spines. The use of neuron tracing software (essentially developed for fluorescence microscopy) is greatly facilitated. The advantage over bright field microscopy for Golgi stains of mouse brains were similar. The method also worked well with several other metallic stains of brain: With Bielschowsky silver stain, it allowed 3-dimensional tracking of closely spaced axons in white matter. Bright luminescence was also obtained with Gallyas (silver stain with gold toning) and Von Braunmuhl (silver) methods, apparently increasing their sensitivities in revealing

pathological structures. The mechanism of this 2P luminescence is not yet elucidated, as its photophysical characteristics are inconsistent with both fluorescence and reflectance. While we are still working to determine the mechanism, the method is already adding startling detail to our picture of human neurons.

Disclosures: M.S. Sonders: None. **G. Rosoklija:** None. **C. Langreck:** None. **T.P. Schnieder:** None. **C. Hikita-Dwork:** None. **A.J. Dwork:** None. **J.A. Javitch:** None.

Poster

174. Anatomic Methods: Image Acquisition II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 174.20/BB60

Topic: I.03. Anatomical Methods

Title: A new neuron confocal reflection super-resolution (CRSR) Golgi-staining imaging technique and its application in neuroinflammation studies

Authors: *Y. KHAW;
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Abstract: Metal-based Golgi staining is an established method used to visualize neurons with great morphological detail in slice preparation. While Golgi staining is a staining method of superior clarity, the current confocal reflection visualization method is diffraction limited, meaning that the resolution is limited to 200 nm. Here, we report a confocal reflection super-resolution (CRSR) technique achieved by minimizing the pinhole size of the optical system. With the application of light that has the short wavelength of 405 nm, the CRSR technique results in ~25% lateral and axial resolution improvement. Deconvolution processing further improves resolution quality by an additional ~30%. Importantly, the application of CRSR on Golgi stained central nervous system samples ensures a more accurate characterization of detailed neuronal morphology such as dendritic spine density and dendritic spine characterization based on volumetric classification. An added advantage to the CRSR technique is that the three-dimensional visualization of Golgi-stained neurons can be spatially combined with confocal fluorescence visualization of immunohistochemistry fluorescent signals. This allows us to simultaneously acquire Golgi-staining-derived neuron morphology data in addition to immunohistochemistry-derived biochemical properties of the given slice preparation with precise spatial relevance. The CRSR technique is especially key to our current study in investigating the effects of direct and indirect interactions between neuron and peripheral immune cells in the context of murine experimental autoimmune encephalitis (EAE) which is a mouse model of multiple sclerosis. Multiple sclerosis is an inflammatory demyelinating autoimmune disease of the central nervous system and is the most common cause of neurological disability in young adults. While multiple sclerosis is known for its demyelination capacity, grey

matter pathology has emerged as a key contributor to long-term disability in multiple sclerosis. Specifically, dendritic spine loss has been well documented in the central nervous system of multiple sclerosis patients and as well as animals induced with EAE. Here, we apply the CRSR technique in combination with confocal fluorescence microscopy on Golgi-stained and immunohistochemistry-processed cryoprotected central nervous system samples of C57BL/6L mice subjected to EAE to understand the spatial relevance of infiltrated peripheral immune cells in the central nervous system on dendritic spine abnormalities.

Disclosures: Y. Khaw: None.

Poster

174. Anatomic Methods: Image Acquisition II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 174.21/BB61

Topic: I.03. Anatomical Methods

Support: NIA 5P30AG013846-22
NIA RO1AG057902
VA National Center for PTSD

Title: Overcoming challenges in neuroscience: Multiplex immunofluorescence and autofluorescence isolation in brain tissue using multispectral imaging

Authors: *B. REMENIUK[1], R. MATHIAS[2,3], K. ROMAN[1], C. COLTHARP[1], B. R. HUBER[2,3];
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Abstract: Objective/ Rationale: Brain tissue often presents a significant challenge when trying to analyze, due in large part to autofluorescence (AF) contribution from lipofuscin. Current tissue preparations to minimize AF can be time consuming and potentially damage precious tissue samples. Additional methodologies employing AF subtraction cannot guarantee complete AF removal and may accidentally remove weak expressing markers. Furthermore, conventional fluorescence microscopy techniques are limited to 3-4 markers of interest. Advancements in the field of multispectral imaging (MSI) have expanded the number of distinguishable markers that can be analyzed while removing tissue AF.
Methods: Formalin-fixed paraffin-embedded healthy human brain tissue (Brain Bank, Boston University) was sliced and mounted to microscope slides. Employing the Phenoptics™ workflow, a 6-plex, 7-color panel using common brain markers (Map2, parvalbumin, PLP, GFAP, Iba-1, smooth muscle actin, and DAPI counterstain) was developed and tissue was stained using Opal™, tyramide signal amplified fluorophores, on a BOND RX. Multispectral

digital scans were acquired on a Vectra Polaris® automated imaging system and analyzed with inForm® and phenoptrReports™ software.
Results: Multispectral scanning and unmixing demonstrated greater AF reduction on all fluorophores in a 6-plex, 7-color panel. Isolation and removal of AF lowered the limit of detection for all markers and improving the dynamic range of the emission channels. Signal-to-noise ratio was also greatly improved compared to conventional staining and scanning methods.
Conclusions: This study demonstrates a proof-of-concept in which MSI was successfully employed to overcome the limitations associated with conventional immunofluorescence staining and imaging in brain tissue. Using this novel multispectral scanning method, the number of markers that were able to be captured on a single brain tissue slide was expanded. More importantly, AF was able to be spectrally unmixed, isolated, and removed without using any additional tissue processing steps. This methodology allows for improved workflow and greater interrogation of neuronal interactions occurring within the brain.

Disclosures: B. Remeniuk: None. **R. Mathias:** None. **K. Roman:** None. **C. Coltharp:** None. **B.R. Huber:** None.

Poster

174. Anatomic Methods: Image Acquisition II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 174.22/BB62

Topic: I.03. Anatomical Methods

Support: NIMH Grant 5R44MH116827-02

Title: ClearScope - The complete light sheet theta microscope for cleared tissue imaging and analysis

Authors: *S. J. TAPPAN[1], W. D. PACK[2], D. S. DENU[2], R. TOMER[3], B. W. HAYDOCK[2], C. THOMAS[2], M. GULENKO[2], B. S. EASTWOOD[2], N. D. LIESE[2], P. J. ANGSTMAN[2], J. R. GLASER[2];
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Abstract: Advances in tissue clearing methods and improved Light Sheet Microscopy (LSM) techniques have opened new avenues of research into brain-wide exploration of connectivity patterns and molecular indicators of behavior and pathology. At present, there are no commercially available light sheet microscopes that can image entire large, thick, cleared brain specimens at cellular resolutions. The Tomer laboratory at Columbia University has recently developed the Light Sheet Theta Microscope (LSTM) which utilizes a unique arrangement of two light sheets oblique to the specimen and detector axes. This arrangement allows LSTM to

overcome the lateral dimensional limitation of specimens that can be imaged.
Here we present our ClearScope technology that refines and improves LSTM by creating a robust commercial technology that can be deployed in most neuroscience laboratories for use with virtually all tissue clearing techniques. The physical dimensions of ClearScope are significantly reduced from the original LSTM system by employing oblique compact illumination arms. Two independent laser sources power these illumination arms giving precise control over the illumination of the specimen from each side. Four laser wavelengths are available for multi-channel imaging of the most common fluorescent labels. ClearScope also provides a wide-field fluorescence imaging mode for quickly obtaining an overview image of a specimen and selecting regions of interest to scan at higher resolutions in light sheet mode. ClearScope introduces improved high-speed acquisition and hardware control, including an intuitive user interface. Researchers can efficiently set image acquisition parameters and easily view their specimens. ClearScope acquires, visualizes, and analyzes the large image volumes created by light sheet microscopy without having to sacrifice image resolution via down-sampling.
High-resolution scans were acquired with ClearScope to demonstrate the image quality and performance capabilities of the system. Here we show these images and the hardware and software features of ClearScope while highlighting the capabilities of LSTM over traditional LSM. ClearScope provides efficient imaging of cleared tissues at cellular resolution without a limitation on tissue size.

Disclosures: **S.J. Tappan:** A. Employment/Salary (full or part-time):; MBF Bioscience. **W.D. Pack:** A. Employment/Salary (full or part-time):; MBF Bioscience. **D.S. Denu:** A. Employment/Salary (full or part-time):; MBF Bioscience. **R. Tomer:** None. **B.W. Haydock:** A. Employment/Salary (full or part-time):; MBF Bioscience. **C. Thomas:** A. Employment/Salary (full or part-time):; MBF Bioscience. **M. Gulenko:** A. Employment/Salary (full or part-time):; MBF Bioscience. **B.S. Eastwood:** A. Employment/Salary (full or part-time):; MBF Bioscience. **N.D. Liese:** A. Employment/Salary (full or part-time):; MBF Bioscience. **P.J. Angstman:** A. Employment/Salary (full or part-time):; MBF Bioscience. **J.R. Glaser:** A. Employment/Salary (full or part-time):; MBF Bioscience.

Poster

174. Anatomic Methods: Image Acquisition II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 174.23/BB63

Topic: I.03. Anatomical Methods

Support: R01-EY025231
U01-EY025477
R01-EY028287

P30-EY026877

Title: Label-free imaging of the photoreceptor mosaic in living mouse eye using confocal adaptive optics scanning light ophthalmoscopy

Authors: *N. SREDAR[1], S. K. CHEONG[1], L. LI[1], S. J. STEVEN[2], T. J. KOWAL[1], Y. SUN[1], Y. HU[1], A. DUBRA[1];
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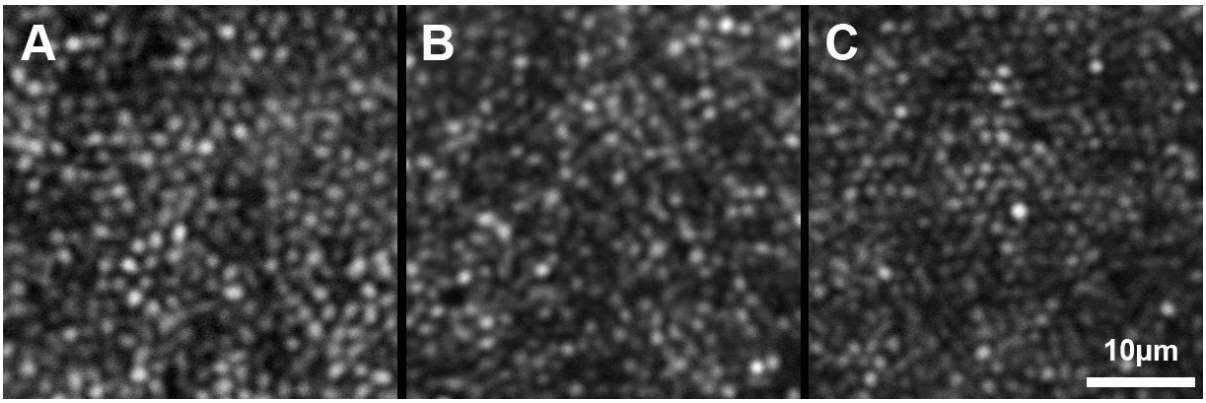
Abstract: Purpose: To demonstrate non-invasive and label-free reflectance imaging of the photoreceptor mosaic in the living mouse retina using a custom adaptive optics scanning light ophthalmoscope (AOSLO).
Methods: 2 male and 1 female C57BL6/J mice that were ~8 weeks old were anesthetized using 0.01 mg xylazine/g and 0.08 mg ketamine/g. Sequences of 200 *en face* images of the photoreceptor mosaic of these mice at the depth of the inner-segment/outer-segment junction were captured using a custom confocal AOSLO in reflectance. The retina was scanned over a 2° field-of-view using a 680 nm superluminescent diode (SLD; 300 μW at the eye) and the single-scattered light was detected through a 0.85 Airy disk diameter confocal pinhole using a photomultiplier tube. The sequence of images was registered and averaged to improve signal-to-noise ratio. Cone centers were manually identified, and the nearest neighbor distance of cone centers were computed. The adaptive optics system consisted of an 850-nm SLD that created a retinal beacon, a custom Shack-Hartmann wavefront sensor with 28 lenslets across 1.5 mm at the mouse pupil that measured the monochromatic aberrations of the mouse eye, and a 97-actuator deformable mirror that corrected these aberrations.
Results: The photoreceptor mosaic in the 3 mice can be consistently resolved (see Figure). Assuming an effective ocular focal length of 1.8 mm, the mean nearest neighbor distance of photoreceptor centers is 1.47 ± 0.14 μm, which is comparable to the diameter of rod outer segments reported in histological studies [1].
Conclusion: Reflectance AOSLO can be used to non-invasively visualize the photoreceptor mosaic in living mice. This imaging has great potential for reducing the number of animals and improving the statistical significance in longitudinal studies of disease models and evaluation of novel therapies.
References: [1] Carter-Dawson, L. D. and Lavail, M. M. (1979), Rods and cones in the mouse retina. I. Structural analysis using light and electron microscopy. J. Comp. Neurol., 188: 245-262.



Disclosures: **N. Sredar:** None. **S.K. Cheong:** None. **L. Li:** None. **S.J. Steven:** None. **T.J. Kowal:** None. **Y. Sun:** None. **Y. Hu:** None. **A. Dubra:** None.

Poster

174. Anatomic Methods: Image Acquisition II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 174.24/BB64

Topic: I.03. Anatomical Methods

Title: Investigation of blood-brain barrier development using atomic force microscopy

Authors: *T. FISCHER[1], B. BRANKIN[2], H. A. MCNALLY[1];
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Abstract: Atomic Force Microscopy (AFM) has been used for numerous biological and biochemistry studies including imaging of DNA, bacteria, viruses and cells. The AFM can also be used to measure mechanical properties such as the unfolding force of membrane proteins. A fine tip at the end of a cantilever is used to sense interaction forces between the tip and the sample. AFM operation is not limited by diffraction allowing for subnanometer resolution in imaging and piconewton force measurements. Operation in liquids make AFMs ideal instruments to investigate biological samples in the living state while in physiologically relevant solutions. The blood-brain barrier (BBB) is the semi permeable tissue regulating the exchange of molecules between the blood and the extracellular fluid of the central nervous system. The BBB is of interest to researchers because of its role in diseases like Multiple Sclerosis, Parkinson's disease and Alzheimer's disease. Atomic force microscopy was utilized in the past during investigations of the distribution and permeability of nanoparticles in and through the BBB.
This AFM investigation of the blood-brain barrier development was conducted on an in-vitro model consisting of human endothelial brain cells. Cells were seeded onto culture dishes and

transwell membranes. AFM samples were fixed daily for two weeks to create a sample set spanning the initial development of the BBB. Optical microscopy and transepithelial/transendothelial electrical resistance (TEER) measurements were used to collect conformational data on the BBB development. Data is presented which describe the structural change of individual cells and their interactions with each other throughout the development of the BBB as confluency is reached. Topographical images of cells and cell groups are provided. Data from AFM force measurements on living models will determine the elasticity of individual cells as well as the forces between cells.

Disclosures: T. Fischer: None. **B. Brankin:** None. **H.A. McNally:** None.

Poster

174. Anatomic Methods: Image Acquisition II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 174.25/BB65

Topic: I.03. Anatomical Methods

Support: NSF Grant 1735252

Title: Diffusion imaging of young and aged rat brain slices on a human clinical MRI scanner

Authors: N. AW[1,2], A. CERJANIC[2,3], ***J. W. MITCHELL**[2,4,5], M. U. GILLETTE[2,3,4,5], B. SUTTON[1,2,3];
[1]Dept. of Electrical and Computer Engin., [2]Beckman Inst. for Advanced Sci. and Technol., [3]Dept. of Bioengineering, [4]Dept. of Cell and Developmental Biol., [5]Neurosci. Program, Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: The use of brain slices is an important tool to perform mechanistic studies in neuroscience. It enables the researcher to isolate aspects of physiological function and to explore the impact of specific signaling pathways, gene promoters, and inhibitors on the structure and function of particular brain regions. Many methods exist to reveal the microstructure of the tissue at the end of the brain slice experiment, such as Nissl staining; however, MRI provides an interesting opportunity to quantify dynamic changes during a longitudinal brain slice experiment. Diffusion tensor imaging (DTI) is frequently used to examine the integrity and myelination of white matter tracts as a function of aging, with decreases in fractional anisotropy (FA) with age in humans (Davis, 2009), but FA increases with age in rats (Yates, 2007). The use of DTI in brain slices enables mechanistic studies of aging, myelination, and the relationship to DTI measures, due to the lack of blood and subject movement as well as end-point histological staining. Given the increasing prevalence of clinical MRI scanners with improved gradient performance, in this work, we examine if DTI can be performed on a brain slice on a clinical MRI scanner. Using a rat coil from Rapid Biomedical and a Siemens 3 T Prisma MRI scanner,

the SNR produced by the diffusion images was adequate to visualize properties of white matter in rat brain slices on a human clinical MRI scanner. Specific brain structures are visible in the FA images that agree with known rat brain atlases, such as the Waxholm Space Sprague Dawley atlas (Papp, 2014). We demonstrated sufficient signal and resolution for detecting differences in brain structure of aging rats, confirming that FA in rat brains increases with age, as previously noted (Yates, 2007). The results indicate that high spatial resolution at a small scale is achievable with a clinical MRI scanner. With the ability to successfully image rat brain slices with diffusion, many opportunities for mechanistic brain slice studies are possible on commonly available clinical scanners.

Disclosures: **N. Aw:** None. **A. Cerjanic:** None. **J.W. Mitchell:** None. **M.U. Gillette:** None. **B. Sutton:** None.

Poster

174. Anatomic Methods: Image Acquisition II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 174.26/BB66

Topic: I.03. Anatomical Methods

Support: NSF Neuronex
McKnight Technical Award for Neuroscience
U01 MH109100

Title: A pipeline from MRI to X-ray to EM for brain imaging

Authors: S. FOXLEY[1], R. VESCOVI[3], V. DEANDRADE[4], A. SOROKINA[5], K. NORWOOD[2], ***N. KASTHURI**[2];
[1]Radiology, [2]Univ. of Chicago, Chicago, IL; [3]Univ. of Chicago/ Argonne Natl. Lab., Chicago, IL; [4]Argonne Natl. Lab., Chicago, IL; [5]Univ. of Chicago/ Argonne Natl. Laborator, Chicago, IL

Abstract: Neuroanatomy is currently studied at orders of magnitude disparate resolutions and volumes: from nanometer reconstructions of small volumes of brains with electron microscopy to millimeter voxel resolution maps of whole brains with magnetic resonance imaging (MRI). Unfortunately, there remains a large gap in our understanding of brain anatomy at the mesoscale - detailing the cellular compositions of entire brains along with the trajectories of the vasculature and the long distance projections of neurons between and within brain regions. Intermediate resolution maps will allow reconciling imaging modalities with disparate resolving powers and describe brain wide differences across animals, development, and disease. In order to address this gap, we describe here a pipeline using small animal MRI (~ 100 micron/voxel resolution), whole mouse brain imaging using synchrotron source X-ray tomography (~ 1 micron/voxel resolution), and automated serial electron microscopy (~ 10 nanometer/voxel resolution) on the

same brains. We conclude that such a multiscale, multimodal pipeline allows for co-registration of these disparate modalities and provides critical orthogonal information across modalities; for example X-ray datasets, which clearly delineate cellular morphology including the shapes and sizes of dendritic arbors and trajectories of myelinated tracts and the vasculature brainwide, can 'ground truth' MRI datasets and provide future nanoscale anatomical biomarkers for disease while MRI can provide the context for the small volumes of brains reconstructed with EM.

Disclosures: S. Foxley: None. **R. Vescovi:** None. **V. DeAndrade:** None. **A. Sorokina:** None. **K. Norwood:** None. **N. Kasthuri:** None.

Poster

174. Anatomic Methods: Image Acquisition II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 174.27/BB67

Topic: I.03. Anatomical Methods

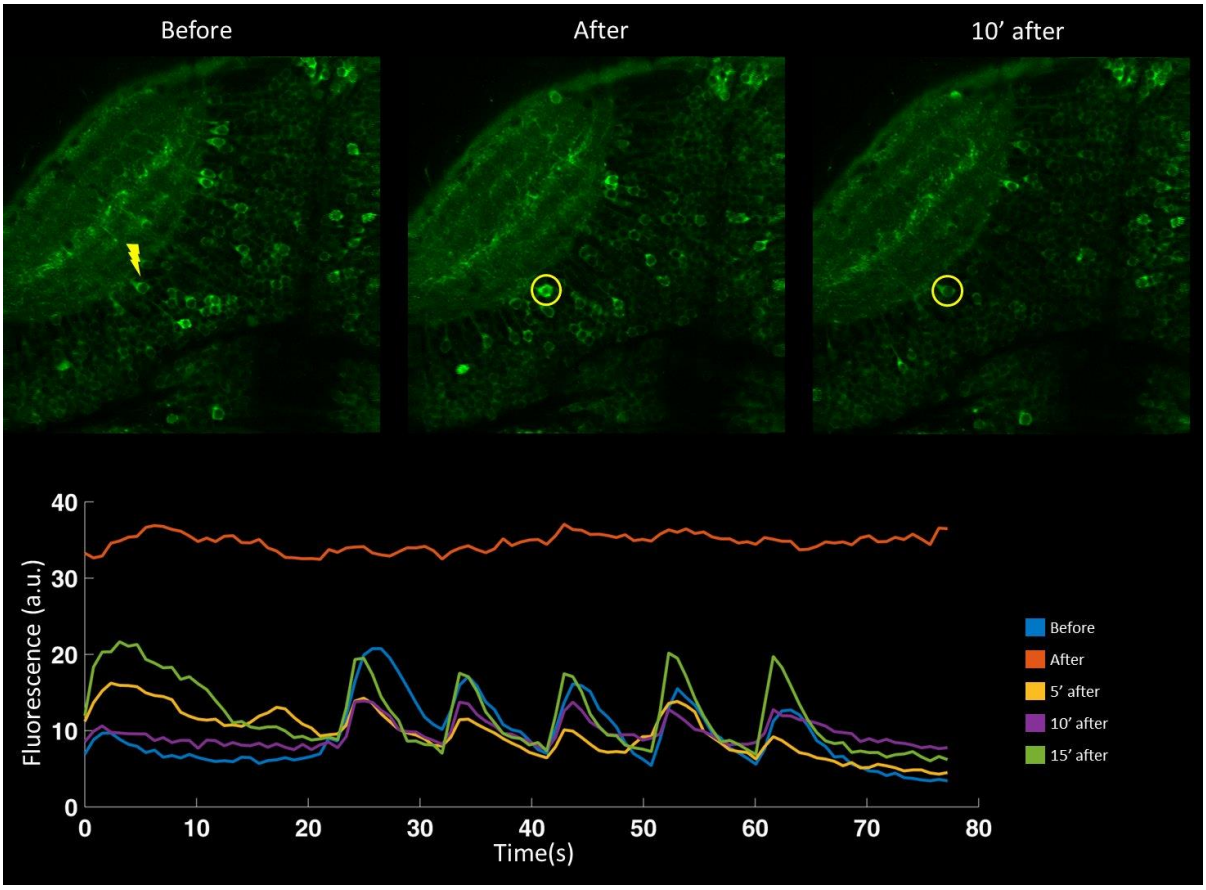
Support: ERC project BrainBit (GA n.692943)

Title: Two photon reversible inactivation and optical highlighting of GCaMP expressing neurons in zebrafish larvae for the study of circuits

Authors: C. FORNETTO[1], L. TURRINI[1], N. TISO[2], ***F. VANZI**[3,1], F. S. PAVONE[1,4]; [1]European Lab. for Non-Linear Spectroscopy, Sesto Fiorentino, Italy; [2]Univ. of Padova, Padova, Italy; [3]Dept. of Biol., [4]Dept. of Physics and Astronomy, Univ. of Florence, Sesto Fiorentino, Italy

Abstract: The transparency and simplicity of the zebrafish larval brain, in combination with the expression of genetically encoded calcium reporters and the development of fast microscopy techniques has led to the possibility of monitoring activity with cellular resolution in the whole brain in real time. The combination of imaging and perturbation techniques (most notably optogenetics) is a powerful tool for the investigation of the circuits involved in response to specific stimuli and information processing. With the intent of investigating the patterns of activity during visual stimulation, and the network connectivity among active cells, we implemented an all-optical method for the temporary inactivation of selected neurons. We demonstrate the use of GCaMP as a reporter of cellular inactivation as indicated by a temporary calcium imbalance in the neuron following high-power irradiation of the cell membrane with the same laser used for two-photon imaging. The figure shows the transient increase in intracellular calcium (possibly due to membrane optoporation at the moment of irradiation), followed by a gradual recovery to the resting level. These results demonstrate a transient loss of function localized specifically in the irradiated cell, without undesired effects on any surrounding cell, thus lending the method to the study of local connectivity. We demonstrate the temporary loss of

function of optic tectum neurons during visual stimulation (the lower panel in the figure shows GCaMP response in the selected neuron before and at different times after irradiation; in each recording the larva is stimulated with five light flashes). The temporary calcium imbalance in the targeted cell also allows tracing its neurites in 3D in neuropil, due to increased fluorescence throughout the cell dendrites and axon. We demonstrate the effectiveness of this approach for a reconstruction of the projections of several neurons in the optic tectum neuropil.



Disclosures: **C. Fornetto:** None. **L. Turrini:** None. **N. Tiso:** None. **F. Vanzi:** None. **F.S. Pavone:** None.

Poster

174. Anatomic Methods: Image Acquisition II

Location: Hall A

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Program #/Poster #: 174.28/BB68

Topic: I.03. Anatomical Methods

Support: EU grant n. 785907 (Human Brain Project)
NIH Grant 1U01MH117023-01

Ente Cassa di Risparmio di Firenze (private foundation)
Eurobioimaging Italian Nodes (ESFRI research infrastructure)
EU grant n. 692943 (BrainBIT)

Title: Quantitative volumetric microscopy of whole mouse brains with a universal autofocus system

Authors: *L. SILVESTRI[1], M. MUELLENBROICH[2], I. COSTANTINI[1], A. DI GIOVANNA[1], G. MAZZAMUTO[1], F. ORSINI[3], A. FRANCESCHINI[1], P. FRASCONI[3], L. SACCONI[1], F. PAVONE[1];
[1]European Lab. For Non-Linear Spectroscopy, Florence, Italy; [2]Sch. of Physics and Astronomy, Univ. of Glasgow, Glasgow, United Kingdom; [3]Dept. of Information Engin., Univ. of Florence, Florence, Italy

Abstract: Light-sheet microscopy is widely used for fast imaging of large clarified specimens, such as entire mouse brains. Albeit in principle this method can easily yield subcellular resolution when a suitable objective lens is used, in practice sample-induced aberrations (mainly defocus) introduce severe blur in the collected images. This significant reduction in resolution prevents effective extraction of quantitative information in general settings, and indeed high-throughput reliable cell counting across the entire brain has been hitherto restricted to specific staining limited to the cell nucleus or soma, or to sparse and strong labeling with viral strategies. Here, we introduce RAPID (Rapid Autofocusing via Pupil-split Image phase Detection), a real-time image-based autofocus system for automated light-sheet imaging of entire mouse brains with subcellular resolution, without any additional optimization time. RAPID-enabled light-sheet microscopy produces high-quality datasets amenable of further quantitative analysis, also in the densely stained volumes typical of standard transgenic labeling, where also axons and dendrites are filled with fluorescent protein. We demonstrate the potential of our method by quantitatively analyzed the full spatial patterning of somatostatin-positive neurons across the entire mouse brain. Our volumetric quantification allows 3D analysis of the arrangement of neurons, highlighting various degrees of spatial clustering in different brain regions.
The inherent scalability of our RAPID-enabled light-sheet microscopy paves the way towards a more comprehensive and unbiased characterization of cytoarchitecture across the entire mouse brain. Our autofocus system is inexpensive and easy to build, and can be introduced into most existing setups. We anticipate that this method could have a significant impact in the community, allowing observing large cleared specimens with reliable quality across the entire sample volume.

Disclosures: **L. Silvestri:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent pending technology. **M. Muellenbroich:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent pending technology. **I. Costantini:** None. **A. Di Giovanna:** None. **G. Mazzamuto:** None. **F. Orsini:** None. **A. Franceschini:** None. **P. Frasconi:** None. **L. Sacconi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent pending technology. **F. Pavone:** E. Ownership Interest (stock, stock

options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent pending technology.

Poster

174. Anatomic Methods: Image Acquisition II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 174.29/BB69

Topic: I.03. Anatomical Methods

Support: EU H2020 grant agreement No. 720270 (Human Brain Project)
EU H2020 grant agreement No. 785907 (Human Brain Project)
EU H2020 grant agreement No. 654148 (Laserlab-Europe)
EU H2020 ERC grant agreement n. 692943 BrainBIT
Italian Ministry for Education, University, and Research (MIUR), Flagship Project NanoMAX
Ente Cassa di Risparmio di Firenze
Italian Ministry for Education, University, and Research (MIUR) in the framework of Eurobioimaging (ESFRI research infrastructure) - Advanced Light Microscopy Italian Node

Title: A software pipeline for image processing and cell segmentation in biomedical microscopy

Authors: *G. MAZZAMUTO[1], F. ORSINI[2], I. COSTANTINI[3,4], M. ROFFILLI[5], P. FRASCONI[2], F. S. PAVONE[1,4], L. SILVESTRI[1,4];
[1]European Lab. For Non-Linear Spectroscopy (LENS), Sesto Fiorentino, Italy; [2]Dept. of Information Engin. (DINFO), Univ. of Florence, Florence, Italy; [3]European Lab. for Non-Linear Spectroscopy (LENS), Sesto Fiorentino, Italy; [4]Natl. Inst. of Optics (INO), Natl. Res. Council (CNR), Sesto Fiorentino, Italy; [5]Bioretics srl, Cesena, Italy

Abstract: In the field of high-resolution biomedical imaging, several challenges arise for what concerns data handling and image processing. In particular, imaging whole organs (e.g. a whole mouse brain) with Light Sheet Fluorescence Microscopy (LSFM) at sub-micron resolution easily results in three-dimensional datasets of several TB in terms of storage. Here we present a software pipeline that we have developed in-house to address the specific needs of efficiently handling such big amounts of data and extract scientifically relevant information out of them (e.g. spatial distribution of cells, etc).
As a first step in the processing pipeline, we perform three-dimensional image stitching using ZetaStitcher, a Python package that we have developed for the specific purpose of stitching such large tomographies in a reasonable time. Indeed, we are able to compute the alignment for a whole mouse brain tomography in less than an hour. The newest version of the package also features an even quicker global optimization algorithm. ZetaStitcher comes with an API that

allows one to query the fused volume in a virtual fashion, so that there is no actual need to produce the fused file and making it possible to feed the whole dataset to the rest of the processing pipeline in small chunks.
Once the alignment of the 3D tiles is computed, we use several techniques based on machine learning for cell segmentation and classification. In the case of a whole mouse brain tomography acquired at the LSFM, we apply semantic deconvolution followed by a mean shift algorithm to reconstruct a point cloud representing the position of each single neuron in the whole brain. One can then perform more complicated studies on this data, such as evaluating cell density and clustering tendencies across the whole volume. In a different kind of sample and experimental setup, i.e. pieces of the human brain cortex imaged with a Two-Photon Fluorescence Microscope, we use a 3-layered Convolutional Neural Network to independently classify each pixel of the images based on the probability for it to be the center of a patch containing the visual pattern of a neuron. A contour finding algorithm is then applied to the heatmaps produced by the classifier to finally obtain an accurate segmentation of the neuronal shape.

Disclosures: **G. Mazzamuto:** None. **F. Orsini:** None. **I. Costantini:** None. **M. Roffilli:** None. **P. Frasconi:** None. **F.S. Pavone:** None. **L. Silvestri:** None.

Poster

174. Anatomic Methods: Image Acquisition II

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 174.30/BB70

Topic: I.03. Anatomical Methods

Support: Human Brain Project - Specific Grant Agreement 2 - agreement n. 785907
Laserlab-Europe 654148
Italian Ministry of Health RF-2013-02355240
Eurobioimaging Italian Nodes (ESFRI research infrastructure)
NIH BRAIN Initiative - Imaging and Analysis Techniques to Construct a Cell Census Atlas of the Human Brain - Grant Number 1U01MH117023-01
Ente Cassa di Risparmio di Firenze (private foundation)

Title: Three dimensional cytoarchitectonic analysis of the human brain

Authors: *I. COSTANTINI[1], G. MAZZAMUTO[2], A. LAURINO[2], E. LAZZERI[2], A. SIMONETTO[3], A. ALLEGRA MASCARO[2], M. ROFFILLI[3], L. SACCONI[1], L. SILVESTRI[2], F. S. PAVONE[2];
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Abstract: Studying the three dimensional architecture of the human neuronal networks in large tissue at subcellular resolution is one of the biggest challenges of our days. Commonly, sampled

slices of the tissue of interest are individually stained and imaged. This approach in addition to being time-consuming does not consider space cell organization and sampling errors, leading, in the best case, to loss of information, and in the worst case to wrong analysis. To overcome 2D imaging's limits, in this study we developed a methodology that allows analyzing the cytoarchitecture of mm^3 of the human brain in three dimensions at high resolution. We successfully integrate the SWITCH immunohistochemistry technique (Murray et al. 2015) with the TDE clearing method (Costantini et al. 2015) to image tissues from different subjects with two-photon fluorescence microscopy. Quantitative analysis of brain cytoarchitecture of the different samples was performed using a machine learning approach that allows an automatic segmentation of neurons in three-dimension with high specificity and sensitivity. The identification and localization of the neurons obtained with this new approach enable to characterize and classify large human brain specimens with a high-resolution optical technique, giving the possibility to expand the histological studies to the third dimension.

Disclosures: I. Costantini: None. **G. Mazzamuto:** None. **A. Laurino:** None. **E. Lazzeri:** None. **A. Simonetto:** None. **A. Allegra Mascaro:** None. **M. Roffilli:** None. **L. Sacconi:** None. **L. Silvestri:** None. **F.S. Pavone:** None.

Poster

175. Drug Delivery

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 175.01/BB71

Topic: I.05. Biomarker and Drug Discovery

Support: DFG-funded Priority Programme SPP 1926 (Next Generation Optogenetics), grant number RU 869/5-1

Title: Silicon probes with buried channels for simultaneous neural recording and drug delivery

Authors: *K. SHARMA, S. D. N. KUMAR, O. PAUL, P. RUTHER;
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Abstract: State-of-the-art tools for a controlled drug delivery into the brain are steel or glass capillaries. Their large size and lack of multifunctionality has necessitated the development of multifunctional neural probes based on microelectromechanical systems (MEMS) technologies. This work reports on the fabrication and characterization of thin silicon (Si) probes with integrated channels and recording electrodes, that hence combine drug delivery and electrophysiological recording functionalities in a single device. Buried channels are realized using vapor-phase, continuous-flow (cf) xenon difluoride (XeF_2) etching of Si through small openings of a masking layer and their subsequent sealing using chemical vapor deposition (CVD) processes. Interconnecting lines and electrodes are deposited and patterned across the

sealed channels. In a final step, probes are thinned to 50 µm using the etching-before-grinding approach. Process characteristics of the integrated channel, e.g XeF_2 etch rate, channel profile, and surface roughness are studied by varying the XeF_2 process parameters and etch mask design. Best etch uniformity and surface quality are obtained at an etch pressure of 2 Torr and an N_2 flow rate of 150 sccm. Depending on the size of the mask openings, etch rates vary from 1.1 µm/min to 4.3 µm/min. Smaller etch openings with a width of 2 µm and a length of 4 µm were found to be optimal for processing stable and smooth channels. Probes with shank lengths up to 24 mm and widths smaller than 165 µm comprising one or two fluidic channels and 32 electrodes are fabricated. Electrical interconnection is achieved by flexible polyimide cables bonded to the probe base while thin polytetrafluorethylene (PTFE) tubings are attached to the fluidic inlets ports. The impedances of IrO_x electrodes (diameter 30 µm) are measured in saline solution to be (35.9±11.8) kΩ at 1 kHz. Water flow through the channels is successfully demonstrated. The hydrodynamic characterization revealed a linear relationship between the applied differential pressure and flow rate, with a slope of 170 nl $min^{-1}bar^{-1}$ for a 15-µm-wide and 9.6-mm-long channel. The electrochemical and fluidic characterization indicates the suitability of the multifunctional probes for in vivo applications, where they can aid in decoding complex neural networks and exploring treatment possibilities for various neural disorders.

Disclosures: K. Sharma: None. **S.D.N. Kumar:** None. **O. Paul:** None. **P. Ruther:** None.

Poster

175. Drug Delivery

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 175.02/BB72

Topic: I.05. Biomarker and Drug Discovery

Title: Development of a high content imaging assay in blood-brain barrier *in vitro* model to monitor protein internalization and transcytosis

Authors: *Q. CHENG[1], M. SINGH[2], D. WAKEFIELD[2], C. HALE[2], D. SIMSEK BUCK[3], X. LUO[2], O. HOMANN[2], S. WANG[4], S. CHAMBERS[2];
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Abstract: Applications of imaging techniques allows us for detailed analyses of transcytosis in brain endothelial cells. We sought to develop a robust, high content image- based in vitro transcytosis assay, in order enabling us to understand the pathways and sorting mechanisms of transcytosis in blood-brain barrier endothelial cells, and to help us to screen and develop targeted generation of “brain shuttle” molecules.
We established an in vitro model system of transcytosis using the hCMEC/D3 immortalized human brain endothelial cell line using a pH sensitive dye / FITC dual labeled anti-transferrin

receptor (TfR) antibody and a pH sensitive dye / Alexa 488 dual labeled transferrin protein (Tf). In this assay, we were able to monitor the protein internalization and whether their destination will involve lysosomale degradation. We also developed an algorithm to analyze the protein movement inside the cells from apical to basolateral for more quantitative, results allowing a higher throughput analysis. Next, we chose to move to a more relevant human in vitro BBB model system using iPSC-derived brain microvascular endothelial like cells (iPSC-dBMECs). The iPSC-dBMECs express many of the expected markers including TJP1 (ZO-1), SLC2A1 (GLUT1), CLDN5, and OCLN, and PECAM-1 (CD31) by immunofluorescence or flow cytometry. When cultured in a trans-well assay, we can reproducibly achieve trans-endothelial electrical resistance (TEER) values above 800. TEER values increase as high as 1500 in the presence of both human brain vascular pericytes and human astrocytes demonstrating increased barrier function. Nearly similar barrier function is observed when the iPSC-dBMECs are cultured with only pericytes, suggesting pericytes alone contributed to much of the increase in barrier integrity. Gene expression analysis of iPSC-dBMECs was performed in the presence of astrocytes and/or pericytes to better understand mechanistically what the relative contribution of each cell type to iPSC-dBMEC barrier formation could be. Finally, multiple approaches were explored and optimized to successfully isolate primary endothelial cells (EC) from mouse brain micro-vessels with the aim to identify novel targets for transcytosis. MACS technologies were applied to further enrich the EC from the tissue prep. We are now combining the iPSC-dBMECs with the
novel image-based target-agnostic transcytosis assay as a proof-of-concept with the purpose to evaluate BBB targets in the primary endothelial cells that could be exploited for RMT.

Disclosures: Q. Cheng: None. **M. Singh:** None. **D. Wakefield:** None. **C. Hale:** None. **D. Simsek Buck:** None. **X. Luo:** None. **O. Homann:** None. **S. Wang:** None. **S. Chambers:** None.

Poster

175. Drug Delivery

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 175.03/BB73

Topic: I.05. Biomarker and Drug Discovery

Support: Marie Sklodowska-Curie Actions
EraNet Neuron TRAINS

Title: Ultrabright lipid droplets light novel opportunities for targeted drug delivery in brain trauma

Authors: *I. KHALIN[1], A. NAGAPPANPILLAI[2], F. HELLAL[1,3], A. KLYMCHENKO[2], N. PLESNILA[1,3];
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Munich, Germany; [2]Lab. de Biophotonique et Pharmacologie, UMR CNRS 7021, Univ. of Strasbourg, Strasbourg, France; [3]Cluster for Systems Neurol., Munich, Germany

Abstract: Nanoscale delivery systems represent a promising research field for the development of new clinical and diagnostic tools. However, delivery of molecules across the blood-brain barrier (BBB) appears to be an unsolved challenge. Lipid nano-droplets (nano-objects with an oil core surrounded by lipidic surfactant) are of particular interest due to their safety, moreover the oily core of the lipid droplets (LD) is a perfect reservoir for the encapsulation of lipophilic molecules. In this study, the opening of the BBB after brain injury has been exploited to investigate the possibility to use LD as a drug carrier for the treatment of brain diseases. We designed LD loaded with cationic rhodamine dye along with the hydrophobic tetraphenyl borate counterions. Control mice prepared with open cranial window and mice subjected to controlled cortical impact (CCI) received LD intravenous injection prior imaging or 1 hour after trauma. We characterized the kinetics and biodistribution of LD using 2-photon *in vivo* imaging, immunohistochemistry and confocal microscopy. Loaded LD provided an excitation energy transfer between the fluorophores resulting in collective quenching and enabling super-brightness optimal for long-term 2-photon *in vivo* imaging. In control mice, we found that over two hours the LD remained stable in the blood circulation without extravasation into the brain parenchyma, as confirmed by confocal microscopy. Moreover, analysis of the liver showed negligible LD incorporation into the liver's perivascular macrophages than FITC-dextran (2000 kDa) suggesting minimal uptake by the reticuloendothelial system. In CCI mice, 30 minutes after the injection, LD were found in the brain parenchyma in the lesion site extravasating from the capillary micro clots as identified by erythrocyte markers. Additionally, we observed LD-positive pericytes around the clots suggesting an extravasation path occurring via these cells. 2 hours after injection, LD were uptaken predominantly by neurons not only within the lesion, but also in the peri-lesion area. Interestingly, we did not observe any incorporation in the microglia. As such, we propose a robust, super-bright and safe platform based on fluorescent for *in vivo* optical imaging of brain trauma and a precise tracking of the nanoparticle kinetics *in vivo* and *ex vivo*. LD are stable in the circulation and uptaken by neurons paving the way for brain-targeted drug delivery in brain injury. Currently we are further characterizing the LD drug-release in the context of traumatic brain injury.

Disclosures: I. Khalin: None. **A. Nagappanpillai:** None. **F. Hellal:** None. **A. Klymchenko:** None. **N. Plesnila:** None.

Poster

175. Drug Delivery

Location: Hall A

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Program #/Poster #: 175.04/BB74

Topic: I.05. Biomarker and Drug Discovery

Support: Council of Scientific & Industrial Research, New Delhi (India) grant number 09/140(0175)/2019-EMR-I.

Title: Lipid based nano-formulation of efavirenz: A strategy to eradicate viral sanctuaries from the brain

Authors: *S. SETHI, V. RANA;
Dept. of Pharmaceut. Sci. & Drug Res., Punjabi Univ., Patiala, India

Abstract: Changes in memory, concentration, attention, and motor skills are common in HIV-infected patients. When not clearly attributable to an alternate cause other than HIV infection, such neurocognitive impairments have been collectively classified as HIV-associated neurocognitive disorders (HAND). The main therapeutic approach to HAND is antiretroviral therapy (ART) but these drugs are ineffective to eradicate the virus from brain mainly due to low penetration across the blood-brain barrier, leading to HIV-encephalitis and antiretroviral drug resistance. Therefore, a novel approach for augmenting the delivery of ART has long been warranted. The objective of this study was to develop and evaluate efavirenz loaded lipid formulation with an aim to enhance brain penetration. It is formulated by maisine 35-1 (20%), transcutol HP (17.7% w/w) and cremophor RH 40:Labrasol (1:1) (62.3% w/w) by employing D-optimal mixture design. Three different formulations (EF1 formulation, EF1 suspension, and Lamivir marketed formulation) each containing 40 mg/Kg efavirenz were administered to adult Wistar rats of either sex (n=6). Drug permission across the blood-brain barrier was assessed through the human brain microvessel endothelial cell line (hCMEC/D3; representative of the blood-brain barrier) and gamma scintigraphy imaging. The in-vivo biodistribution studies show significantly higher concentration ($P < 0.05$) of efavirenz in brain administered with EF1 formulation as compared to suspension and marketed formulation groups. The cell line and gamma scintigraphy imaging conclusively demonstrated higher drug transport in the brain of rats administered with EF1 formulation as compared to suspension and marketed formulation. Collectively, these findings suggest that lipid-based nanoformulation may enhance brain delivery of the potent and frequently used antiretroviral drug efavirenz.

Disclosures: S. Sethi: None. **V. Rana:** None.

Poster

175. Drug Delivery

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 175.05/BB75

Topic: I.05. Biomarker and Drug Discovery

Title: Bolus volume effect on the distribution and silencing of intrathecally delivered siRNA conjugates in the central nervous system

Authors: *J. A. GILBERT, S. LEBLANC, S. AGARWAL, S. MILSTEIN, E. FISHILEVICH, T. NGUYEN, R. DEGAONKAR, A. CASTORENO, S. CHIGAS, J. NAIR, V. JADHAV, M. MAIER, M. JANAS, K. BROWN;
Alnylam, Cambridge, MA

Abstract: Diseases of the central nervous system (CNS) represent some of the highest unmet medical need and greatest therapeutic challenges. Multiple diseases of the CNS have been associated with dominant mutations, making them suitable candidates for an RNA interference (RNAi)-based approach. However, accessing the CNS broadly and effectively has been a challenge. Our recent work that combines stable siRNA designs with ligand conjugation strategies enables intrathecal delivery that yields robust and long-lasting silencing across the CNS in rodents. We show that in rats a single intrathecal bolus of conjugated short interfering RNA (siRNA) targeting the ubiquitously expressed *superoxide dismutase 1* (*SOD1*), leads to silencing of the target transcript throughout the brain and spinal cord. To explore the effects of bolus size on siRNA efficacy and distribution, we dosed rats with a single intrathecal bolus of 30 µL or 10 µL, each containing 0.3 mg of siRNA, and collected tissues 7 days and 14 days post dose. Comparable knockdown of the target mRNA was achieved across the dose volumes and timepoints in all tested regions of the brain and spinal cord. These results suggest that at the tested dose, bolus volume does not significantly affect the distribution and knockdown efficacy of siRNAs in rodent CNS.

Disclosures: J.A. Gilbert: None. **S. Leblanc:** None. **S. Agarwal:** None. **S. Milstein:** None. **E. Fishilevich:** None. **T. Nguyen:** None. **R. Degaonkar:** None. **A. Castoreno:** None. **S. Chigas:** None. **J. Nair:** None. **V. Jadhav:** None. **M. Maier:** None. **M. Janas:** None. **K. Brown:** None.

Poster

175. Drug Delivery

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 175.06/BB76

Topic: I.05. Biomarker and Drug Discovery

Support: RTI Internal Research and Development

Title: Discovery of a small molecule antagonist scaffold for the relaxin-3/RXFP3 System

Authors: C. JIN[1], ***E. A. GAY**[1], K. M. MATHEWS[1], D. F. LOVELOCK[2], J. BESHEER[3,2];
[1]Ctr. for Drug Discovery, Res. Triangle Inst., Research Triangle Park, NC; [2]Bowles Ctr. for Alcohol Studies, [3]Dept. of Psychiatry, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Relaxin-3 is a newly identified neuropeptide, belonging to the relaxin/insulin superfamily. The cognate receptor of relaxin-3 is RXFP3 (formally GPR135), a class A GPCR

coupled to $G\alpha_{i/o}$ proteins. Relaxin-3 is expressed predominantly in the brainstem nucleus incertus (NI) GABAergic neurons that project to a broad range of RXFP3-rich forebrain areas. Emerging evidence suggests that the relaxin-3/RXFP3 system modulates a number of physiological processes including stress response, feeding, motivation for reward, and circadian rhythm. Compounds that can modulate the relaxin-3/RXFP3 system, particularly antagonists, have the therapeutic potential to treat several diseases such as stress-associated disorders, obesity, and alcohol addiction. To our knowledge, small molecule RXFP3 antagonists have not been disclosed. In this poster, we present the discovery of the first small molecule antagonists at the RXFP3 receptor through a high throughput screening campaign. Focused structure-activity relationship studies of the hit compound have resulted in RTI-RLX-33 that has a submicromolar potency and is at least 100-fold selective for RXFP3 over RXFP1. RTI-RLX-33 is a promising lead for the development of antagonist probes to further characterize the nature of central relaxin-3/RXFP3 functions.

Disclosures: C. Jin: None. **E.A. Gay:** None. **K.M. Mathews:** None. **D.F. Lovelock:** None. **J. Besheer:** None.

Poster

175. Drug Delivery

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 175.07/BB77

Topic: I.05. Biomarker and Drug Discovery

Title: Anesthetic complications during surgery in a juvenile nonhuman primate model for safety assessment of therapeutics with CNS indications

Authors: L. D. KRUEGER[1], M. P. BRADLEY[2], J. W. VEENSTRA[2], S. L. ADRIAN[2], M. D. JOHNSON[3], *B. W. GUNTER[3];
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Abstract: Physiologic differences exist between adult and pediatric patients that can result in different therapeutic safety profiles which must be assessed preclinically. Juvenile animals are important for evaluating therapeutics for the clinical pediatric population and considerations for their use during the drug discovery process are discussed in regional and international guidelines. Some therapeutics with CNS indications have limited bioavailability and efficacy after delivery via traditional routes of administration. Thus, it is necessary to surgically administer the therapeutic to the CNS under anesthesia. Nonhuman primates (NHPs) display functional, developmental, and anatomic similarity to humans and are often chosen as the juvenile animal model for safety assessment of therapeutics for CNS indications. However, utilization of this model for targeted surgical delivery of the therapeutic in the CNS poses unique challenges due to

the size and age of the animals. In several preclinical neurosurgeries conducted at our facility, we have observed peri- and post-anesthetic complications that may arise during surgery in juvenile cynomolgus macaques (*Macaca fascicularis; n= 26 male, 16 female;* age = 9-12 mo; weight = 1.0-2.0 kg). The noted complications include hypoventilation and apnea, cardiac and respiratory arrest, hyperkalemia, hypoglycemia, and emesis. We sought to assess and refine the anesthetic protocol to reduce the incidence of procedural complications and, ultimately, experimental variability. Possible refinements to these procedures included swapping anesthetic delivery systems, using benzodiazepines in the pre-medication, adding dextrose to the fluid therapy, and administering an antiemetic prior to recovery. Several of these refinements have been implemented in ongoing and in future targeted drug delivery studies utilizing juvenile NHPs for safety assessment of therapeutics with CNS indications.

Disclosures: **L.D. Krueger:** None. **M.P. Bradley:** A. Employment/Salary (full or part-time):; Charles River Laboratories. **J.W. Veenstra:** A. Employment/Salary (full or part-time):; Charles River Laboratories. **S.L. Adrian:** A. Employment/Salary (full or part-time):; Charles River Laboratories. **M.D. Johnson:** A. Employment/Salary (full or part-time):; Charles River Laboratories. **B.W. Gunter:** A. Employment/Salary (full or part-time):; Charles River Laboratories.

Poster

175. Drug Delivery

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 175.08/BB78

Topic: I.05. Biomarker and Drug Discovery

Support: NIH Grant MH103708
NIH Grant AA026820

Title: Design, synthesis and pharmacological characterization of GPR88 agonists with improved metabolic stability

Authors: C. JIN[1], D. A. PERREY[1], M. T. RAHMAN[1], *A. M. DECKER[1], T. L. LANGSTON[1], W. MA[2], E. DARCQ[2], B. L. KIEFFER[2];
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Abstract: GPR88 is an orphan G-protein-coupled receptor (GPCR) and has a high expression in both dorsal and ventral areas of the striatum. GPR88 has also been found in other regions of the brain, including the cerebral cortex, amygdala, and hypothalamus. In the striatum, GPR88 is expressed at postsynaptic sites in medium spiny neurons (MSNs) of both direct and indirect pathways. A number of studies using GPR88 knockout (KO) mice have suggested that genetic

ablation of GPR88 induces a state of hypersensitivity to the dopamine system, and the receptor is a promising drug target for a number of disease states such as schizophrenia and drug addiction. We have previously reported the discovery of the first potent, selective, and brain-penetrant GPR88 agonist RTI-13951-33 that significantly reduced alcohol self-administration and alcohol intake in rats without effects on locomotion and sucrose self-administration when administered intraperitoneally. In the pharmacokinetic (PK) studies, RTI-13951-33 demonstrated a moderate oral bioavailability and a short half-life. Therefore, further optimization is required to obtain a more drug-like probe to understand the GPR88 system for both acute and chronic effects. This poster presents the synthesis, pharmacological characterization, and *in vitro* ADME of RTI-13951-33 analogs that are designed to block the metabolically vulnerable sites in the aim of enhancing metabolic stability.

Disclosures: C. Jin: None. **D.A. Perrey:** None. **M.T. Rahman:** None. **A.M. Decker:** None. **T.L. Langston:** None. **W. Ma:** None. **E. Darcq:** None. **B.L. Kieffer:** None.

Poster

175. Drug Delivery

Location: Hall A

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Program #/Poster #: 175.09/BB79

Topic: I.05. Biomarker and Drug Discovery

Support: Central Michigan University Neuroscience program
Central Michigan University College of Medicine
Central Michigan University Department of Psychology
Central Michigan University Department of Chemistry and Biochemistry
Central Michigan University Field Neurosciences Institute
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American Heart Association 18AIREA33990094

Title: Investigation of safety profile and potential diapedesis of G4 PAMAM dendrimers across the blood brain barrier following systemic injection in healthy rats

Authors: *M. R. RESK[1,2], M. M.-M. ANDREWS[1,2,3], B. SRINAGESHWAR[1,2,3], A. K. TOTH[1,2], S. KONERU[1,2], J. GALLIEN[1,2], D. SWANSON[4], G. L. DUNBAR[1,2,5,6], A. SHARMA[4], J. ROSSIGNOL[1,2,3];
[1]Field Neurosciences Inst. Lab. for Restorative Neurol., [2]Program in Neurosci., [3]Col. of Med., [4]Dept. of Chem. & Biochem., Central Michigan Univ., Mount Pleasant, MI; [5]Field Neurosciences Inst., Central Michigan Univ., Saginaw, MI; [6]Dept. of Psychology Central Michigan Univ., Central Michigan Univ., Mount Pleasant, MI

Abstract: Dendrimers are nanostructured macromolecules, made up of artificial polymers that help carry therapeutic drugs and DNA in biomedical fields. Dendrimers exist in generations (G), referring to the amount of branching; as the generations increase with size. Only generation 4 dendrimers, or larger, are 3-dimensional and have the potential to entrap/encapsulate drugs and biomolecules. Therefore, we chose to use a generation 4 (G4) dendrimer for this study. The basic structure of a dendrimers surface has a high number amines (100%) which increases the toxicity both *in vitro* and *in vivo* due to the high positive charge. However, with the modification of the surface groups, the toxicity can be decreased. These dendrimers have been modified to have 90% hydroxyl and 10% amine surface groups to decrease the toxicity of the dendrimers on cells by reducing the total amounts of positive charge. Currently, dendrimers are being tested as a method of biomolecule (large plasmids) and drug delivery via intracranial injections. The invasive nature of this method limits the clinical application for potential therapies, shifting the current focus to determining if these studies can give dendrimers via a systemic injection instead. The purpose of this study is to look at the ability for the G4 dendrimer nanoparticles to cross the blood brain barrier (BBB) and colocalize with various cell types in healthy rat brain when given as a single injection or as multiple injections via the tail vein. We also investigated if any apoptotic markers resulted from these systemic injection, both in the brain and the peripheral organs. In this study, 45 day old rats were given a lateral tail vein injection of dendrimer, either at one time point (6μg/g; n=8) or at three separate time points (2μg/g; n=8), every other day. After 7 days, the animals were sacrificed and organs were collected for further testing. Our results showed that the dendrimers are crossing the BBB and are taken up by neurons and glial cells. We also observed, via the *In Vivo* Imaging System (IVIS), that the dendrimers accumulated in the peripheral organs following injection, including liver and kidneys in addition to the dendrimers found in the brain.

Disclosures: M.R. Resk: None. **M.M. Andrews:** None. **B. Srinageshwar:** None. **A.K. Toth:** None. **D. Swanson:** None. **G.L. Dunbar:** None. **A. Sharma:** None. **J. Rossignol:** None. **S. Koneru:** None. **J. Gallien:** None.

Poster

175. Drug Delivery

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 175.10/BB80

Topic: I.05. Biomarker and Drug Discovery

Support: FDN 154272

Title: Influence of tight junction protein density on focused ultrasound-mediated blood-brain barrier permeability enhancement measured by dynamic contrast-enhanced MRI

Authors: *D. MCMAHON[1,2], K. HYNYNEN[1,2];
[1]Physical Sci., Sunnybrook Res. Inst., Toronto, ON, Canada; [2]Med. Biophysics, Univ. of Toronto, Toronto, ON, Canada

Abstract: Focused ultrasound (FUS), in conjunction with circulating microbubbles (MBs), can be used to enhance blood-brain barrier (BBB) permeability in a targeted and transient manner, providing an avenue for the delivery of therapeutic agents from systemic circulation into the brain. Preclinical research has demonstrated efficacy in a variety of disease models, however, detailed understanding of the biological factors that influence cerebrovasculature permeability following FUS+MB exposure is lacking. Tight junction (TJ) protein trafficking away from interendothelial clefts has been observed 1-2 hours following sonication, accompanied by increased paracellular leakage. While this observation implicates TJ integrity in FUS+MB-mediated BBB permeability enhancement, it is unclear how pre-sonication TJ protein density influences the subsequent effects of exposure. This is especially relevant for FUS+MB exposures in the context of diseases where TJ protein density is altered, such as for glioblastoma. To investigate the role of TJ protein density on post-sonication BBB permeability, rats were treated with dexamethasone (DEX; n = 5) or saline (n = 5) for 3 days prior FUS+MB exposure. The dose of DEX administered (3 mg/kg/day, ip) in this study has previously been shown to increase the expression of occludin and to reduce BBB permeability in rats. Each animal was sonicated in 6 locations using a single element focused transducer (580 kHz). Pressure was calibrated based on ultraharmonic emissions. BBB permeability (K^{trans}) was assessed by dynamic contrast-enhanced MRI. Results indicate that the correlation between second harmonic emissions - a measure of MB activity - and the K^{trans} of gadolinium in DEX-treated animals was significantly different than in saline-treated animals. At equal magnitudes of exposure-averaged second harmonic emissions, K^{trans} was higher in DEX-treated rats. It is unclear from this result whether second harmonic emissions are dampened in DEX-treated animals, perhaps due to a stiffening of vasculature, or if the response of vasculature to MB stimulation is altered, possibly relating to increased TJ protein density. This result, however, highlights the importance of considering the influence of biological factors on FUS+MB-mediated BBB permeability enhancement.

Disclosures: D. McMahon: None. **K. Hynynen:** None.

Poster

175. Drug Delivery

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 175.11/DP14/BB81

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Topic: I.05. Biomarker and Drug Discovery

Support: Picower Postdoctoral Fellowship
The Department of Anesthesia, Critical Care and Pain Medicine at MGH
Institute for Medical Engineering and Sciences
Department of Brain and Cognitive Sciences
Picower Center for Learning and Memory MIT
NIH Director's Pioneer Award
NIH Director's Transformative Research Award

Title: A non-human primate model for closed-loop control of propofol-induced unconsciousness

Authors: *S. CHAKRAVARTY[1], J. A. DONOGHUE[2], A. WAITE[1], M. K. MAHNKE[1], I. C. RICE[3], E. K. MILLER[1], E. N. BROWN[4];
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Abstract: General anesthesia is a drug-induced reversible state characterized by antinociception, unconsciousness, amnesia and akinesia along with maintenance of physiological stability. The anesthetic agents maintain these behavioral states by binding to the specific receptors and inducing oscillations that disrupt communication among brain regions. The oscillations are specific to the mechanism of action of the anesthetic. For example, the target receptors for the widely used anesthetic propofol, are $GABA_A$ receptors. Hence, the anesthetic state can be controlled by controlling oscillatory dynamics. We have previously reported closed-loop control of the anesthetic state of burst suppression using propofol in a rodent model. In order to develop a system that is more closely applicable to humans, we have developed a closed-loop anesthesia delivery (CLAD) system in a non-human primate (rhesus macaque) model using propofol. The oscillatory dynamics induced by propofol are tracked in real time from local field potentials (LFPs) recorded from the pre-frontal cortex. The system consists of a computer controlled infusion pump, the control algorithm and the LFP recording system. We defined as our control target the power in the 12-34 Hz band of the LFP. The LFP is recorded in real time and the 12-34 Hz power is computed. We first tested manual maintenance of the target near the specified target levels. We then let the CLAD system take over to maintain closed-loop control. The propofol infusion rate is adjusted up or down automatically by the controller depending on the difference between the actual and the target value of the 12-34 Hz power. In the closed-loop mode, the CLAD system tracked the target with minimal error. We successfully repeated the same experiment using closed-loop control, preceded by open-loop control, 3 times. Each time, we maintained close-loop control for a minimum of 70 minutes. These results establish the feasibility of maintaining CLAD for humans.

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options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MGH has licensed intellectual property for EEG monitoring co-developed by Dr. Brown to Masimo. Dr. Brown hold interests in PASCALL, a start-up company.

Poster

175. Drug Delivery

Location: Hall A

Time: Sunday, October 20, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 175.12/BB82

Topic: I.05. Biomarker and Drug Discovery

Title: MRI-guided injections in the brain: A method for gene therapy delivery for NHP and humans

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Abstract: Selective gene delivery is a powerful approach for treatment of currently incurable diseases including several rare and orphan diseases. Huntington disease (HD) is an autosomal dominant neurodegenerative disorder caused by an expanded polyglutamine repeat in the huntingtin (HTT) protein, affecting numerous cellular processes. Gene silencing of mutant HTT can be a beneficial therapeutic strategy. The gene silencing therapy developed by uniQure is based on a microRNA targeting human HTT delivered with adeno-associated viral vector serotype 5 (AAV5-miHTT) directly into affected brain structures by MRI-guided CED delivery. The Renishaw drug delivery system (tooling and planning software (neuroinspire™)) was used for MRI-guided convection-enhanced delivery (CED) for gene therapy delivery to the striatum in cynomolgus macaques. Animals were mounted in a stereotaxic frame, and T1 and T2 weighted anatomical scans were performed at 3 Tesla. Animals remained in the fixation frame and were transferred to the surgical room. In parallel, the surgical trajectories were calculated based on the MRI. After removal of the fiducial arc, a CRW stereotactic frame was attached to the fixation frame. Using the derived coordinates, the animals were surgically implanted with bilateral microstep catheters. After implantation, the wound was temporarily sutured allowing access to the connecting port. The CRW frame was removed from the fixation frame and the animal was transferred back to the MR scanner where the infusion lines were connected to the port. Target acquisition was confirmed by a post-implant scan. All catheters and infusion pump were started simultaneously. Infusion rates were gradually increased from 0.8 µl/min to 3 µl/min in 40 minutes and were then maintained at this rate. In parallel, MR scans of approximately 5 min duration were repeated throughout infusions. A total of 100 µl of AAV5-miHTT was injected in

each targeted brain structures. A final MR scan was performed to document the test material formulation distribution in the brain. The injection method as well as the AAV5-miHTT treatment were well tolerated. Administration of AAV5-miHTT into the striatum showed widespread distribution of the vector in the striatal and cortical structures, brain regions affected by Huntington disease. Follow-up studies demonstrated that the injection method could be successfully performed with a mobile 1.5 T MRI scanner and that AAV5-miHTT was well tolerated in a GLP biodistribution and toxicity study. As such, the method is also available for drug discovery and safety organizations without access to an in-house MR scanner. The method is translatable for application in humans.

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Poster

175. Drug Delivery

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Program #/Poster #: 175.13/BB83

Topic: I.05. Biomarker and Drug Discovery

Support: Health Research Council of New Zealand grant
Brain Research New Zealand PhD scholarship

Title: Investigating enhanced gene transfer to the mouse central nervous system using modified viral vectors

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Abstract: Methods that effectively deliver therapeutics to the central nervous system (CNS), particularly minimally invasive methods, are crucial for the treatment of neurological diseases.

Adeno-associated viral (AAV) vectors used in gene therapy are an attractive approach as some vectors can cross the blood-brain barrier, allowing them to be administered via systemic routes. A novel AAV vector recently evolved *in-vivo*, AAV-PHP.eB, has been reported to produce more effective CNS transduction than existing AAV vectors in some strains of mice, but not in others. Here, we compared the efficacy of two AAV vectors, AAV-PHP.eB and AAV9, in targeting mouse CNS and peripheral tissues after administration via various routes, and in two different mouse strains. C57BL/6 mice were administered a combination of AAV-PHP.eB and AAV9 encoding different coloured fluorescent reporter proteins, either by intravenous injection (n = 6), intranasal injection (n = 6), or intrahippocampal injection (n = 5). B6C3 mice (n = 4) also received the same vectors via intravenous injection. Four weeks after vector administration, tissue sections were examined for reporter protein expression and cell-type transduction. We observed that in C57BL/6 mice, AAV-PHP.eB was more effective at transducing CNS tissues than AAV9, but was less effective at transducing peripheral tissues. The two vectors showed similar, low efficacy at transducing the CNS in B6C3 mice. Both vectors showed similar efficacy after intranasal injection, where the vectors did not spread beyond the olfactory bulb, and intrahippocampal injection. AAV-PHP.eB transduced more neuronal than glial cells. Our results indicate that intravenous administration of modified viral vectors for CNS gene therapy may be a viable method for overcoming the challenges of invasive administration. However, there appears to be critical blood brain barrier differences across mouse strains that determine the way these modified vectors are transported from the bloodstream to the CNS. Caution will be required when considering translation of these vectors to primate and human studies.

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