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Poster

PSTR381. Synaptic and Cellular Mechanisms of Autism I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR381.01/A1

Topic: A.07. Developmental Disorders

Support: Colorado State University

Title: Prenatal exposure to valproic acid reduces synaptic δ-catenin levels and disrupts ultrasonic vocalization in neonates

Authors: *S. ROH, H. MENDEZ-VAZQUEZ, M. SATHLER, M. DOOLITTLE, A. ZAYTSEVA, H. BROWN, M. SAINSBURY, S. KIM;
Colorado State Univ., Fort Collins, CO

Abstract: Valproic acid (VPA) is an effective and commonly prescribed drug for epilepsy and bipolar disorder. However, children born from mothers treated with VPA during pregnancy exhibit an increased incidence of autism spectrum disorder (ASD). Although VPA may impair brain development at cellular level, the mechanism of VPA-induced ASD has not completely addressed. The previous study has found that VPA treatment strongly reduces neuronal δ-catenin mRNA level. δ-catenin is important for the control of glutamatergic synapses and strongly associated with ASD. Interestingly, VPA inhibits dendritic morphogenesis in developing neurons, which is also found in neurons lacking δ-catenin expression. We thus hypothesize that prenatally exposure to VPA significantly reduces δ-catenin levels in the brain, which disrupts glutamatergic synapses, contributing to the development of ASD. Here, we found that VPA impaired the development of cultured mouse cortical neurons, which was reversed by elevating δ-catenin expression. Prenatally exposure to VPA significantly reduced synaptic δ-catenin levels and impaired ultrasonic vocalization (USV) in newly born pups. Importantly, we found that prenatal VPA treatment significantly decreased neuronal activation in the arcuate nucleus of the hypothalamus, which is important for the production of animals' USVs following isolation from the nest. Finally, VPA significantly reduced the levels of AMPA receptors and postsynaptic density 95 (PSD-95), a key scaffolding protein in excitatory synapses, in mouse newborns, which likely contributed to reduced neuronal activation. Therefore, these results suggest that VPA-induced ASD pathology can be mediated by loss of δ-catenin functions.

Disclosures: S. Roh: None. **H. Mendez-Vazquez:** None. **M. Sathler:** None. **M. Doolittle:** None. **A. Zaytseva:** None. **H. Brown:** None. **M. Sainsbury:** None. **S. Kim:** None.

Poster

PSTR381. Synaptic and Cellular Mechanisms of Autism I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR381.02/A2

Topic: A.07. Developmental Disorders

Support: Colorado State University
NIH/NCATS Colorado CTSA Grant UL1 TR002535
Boettcher Foundation's Webb-Waring Biomedical Research Program
NIH Grant 1R03AG072102

Title: The autism-associated loss of δ-catenin functions disrupts social behavior

Authors: *R. ROACH[1], H. MENDEZ-VAZQUEZ[1], K. NIP[1], S. CHANDA[1], M. SATHLER[1], T. GARVER[1], R. DANZMAN[1], M. MOSELEY[1], J. ROBERTS[1], O. KOCH[1], A. STEGER[2], R. LEE[1], J. ARIKKATH[3], S. ROH[1], S. KIM[1];
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Abstract: δ-catenin is expressed in excitatory synapses and functions as an anchor for the glutamatergic AMPA receptor (AMPAR) GluA2 subunit in the postsynaptic density. The glycine 34 to serine (G34S) mutation in the δ-catenin gene has been found in autism spectrum disorder (ASD) patients and results in loss of δ-catenin functions at excitatory synapses, which is presumed to underlie ASD pathogenesis in humans. However, how the G34S mutation causes loss of δ-catenin functions to induce ASD remains unclear. Here, using neuroblastoma cells, we identify that the G34S mutation increases glycogen synthase kinase 3β (GSK3β)-dependent δ-catenin degradation to reduce δ-catenin levels, which likely contributes to the loss of δ-catenin functions. Synaptic δ-catenin and GluA2 levels in the cortex are significantly decreased in mice harboring the δ-catenin G34S mutation. The G34S mutation increases glutamatergic activity in cortical excitatory neurons while it is decreased in inhibitory interneurons, indicating changes in cellular excitation and inhibition. δ-catenin G34S mutant mice also exhibit social dysfunction, a common feature of ASD. Most importantly, pharmacological inhibition of GSK3β activity reverses the G34S-induced loss of δ-catenin function effects in cells and mice. Finally, using δ-catenin knockout mice, we confirm that δ-catenin is required for GSK3β inhibition-induced restoration of normal social behavior in δ-catenin G34S mutant animals. Taken together, we reveal that the loss of δ-catenin functions arising from the ASD-associated G34S mutation induces social dysfunction via alterations in glutamatergic activity and that GSK3β inhibition can reverse δ-catenin G34S-induced synaptic and behavioral deficits.

Disclosures: R. Roach: None. **H. Mendez-Vazquez:** None. **K. Nip:** None. **S. Chanda:** None. **M. Sathler:** None. **T. Garver:** None. **R. Danzman:** None. **M. Moseley:** None. **J. Roberts:** None. **O. Koch:** None. **A. Steger:** None. **R. Lee:** None. **J. Arikkath:** None. **S. Roh:** None. **S. Kim:** None.

Poster

PSTR381. Synaptic and Cellular Mechanisms of Autism I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR381.03/A3

Topic: A.07. Developmental Disorders

Support: NIH/NIAAA R03AA022479
NIH/NIGMS (2 SO6 GM08016-39)

Title: Butyrate Protects Against Propionic Acid-Induced Toxicity in SH-SY5Y Cells: Implications for Autism Spectrum Disorder

Authors: *A. K. BHATTI[1], B. GETACHEW[2], R. L. COPELAND[2], Y. TIZABI[2];
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Abstract: Autism Spectrum Disorder (ASD), a neurodevelopmental disorder, is characterized by persistent deficits in social interaction, social communication, and manifests in early childhood. It is followed by significant impairment in social and occupational functions in adolescence and adulthood. Although genetics have been implicated, the exact causes of ASD have yet to be fully elucidated. New evidence suggests that dysbiosis or perturbation in gut microbiota may play an important role in ASD etiology. Short chain fatty acids (SCFAs) such as butyrate, acetate, and propionate are metabolites produced by gut bacteria during the fermentation of dietary fibers. They play a crucial role in the bidirectional communication between the gut and the brain, commonly referred to as “gut-brain axis” (GBA). These compounds can modulate various functions including neurotransmitter synthesis, energy metabolism, and inflammation. However, high levels of PPA can have neurotoxic effects and is used to generate a rodent model of ASD. Indeed, elevated PPA levels have been detected in children suffering from ASD. Butyrate, on the other hand, has been shown to have protective effects against various toxicants. Butyrate acts through G protein-coupled receptors (Gi/o and Gq) and is a well-known histone deacetylase inhibitor. Its important role in maintaining homeostasis of GBA is well established. In this study, we sought to determine whether butyrate may also protect against PPA-induced toxicity in SH-SY5Y cells. These cells, derived from human neuroblastoma cells, are considered representative of dopaminergic neurons, a neurotransmitter that is also affected in ASD. Exposure of SH-SY5Y cells to 2mM PPA for 24h, resulted in a concentration-dependent toxicity and approximately 25% cell death was observed. This toxicity was completely blocked by pretreatment with 20μM butyrate applied one hour prior to PPA. These preliminary findings render further support to the protective properties of butyrate and provide a conceptual basis for deeper investigation of potential therapeutic use of this SCFA in ASD. Future directions should include further elucidation of mechanism(s) of action of butyrate and its involvement in GBA, as well as in-vivo evaluation of its effectiveness in animal models of ASD.

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Poster

PSTR381. Synaptic and Cellular Mechanisms of Autism I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR381.04/A4

Topic: A.07. Developmental Disorders

Title: Impact's role as a translational regulator in memory, social behavior, and neural plasticity

Authors: *C. WALTERS[1], A. NAGPAL[2], Z. HUANG[3], R. CAGNETTA[3], S. WIEBE[3], E. MATTA-CAMACHO[4], S. UTTAM[3], J. POPIC[5], C. G. GKOGKAS[5], A. KHOUTORSKY[7], M. JAFARNEJAD[8], I. GANTOIS[3], N. SONENBERG[6];
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Abstract: Translational control is an important regulatory mechanism in neural plasticity, particularly during neurodevelopment. Dysregulation of this critical step is implicated in several pathologies, including Autism Spectrum Disorder (ASD). The IMPrinted and AnCienT gene (IMPACT) is a protein coding gene with a highly conserved RWD domain across eukaryotic species. IMPACT is a translational regulator via a negative regulation of General Control Nonderepressible 2 (GCN2) kinase activity. GCN2 phosphorylates the alpha subunit of Eukaryotic Initiation Factor 2 (eIF2α) as a cellular stress response. GCN2's phosphorylation of eIF2α serves as an inhibitor of eIF2 and its ternary complex, which causes a repression of general translation of mRNAs and decreased global protein synthesis. Paradoxically, a small subset of mRNAs are up-regulated. This translational regulatory mechanism also plays a critical role in the nervous system under physiological conditions, such as neural wiring, synaptic plasticity, long-term memory consolidation and learning. However, the impact of chronic phosphorylation of eIF2α throughout neurodevelopment has not been explored. In this *in vivo* study, we investigated the neurological effects due to loss of IMPACT, using a transgenic mouse model which removed the expression of IMPACT ($IMP^{-/-}$) and therefore its regulatory effect on GCN2. This in turn led to increased GCN2 expression and increased phosphorylation of eIF2α. We found that loss of the IMPACT protein causes deficits in social novelty preference, elicits repetitive behavior, and impairs long term memory. It also triggers anxiety and impairs memory extinction and spatial memory. Many of these phenotypes are present in patients with neurodevelopmental disorders, particularly ASD. We also identified specific mRNAs translationally regulated by IMPACT in the hippocampus and in the prefrontal cortex. Of particular note, Cytoplasmic FMR1 Interacting Protein 2 (CYFIP2) expression is increased in the hippocampus in $IMP^{-/-}$ compared to wild type mice. Taken together, our research supports the hypothesis that IMPACT is an important translational regulator in neurodevelopment, and mutations that impair its function may have implications in neurodevelopmental disorders such as Autism Spectrum Disorder.

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Poster

PSTR381. Synaptic and Cellular Mechanisms of Autism I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR381.05/A5

Topic: A.07. Developmental Disorders

Support: NIMH R01 MH115012
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NIH NIMH K99/R00 (K99 MH119319P)
Stanford Center for Innovation in In vivo Imaging
New York Stem Cell Foundation (NYSCF)
CZI Ben Barres Investigator
CZ Biohub

Title: A targeted antisense oligonucleotide therapeutic approach for Timothy syndrome

Authors: *X. CHEN[1], F. BIREY[2], M.-Y. LI[1], O. REVAH[3], R. LEVY[1], M. THETE[1], N. REIS[1], N. SAKAI[1], K. KAGANOVSKY[1], S. NISHINO[1], J. HUGUENARD[1], S. PASCA[1];
[1]Stanford Univ., Stanford, CA; [2]Emory Univ., Atlanta, GA; [3]Hebrew Univ., Hebrew, Israel

Abstract: Timothy syndrome (TS) is a debilitating multi-system disorder characterized by cardiac long QT syndrome, autism, epilepsy, and other long-term neuropsychiatric conditions. TS is caused by pathogenic variants in the *CACNA1C* gene encoding the L-type calcium channel. TS type 1 (TS1) is associated with a heterozygous gain-of-function variant in the alternately spliced and developmentally enriched *CACNA1C* exon 8A, as opposed to its counterpart exon 8. We reported cellular defects in TS1 patient-derived neural cells, including extended expression of the mutant 8A in human neural cells likely due to splicing interference. Therefore, switching expression of *CACNA1C* from the exon 8A to the postnatally enriched exon 8 isoform emerges as a potential therapeutic strategy. Here, we developed a promising therapeutic strategy by effectively decreasing the exon 8A isoform of *CACNA1C* using antisense oligonucleotides (ASO) in human cells *in vitro* and, following transplantation, in rodents. The ASO-mediated switch from 8A to 8 isoforms of *CACNA1C* can robustly rescue the channel inactivation defect and depolarization-induced calcium rise in patient-derived cortical organoids, and can effectively rescue cortical interneuron migration in TS1 forebrain assembloids. By leveraging our transplantation platform in which human neurons mature and integrate into rat brain circuit, we

demonstrate that a single intrathecal ASO administration can genetically and functionally rescue defects in human patient-derived cortical neurons in vivo. These results suggest suppression of the mutation-carrying isoform of *CACNA1C* could be developed as a treatment for TS1 in humans. Moreover, these experiments highlight the potential of a multi-level, *in vivo* and *in vitro* stem cell model-based approach for identifying strategies to reverse disease-associated pathophysiology.

Disclosures: X. chen: None. **F. Birey:** None. **M. Li:** None. **O. Revah:** None. **R. Levy:** None. **M. Thete:** None. **N. Reis:** None. **N. Sakai:** None. **K. Kaganovsky:** None. **S. Nishino:** None. **J. Huguenard:** None. **S. Pasca:** None.

Poster

PSTR381. Synaptic and Cellular Mechanisms of Autism I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR381.06/A6

Topic: A.07. Developmental Disorders

Title: Altered Chandelier Cell Within the Autism-Associated 16p11.2 Microdeletion Mouse Model

Authors: *A. A. BENN;
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Abstract: Autism spectrum disorder (ASD) describes a family of neurodevelopmental disorders (NDDs) that comprise a broad range of conditions such as abnormal reaction to sensory stimuli, deficits in social interaction and communication, as well as restricted behaviors. The exact cause of ASD is unknown; however, numerous genes and environmental factors have been identified as contributing to the disorder. It is known that recurrent copy number variations in the human 16p11.2 locus have been linked to ASD phenotypes. A plausible model for autism proposes that alterations to cortical interneurons contribute to synaptic excitatory/inhibitory imbalance within the autistic cerebral cortex, whereas hyperexcitability, increased spiking, and cortical circuitry noise induce ASD symptoms. Gamma-aminobutyric acid (GABA) inhibitory neurotransmission is required for the regulation of brain rhythm and spontaneous neuronal activities during development. Strikingly, the number of GABAergic parvalbumin-expressing interneurons specifically chandelier cells (ChCs) is reduced in the pre-frontal cortex (PFC) of individuals with ASD. Furthermore, fMRI studies comparing humans with the 16p11.2 microdeletion and mice with similar genetic deficiency indicate that both species have diminished PFC neuronal connectivity. Altogether, the prior evidence suggests that the 16p11.2 microdeletion and a reduction in the number of ChCs are potential risk factors contributing to the development of ASD.Overall, the goal of the study was to test the link between the 16p11.2 microdeletion and its impact on ChCs in the cortex of a transgenic 16p11.2 $^{del/+}$ mouse model. Results demonstrate that there are cortical region-specific differences in the number and size of ChC boutons which

innervation pyramidal neurons within the 16p11.2 $^{del/+}$ mouse. Additionally, the number of ChCs across cortical regions and layers differ between the wild-type and the 16p11.2$^{del/+}$ mice.

Disclosures: A.A. Benn: None.

Poster

PSTR381. Synaptic and Cellular Mechanisms of Autism I

Location: WCC Halls A-C

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Program #/Poster #: PSTR381.07/A7

Topic: A.07. Developmental Disorders

Support: UCLA BSCRC and Rose Hills Foundation Predoctoral Training Fellowship
NIMH Grant R01MH130061
NICHD P50HD103557

Title: A reduction in the autism associated gene Lysine Demethylase 6B (KDM6B) alters cortical development in human pluripotent stem cell derived cell types and brain organoids

Authors: *J. E. BUTH, H. GULESSERIAN, S. RYAN, A. DURRANI, T. ISHIDA, F. TURCIOS, B. G. NOVITCH;
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Abstract: Autism spectrum disorder (ASD) is a heritable lifelong developmental disability that affects 1-2% of the population worldwide. Individuals experience challenges with social interaction, communication, and repetitive behaviors. Treatments include behavioral therapy, medications, and assistive technology focused on improving the patient's quality of life. Pathological studies of ASD brains point to changes in cortical cytoarchitecture, synapse formation/pruning, altered excitatory/inhibitory neuron balance, and microglial activation, but the mechanisms of how this occur remains unclear. 100s of ASD-risk genes have been identified, many of which modify histones/chromatin. KDM6B is a histone modifier that removes repressive tri-methyl marks on lysine 27 of histone 3 to promote gene expression; a heterozygous loss of function is associated with ASD. Previous studies have shown KDM6B is highly expressed during neurodevelopment in all cell types in the brain. In mice, overexpression results in upregulation of neuronal/astroglial genes and knockdown results in a microcephalic phenotype with fewer cortical neurons. In microglia, KDM6B knockdown exaggerates inflammation leading to neuronal death in vitro. As many clinical trials for neurological disorders based on mouse data fail, findings need to be validated with human models. To determine whether the reduction in cortical neurons was due to changes in neural progenitors (NPC) or microglia, we compared the effect of KDM6B inhibition or CRISPR knockdown using human pluripotent stem cell (hPSC)-derived NPCs, brain organoids, and microglia. Many KDM6B targets are neuronal, so we hypothesized a reduction in KDM6B would result in reduced production of neurons. We found KDM6B inhibition in NPCs maintained NPC identity and reduced neuron differentiation.

To better recapitulate human development, we then assessed changes in brain organoids and found the ratio cortical cell types was altered. As KDM6B also plays a role in inflammation, we hypothesized a reduction in KDM6B would lead to activation of microglia. We tested how KDM6B activity would affect microglia (1) gene expression, (2) migration, and (3) phagocytosis. We found a reduction in KDM6B altered microglial expression of activation markers, increased migration, and reduced phagocytosis. As microglia/neurons interact during development, future studies will incorporate microglia into the organoid model to assess how knockdown in the organoid, microglia, or both effects development and maturation. These results provide a better understanding of developmental changes leading to ASD and help narrow the focus of possible treatment targets.

Disclosures: **J.E. Buth:** A. Employment/Salary (full or part-time):; Department of Neurobiology, David Geffen School of Medicine, UCLA, Los Angeles, CA, USA., Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, UCLA, Los Angeles, CA, USA., Intellectual and Developmental Disabilities Research Center, UCLA, Los Angeles, CA, USA. **H. Gulesserian:** A. Employment/Salary (full or part-time):; Department of Neurobiology, David Geffen School of Medicine, UCLA, Los Angeles, CA, USA., Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, UCLA, Los Angeles, CA, USA., Intellectual and Developmental Disabilities Research Center, UCLA, Los Angeles, CA, USA. **S. Ryan:** A. Employment/Salary (full or part-time):; Department of Biology, CSUN, Northridge, CA, USA.. **A. Durrani:** None. **T. Ishida:** None. **F. Turcios:** A. Employment/Salary (full or part-time):; Department of Neurobiology, David Geffen School of Medicine, UCLA, Los Angeles, CA, USA., Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, UCLA, Los Angeles, CA, USA., Intellectual and Developmental Disabilities Research Center, UCLA, Los Angeles, CA, USA. **B.G. Novitch:** A. Employment/Salary (full or part-time):; Department of Neurobiology, David Geffen School of Medicine, UCLA, Los Angeles, CA, USA., Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, UCLA, Los Angeles, CA, USA., Intellectual and Developmental Disabilities Research Center, UCLA, Los Angeles, CA, USA..

Poster

PSTR381. Synaptic and Cellular Mechanisms of Autism I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR381.08/A8

Topic: A.07. Developmental Disorders

Support: MEXT KAKENHI JP19H05218
MEXT KAKENHI JP21H00213

Title: Autism-associated ANK2 regulates development of cortical neurons

Authors: *S. KAWANO[1], S. KATAYAMA[1], D. MIURA[1], H. FUKUSHIMA[1], H. HASHIMOTO[2,3,4,5,6], T. NAKAZAWA[1,2];

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Abstract: Autism spectrum disorders (ASDs) are neurodevelopmental conditions characterized by altered social communication, stereotyped behaviors, and restricted interests. Although molecular and cellular pathogenesis of ASD remains largely unclear, impaired neural development is likely to contribute to ASD. Recent genetic studies have suggested that de novo mutations occurring in children of patients, which are not present in their parents' genomes, may be involved in the risk of ASD and that 102 recurrently de novo mutated genes may be critically associated with ASD. Among these risk genes, the ANK2 gene is classified as a high-confidence ASD gene in the SFARI database. The ANK2 gene encodes Ankyrin-B, a scaffolding protein that regulates the intracellular localization of specific membrane proteins. Currently, the roles of ANK2 in early neural development in the embryonic stages, during which abnormalities have been suggested in patients with ASD, remain unclear. In this study, we analyzed the roles of ANK2 in early neural development. Firstly, we found that Ank2 was highly expressed at embryonic day 14 to 18, when neurons are extensively generated, suggesting that ANK2 may regulate early neural development. Then we analyzed the function of ANK2 in early neural development using in utero electroporation and found that ANK2 knocked down cells were remained in the intermediate zone and subventricular zone. These results argue that ANK2 knock down impairs early neural development in the cerebral cortex. We then examined the effect of ANK2 knockdown on neurite outgrowth using the neuroblastoma Neuro2A cell line, which is widely used as a model for evaluating neuronal development, and observed that ANK2 knockdown decreased total neurite length per cell. Finally, to analyze the molecular mechanisms underlying the developmental abnormalities caused by ANK2 knockdown, we performed RNA sequencing using total RNAs extracted from ANK2 knocked down Neuro2A cells. We performed Gene Ontology (GO) analysis using differentially expressed genes between ANK2 and mock knockdown cells and found a significant enrichment of GO terms related to neuronal development and differentiation. Taken together, these results suggest that ANK2 regulates early neural development during embryonic stages and that de novo mutations in the ANK2 gene impair the neural development, which may be associated with the onset of ASD. Our current results contribute not only to elucidate the molecular and cellular pathogenesis of ASD but also to identify intermediate phenotypes of ASD and eventually to stratify patients with ASD based on the molecular and cellular pathogenesis.

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Poster

PSTR381. Synaptic and Cellular Mechanisms of Autism I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR381.09/A9

Topic: A.07. Developmental Disorders

Support: NIMH Intramural Research Program (ZIAMH002959)

Title: Reduction of SYNGAP1 in Parvalbumin (PV)-positive GABAergic neurons impairs long-range cortico-cortical connectivity

Authors: *S. NASKAR[1], P. STEVENSON[2], J. QI[3], S. LEE[4];
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Abstract: Impairment of long-range connectivity has been attributed to a wide range of neurodevelopmental disorders (NDDs). Previously, we reported that diverse long-range inputs from different brain areas recruit specific types of GABAergic INs in the primary somatosensory cortex (S1). This input-area-dependent recruitment of specific GABAergic INs plays an important role in active sensory processing. Abnormal sensory perception is common in NDDs, and the mechanisms that underlie impaired sensory processing associated with NDDs are not well studied. Haploinsufficiency of the *SYNGAP1* (Synaptic Ras GTPase Activating Protein1) gene has been shown to impair sensory processing and cognition in both humans and mice. Reduction of SynGAP1 in mice causes cognitive deficits by enhancing glutamatergic transmission and increasing excitatory connectivity. While the importance of SynGAP1 in excitatory neurons has been reported, the role of SynGAP1 in cortical GABAergic neurons is largely unknown. Here, we asked whether and how the disruptions of SynGAP1 in different GABAergic IN subtypes lead to the impairment of input-area-dependent corticocortical communication. Using optogenetics and *ex vivo* electrophysiology with specific deletion of *SYNGAP1* in Parvalbumin (PV)-positive GABAergic neurons, we found that the long-range cortical inputs to PV INs in S1 are altered. In PVCre: SynGAP1$^{flox/+}$ mice, PV neurons in S1 receive abnormally strong excitatory inputs from the whisker-related primary motor cortex (wM1). Since it is known that SynGAP1 regulates the level of AMPA receptors at the postsynaptic membrane of excitatory neurons, we asked whether the NMDA and AMPA conductance are altered for wM1 inputs to S1 in PVCre: SynGAP1$^{flox/+}$ mice. We found that the NMDA/AMPA ratio from PV INs was significantly higher in PVCre: SynGAP1$^{flox/+}$ mice compared to that of the control upon stimulation of wM1 inputs. Interestingly, high-frequency optical stimulation (HFS) of wM1 inputs led to a significant reduction of NMDA current amplitude in PVCre: SynGAP1$^{flox/+}$ mice, indicating a lack of NMDAR-mediated plasticity. Indeed, PVCre: SynGAP1$^{flox/+}$ mice failed to discriminate between a novel and familiar texture when subjected to a novel texture discrimination task, indicating a lack of learning. Together, our data suggest that *SYNGAP1* plays a critical role in regulating the excitatory synaptic strength from long-range inputs to cortical GABAergic neurons. Our results also suggest that PV-specific SynGAP1 haploinsufficiency impairs NMDAR-mediated plasticity, which may negatively impact learning.

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Poster

PSTR381. Synaptic and Cellular Mechanisms of Autism I

Location: WCC Halls A-C

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Program #/Poster #: PSTR381.10/A10

Topic: A.07. Developmental Disorders

Support: 2R01MH116500-06A1
5R01MH116500-05

Title: Impaired visual experience-dependent oscillations and underlying interareal circuit in the visual cortex of Fmr1 KO mice

Authors: *X. CHENG[1], S. NAREDDULA[1], H.-C. GAO[2], Y. CHEN[3], T. XIAO[3], P. A. EDENS[1], A. J. KIMBROUGH[3], F. HUANG[2], A. A. CHUBYKIN[1];
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Abstract: Fragile X syndrome (FXS) is the most prevalent heritable autism spectrum disorder (ASD), characterized by hypersensitivity and difficulty adapting to new sensory stimuli. Individuals with FXS often exhibit visual perception and learning impairments. Previous studies have described attenuation of 4-8 Hz oscillations, aberrant functional connectivity in Fmr1 KO mice, and impaired short-term (STP) in Fmr1 KO mice, the model of FXS. The reciprocal connections between the primary visual cortex (V1) and higher visual areas are crucial in cognitive processes. However, the impact of FXS on inter-areal connectivity remains poorly understood. To shed light on this phenomenon, we developed a new perceptual experience paradigm that induced familiarity-specific 4-8 Hz oscillations in V1 and the lateromedial area (LM), a part of the ventral pathway in mice. Using *in vivo* simultaneous silicon probes recordings and channelrhodopsin-2-assisted circuit mapping (CRACM) in acute brain slices, we investigated the strength and characteristics of long-range functional connections between V1 and LM in wildtype (WT) and Fmr1 KO mice before and after the visual experience. Simultaneous recordings of V1 and LM showed that the 4-8 Hz visual experience-dependent oscillations of both local field potentials and single-unit activity were lower in power and shorter in duration, and unit population firing rates were also lower in the V1 and LM of Fmr1 KO mice, which indicated deficits in communication between LM and V1. CRACM of feedforward projections revealed increased synaptic strength from V1 onto pyramidal cells (PCs) in all cortical layers of LM after visual experience in WT, while only a mild increase in the superficial layer II/III in Fmr1 KO mice. CRACM of feedback projections revealed decreased synaptic strength from LM onto PCs in layer II/III of V1 and increased strength in deep layer V after experience in WT, but no changes in Fmr1 KO mice. The visual experience also induced dendritic spine morphology plasticity we observed using super-resolution imaging and increased c-Fos expression in the visual cortex in WT but not in Fmr1 KO mice. Interestingly, some of the synaptic properties of the feedback projections in Fmr1 KO mice appeared to improve following visual experience, such as the paired-pulse ratios as a measurement of STP. Our findings provide the first measurements of the inter-areal synaptic connectivity before and after visual experience

in WT and Fmr1 KO mice, along with simultaneous *in vivo* recordings of neural activity, and indicate that visual training may serve as a promising therapeutic intervention for ASD.

Disclosures: X. Cheng: None. **S. Nareddula:** None. **H. Gao:** None. **Y. Chen:** None. **T. Xiao:** None. **P.A. Edens:** None. **A.J. Kimbrough:** None. **F. Huang:** None. **A.A. Chubykin:** None.

Poster

PSTR381. Synaptic and Cellular Mechanisms of Autism I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR381.11/A11

Topic: A.07. Developmental Disorders

Support: Spanish Research Agency PID2021-122723OA-I00 to MDMS

Title: Autism-associated CNTNAP2 mutations modulate neuronal connectivity through ectodomain shedding

Authors: L. E. DIONISIO[1], E. L. MCCOIG[2], N. H. PIGUEL[2], B. P. SPIELMAN[2], M. LOBETE[3], C. BOERS[4], M. DOS SANTOS[2], R. GAO[2], D. COMOLETTI[5], P. PENZES[6], ***M. D. MARTIN-DE-SAAVEDRA**[3];
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Abstract: Contactin-associated protein-like 2, CNTNAP2 or Caspr2, is a cell adhesion molecule that belongs to the neurexin superfamily. Genetic variation in *CNTNAP2* is associated in neurodevelopmental conditions such as Autism spectrum disorders, schizophrenia, and cortical dysplasia focal epilepsy syndrome (CDFE). At the cellular level, CNTNAP2 plays a crucial role in modulating structural plasticity, including dendritic arborization and dendritic spines. Recent studies have revealed that CNTNAP2 undergoes ectodomain shedding, resulting in the release of a soluble ectodomain (sCNTNAP2), which regulates neuronal synchrony. However, it is still unknown if CNTNAP2 ectodomain shedding impacts dendritic architecture.
In this study, we show that ASD-associated CNTNAP2 mutations affect sCNTNAP2 levels, due to retention in the endoplasmic reticulum or altered processing by MMP9. We demonstrate that CNTNAP2 mutants display alterations in dendritic arborization, probably derived from modified levels of shed ectodomain. Our study sheds light on the mechanisms underlying the role of CNTNAP2 ectodomain shedding in neuronal connectivity. These findings may have significant implications for the development of targeted therapies aimed at modulating CNTNAP2 function and improving neuronal connectivity in individuals with neurodevelopmental disorders.

Disclosures: L.E. Dionisio: None. **E.L. McCoig:** None. **N.H. Piguel:** None. **B.P. Spielman:** None. **M. Lobete:** None. **C. Boers:** None. **M. Dos Santos:** None. **R. Gao:** None. **D. Comoletti:** None. **P. Penzes:** None. **M.D. Martin-de-Saavedra:** None.

Poster

PSTR381. Synaptic and Cellular Mechanisms of Autism I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR381.12/A12

Topic: A.07. Developmental Disorders

Support: NRF Grant 2022R1A6A3A13072676
NRF Grant 2021M3E5D9021884

Title: Autism-associated EXOC4 deficiency reveals dysregulation of cortical and synapse development in a cerebral organoid model

Authors: *J. KIM[1], S. JEON[1], Y. LEE[1], M. LEE[1], K. KIM[1,2], J. LEE[1,2];
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Abstract: *EXOC4* (exocyst complex component 4), which encodes the EXOC4 protein, is a mutated gene found in patients with autism spectrum disorder (ASD). EXOC4 is known to play an essential role in rapid membrane expansion, which occurs during the outgrowth of neurons and synaptogenesis. However, the mechanism by which mutations in the *EXOC4* gene lead to the ASD phenotype remains unclear. In the current study, cerebral organoids derived from induced pluripotent stem cells (iPSCs) with various *EXOC4* mutations generated by CRISPR-Cas9 gene editing were used. We found that *EXOC4* haploinsufficiency induces enlargement of cerebral organoids and delayed generation of cortical neurons, serving as an *in vitro* correlate of patients' macrocephaly and cognitive impairment. Remarkably, we also discovered defects in dendrite arborization and synapse development that recapitulate *EXOC4*-specific traits. Our results uncover that *EXOC4*-dependent molecular deficits provoke ASD characteristics and confirm the potential utility of CRISPR engineering in cerebral organoids for neurodevelopmental disease modeling.

Disclosures: J. Kim: None. **S. Jeon:** None. **Y. Lee:** None. **M. Lee:** None. **K. Kim:** None. **J. Lee:** None.

Poster

PSTR382. Down Syndrome

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR382.01/A13

Topic: A.07. Developmental Disorders

Support: NIH NINDS Grant R01 NS105138

Title: The variety and activity of neural precursors in a dorsal forebrain spheroid model of Down syndrome

Authors: *A. AYOUB[1,2], T. F. HAYDAR[1,2];
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Abstract: Down syndrome (DS) is the most common genetic form of intellectual disability (ID). Early events in fetal corticogenesis are preferentially affected in DS and possibly contribute to the lifelong cognitive impairments associated with ID. The major determinant of neocortical size is the properly timed genesis of neurons and glia from neural precursor cell (NPC) populations. While several classes of NPCs have been identified, how they contribute to neocortical growth during normal development and their roles in DS remain to be elucidated. Here we investigate the diversity, proliferation potency and differentiation of NPCs during early cortical development in DS. We differentiated isogenic lines of induced pluripotent stem cells (iPSCs) derived from people with DS into human cortical spheroids with dorsal forebrain characteristics using small molecules and growth factors to mimic *in vivo* development and drive regional specificity. We perform high-resolution 3D imaging of entire cleared spheroids using 2-photon microscopy and a combination of immunohistochemical markers to characterize the architectonic features, size, and cellular composition of rosette structures to determine how these proliferative rosettes relate to overall spheroid size by genotype. EdU proliferative assays and co-immunofluorescent staining are performed to label dividing precursor cells and assess the relative degree to which proliferating cells undergo self-renewal or differentiation. Single-nuclei RNA sequencing will be performed to assay the heterogeneity and proportion of NPCs, and the different classes of neurons and glia contemporaneously generated. These experiments will temporally characterize the development of cortical progenitors and their progeny during the first month of development. We have demonstrated successful generation and 3D imaging of trisomic and euploid dorsal forebrain spheroids. We find that trisomic spheroids are consistently smaller in size and display altered gross morphology compared to isogenic euploid controls. We will investigate differences in the parameters and cellular composition of each rosette as well as the NPC proliferation kinetics between genotypes. Specifically, trisomic spheroids show altered precursor cell specification, proliferation, and distribution as well as reduced neurogenesis compared to euploid. By identifying specific changes in NPC dynamics in trisomic cells, we will elucidate some of the cellular changes underlying the neuroanatomical deficits occurring during very early development in DS.

Disclosures: A. Ayoub: None. **T.F. Haydar:** None.

Poster

PSTR382. Down Syndrome

Location: WCC Halls A-C

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Program #/Poster #: PSTR382.02/A14

Topic: A.07. Developmental Disorders

Support: University of Michigan Protein Folding Disease Initiative
Career Training in the Biology of Aging (T32-AG000114)

Title: Normalization of DSCAM in the Ts65Dn mouse model of Down Syndrome ameliorates dendritic phenotypes in the dentate gyrus of the hippocampus

Authors: *R. NEFF[1,3], T. HERGENREDER[3,4], B. YE[2,3,4], G. G. MURPHY[1,3];
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Abstract: Down Syndrome (DS) results from trisomy of human chromosome 21 (HSA21) and is characterized by physical and psychomotor impairments, and neurodevelopmental delays. Determining the genetic substrates that underlie the impairments observed in DS will not only lead to viable treatments for DS individuals, but also provide insight into the role of those genes in normal cognition. The Down Syndrome Cell Adhesion Molecule (DSCAM) is located on HSA21 and has been shown to be crucial for mediating neurodevelopmental processes such as presynaptic growth. It remains unknown as to whether DSCAM contributes to cognitive phenotypes and neurodevelopmental changes associated with the hippocampus, a region critical for learning and memory. The Ts65Dn mouse model of DS replicates many of the human phenotypes, including abnormalities in neuronal structure within the hippocampus. Through genetic correction of DSCAM (i.e., normalization) in the Ts65Dn, we aim to determine the relative contribution of DSCAM overexpression to the cognitive phenotypes in DS. In this study, we utilized Golgi-Cox staining in fixed brain tissue from 5-6-month-old mice to study the morphology of dendritic spines in the CA1 and dentate gyrus (DG) regions of the dorsal hippocampus. We observed regional differences in DS spine density, with an increase seen in CA1 pyramidal cells and a decrease in granule cells of the DG. Additionally, there was an enlargement of DS spines across all regions examined. We hypothesized that spine enlargement might be a compensatory effect in response to the enhanced inhibition and altered excitatory-inhibitory imbalance observed in the Ts65Dn mouse model of DS. Using immunostaining and confocal microscopy followed by unbiased analysis, we observed an increase in the number of inhibitory synapses on the pyramidal cell soma within the DS CA1 hippocampus. This phenotype was then ameliorated by DSCAM normalization. Furthermore, we saw that DSCAM normalization reduced spine length and area in CA1 and DG, correcting the spine enlargement seen in Ts65Dn. We also show that the deficit in DS granule cell spine density in the DG was restored with DSCAM normalization. As adult neurogenesis is deficient in DS, and the DG is one of the few brain regions where this process takes place, we are now quantifying newborn neurons in the DG using doublecortin (DCX) immunostaining to determine to what extent DSCAM normalization impacts adult neurogenesis in the Ts65Dn mouse model of DS. Overall, our findings provide evidence for DSCAM overexpression as a central contributor to the abnormalities in neuronal structure within the DS brain and suggest a promising therapeutic target for DS research.

Disclosures: R. Neff: None. **T. Hergenreder:** None. **B. Ye:** None. **G.G. Murphy:** None.

Poster

PSTR382. Down Syndrome

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR382.03/A15

Topic: A.07. Developmental Disorders

Title: Prenatal non-invasive 40Hz sensory stimulation treatment rescues Down syndrome phenotypes in embryonic cortex

Authors: *D. PARK[1], J. YANG[2], B. SCHATZ[1], R. THOMAS[2], M. KELLIS[2], L.-H. TSAI[1]; [1]MIT - Picower Inst. For Learning & Memory, Cambridge, MA; [2]MIT, Cambridge, MA

Abstract: Prenatal non-invasive 40Hz sensory stimulation treatment rescues Down syndrome phenotypes in embryonic cortex
Dong Shin Park, Jackie Yang, Raina Thomas, Brooke Schatz, Kyriakitsa Galani, Manolis Kellis, Li-Huei Tsai
Key words: GENUS, neurogenesis, embryo, Down syndrome, prenatal, Ts65Dn
Non-invasive sensory stimulation using light and sound at 40 Hz (Gamma ENtrainment Using Sensory stimuli, or GENUS) has been shown to reduce the pathological features of Alzheimer's disease. Unpublished work from our lab further showed that GENUS can be extended to treat other neurological disorder such as Down Syndrome (DS). We show here that GENUS promotes hippocampal neurogenesis and increased synaptic density in adult DS mouse model known as Ts65Dn. Since DS is a developmental disorder, we further tested whether prenatal GENUS treatment can rescue DS phenotypes that appear at the embryonic stage. We exposed the pregnant dam to GENUS and showed that it increased brain size and the thickness of cortical plate in the embryonic brain. Single nuclus RNA-seq experiments further demonstrated that prenatal GENUS treatment increased the expression levels of the genes that are downregulated in Ts65Dn embryonic brain. Many of these genes are involved in neurogenesis and cell migration. Overall, our data suggest that prenatal GENUS treatment could be a potential therapeutic tool for DS.

Disclosures: D. Park: None. **J. Yang:** None. **B. Schatz:** None. **R. Thomas:** None. **M. Kellis:** None. **L. Tsai:** None.

Poster

PSTR382. Down Syndrome

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Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR382.04/A16

Topic: A.07. Developmental Disorders

Support: FPU19/04789

Title: Unraveling the role of somatostatin hilar interneurons in engram formation in Down syndrome.

Authors: *M. SABARIEGO NAVARRO, C.-L. SIERRA NOGUERA, A. ZAMORA MORATALLA, M. DIERSSEN;
Systems Biol., Ctr. for Genomic Regulation (CRG), Barcelona, Spain

Abstract: Down syndrome is the most prevalent genetic cause of intellectual disability but the mechanisms underlying cognitive impairment remain obscure. Our research has shown that engram formation is impaired in a mouse model of Down syndrome, the Ts65Dn, but the underlying causes of this deficit have not yet been fully understood. We here hypothesized that alterations in inhibitory microcircuits leading to overinhibition could contribute to the disrupted formation of hippocampal engrams in Down syndrome. Single-nuclei RNA sequencing revealed changes in cell composition with significant increases in specific subtypes of inhibitory interneurons in the Ts65Dn hippocampus, including somatostatin (SST+), vasoactive intestinal peptide-expressing (VIP+), and calretinin+ interneurons. Moreover, these interneurons exhibited an altered transcriptomic profile, particularly the SST+ cells, with alterations in genes which modulates the excitability of these cells as *Hcn1* and *Grik1*. To investigate the role of SST+ neurons in Down syndrome, we crossbred trisomic mice with transgenic mice expressing the Cre recombinase under the SST promoter. Through Contextual Fear Conditioning (CFC) experiments and using cFos as a measure of neuronal activity, we discovered that there was an increased number of cFos expressing SST+ cells upon CFC training. To establish a causal link between this phenotype and memory deficits in trisomic mice, we employed viral constructs containing chemogenetic tools to modulate the activity of SST neurons during engram formation. Remarkably, when we inhibited hilar SST+ cells during training, we observed a rescue in CFC performance, suggesting that the increase of cFos expressing (activated) SST+ interneurons impairs the formation of associative memories in the Down syndrome mouse model. Taken together our results suggest that in Down syndrome SST+ cells are more excitable and more prone to be activated upon learning, impairing engram formation and, ultimately, memory processes.

Disclosures: M. Sabariego Navarro: None. **C. Sierra Noguera:** None. **A. Zamora Moratalla:** None. **M. Dierssen:** None.

Poster

PSTR382. Down Syndrome

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR382.05/A17

Topic: A.07. Developmental Disorders

Support: Wellcome Trust Grant

Title: Dissecting the cellular landscape of Down syndrome hippocampus

Authors: *M. PEREZ GONZALEZ[1], J. CONSTABLE[1], M. KUROSAWA[1], P. M. MUZA[1], S. J. WEST[2], V. TYBULEWICZ[3], E. M. FISHER[1];
[1]Univ. Col. London, London, United Kingdom; [2]Sainsbury Wellcome Ctr., London, United Kingdom; [3]Francis Crick Inst., London, United Kingdom

Abstract: Down syndrome (DS) is caused by trisomy of human chromosome 21 (Hsa21). Defective cell proliferation and neurogenesis but increased number of glial cells, have been found in DS brains. These alterations are believed to disturb homeostasis and affect brain development contributing to intellectual disability (Lee et al., 2016). Here, we aim to study the cellular composition of the hippocampus of DS mouse models, ultimately to identify candidate genes and their contribution to cognitive impairment. For this, we work with mouse models with segmental duplications of Hsa21 orthologous genes on mouse chromosome 16 (Mmu16). Cell counts are assayed in Dp(16Lipi-Zbtb21)1Tyb (Dp1Tyb) mice at 3-4 months of age, which have cognitive deficits (Chang et al., 2020). If a difference from wildtype (WT) littermates is identified with counts of individual cell types, we also assayed those cells in 3-4 month Dp(16Mir802-Zbtb21)3TybEmcf (Dp3Tyb), Dp(16Lipi-Hunk)9TybEmcf (Dp9Tyb) and Dp(16Mis18a-Runx1)2TybEmcf (Dp2Tyb) - which form a genetic mapping panel of the duplicated region in Dp1Tyb mice. Cell counting is performed using thick coronal sections from the dorsal hippocampus, which are optically cleared and immunolabelled using the ABSOC protocol (Muza et al., 2023). Dp1Tyb mice have higher hippocampal density of neuropeptide Y (NPY)+ and calretinin+ cells compared to WT mice but no changes in parvalbumin+ cells, indicating that there is increased numbers of specific subtypes of interneurons in these mice, consistently with an hypothesis postulating that excessive GABAergic signaling in DS contributes to cognitive impairment (Contestabile et al., 2017). Dp1Tyb mouse hippocampus has higher levels of IBA1+ cells and augmented density of S100B+ but not GFAP+ cells, suggesting increased density of microglia and certain astrocyte subpopulations. As S100B is also expressed in oligodendrocyte precursor cells (OPCs), density of NG2+ cells was also assessed, and indeed Dp1Tyb mice present increased numbers of OPCs. Using a genetic mapping approach, we have determined that the increased NPY cell density observed in Dp1Tyb mice was present in Dp3Tyb mice but not in Dp9Tyb or Dp2Tyb so there must be at least one gene present between Mir802 - Zbtb21 on Mmu16 causing an increase in hippocampal NPY+ interneuron density. Alterations in cell populations are likely to contribute to cognitive impairment in DS. Identifying the genes causing these alterations is essential to develop effective treatments for cognitive dysfunction in DS. Here, we have mapped a dosage-sensitive gene(s) causing an increase in NPY interneurons to the Dp3Tyb region that is duplicated for just 39 coding-genes.

Disclosures: M. Perez Gonzalez: None. **J. Constable:** None. **M. Kurosawa:** None. **P.M. Muza:** None. **S.J. West:** None. **V. Tybulewicz:** None. **E.M. Fisher:** None.

Poster

PSTR382. Down Syndrome

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR382.06/A18

Topic: A.07. Developmental Disorders

Support: NIH Grant RO1AR078663-01
Clinical and Translational Sciences Institute of Indiana

Title: Effects of a postnatal Dyrk1a functional copy number reduction in the Ts65Dn mouse model of Down syndrome on sensorimotor development

Authors: *L. E. HAWLEY[1], L. JOHNSON[1], K. HEAGY[1], C. R. GOODLETT[2], R. J. ROPER[1]; [1]Dept. of Biol., [2]Dept. of Psychology, Indiana University-Purdue Univ. Indianapolis, Indianapolis, IN

Abstract: Down syndrome (DS) is the triplication of human chromosome 21 (Hsa21) and causes abnormal brain and behavior development. Overexpression of *DYRK1A*, triplicated in DS, causes impairments in neuronal proliferation, differentiation, and cell cycle progression. Inhibition of DYRK1A is a candidate therapeutic for DS, but questions remain regarding when to start treatments. The Ts65Dn DS mouse model contains an extra segmental chromosome, adding a third copy of ~ half of Hsa21 orthologs. We found amplified DYRK1A expression in brain tissues of trisomic pups on postnatal day (P) 6, suggesting P6 may be a critical stage of altered brain development in this DS mouse model. We hypothesized that a functional reduction of *Dyrk1a* gene copy number before P6 in otherwise trisomic pups would rescue sensorimotor delays typical in Ts65Dn growth. A doxycycline-induced truncation of one *Dyrk1a* allele initiated on P0 resulted in euploid (Eu), trisomic (Ts,*Dyrk1a*$^{+/+/+}$), and trisomic mice with 2 functional copies of *Dyrk1a* (Ts,*Dyrk1a*$^{+/+/-}$). PCR confirmed excision with a novel primer probing the DNA bridge created by this deletion. Pups were tested daily from P3 to P21 with a battery of 14 neurobehavioral milestone assessments. Ts,*Dyrk1a*$^{+/+/+}$ pups consistently weighed less than Eu matched littermates, a disparity that widened with age through P21. Reducing *Dyrk1a* copy number in Ts,*Dyrk1a*$^{+/+/-}$ did not significantly improve these deficits. For cliff aversion and negative geotaxis, there were no significant differences among groups in mean age meeting criterion, but the Eu mice showed a wider range of ages (positively correlated with body weight) while the Ts,*Dyrk1a*$^{+/+/+}$ and Ts,*Dyrk1a*$^{+/+/-}$ pups had a smaller range at criterion uncorrelated with body weight. Locomotor activity was assessed in 10-minute sessions at P10, P13, P16, and P19. Total distance traveled increased in all groups across ages, but the magnitude of increase varied according to genotype. Eu pups opened their eyes significantly earlier ($\bar{x}$=13.4) than Ts,*Dyrk1a*$^{+/+/+}$ ($\bar{x}$=15.1) or Ts,*Dyrk1a*$^{+/+/-}$ ($\bar{x}$=15.4) pups; a noteworthy increase in P13 Eu pup activity level may be due to this earlier eye opening. Ts,*Dyrk1a*$^{+/+/-}$ pups had significantly lower activity levels at the three earlier ages compared to Euploid littermates. At P19, mean activity levels of female Ts,*Dyrk1a*$^{+/+/+}$ mice were significantly higher than female Eu and Ts,*Dyrk1a*$^{+/+/-}$littermates. In contrast, mean activity levels of P19 males did not differ among the three genotype groups. These findings to date suggest that certain sexually dimorphic trisomic phenotypes that are sensitive to *Dyrk1a* dosage in Ts65Dn mice may emerge during postnatal development.

Disclosures: L.E. Hawley: None. **L. Johnson:** None. **K. Heagy:** None. **C.R. Goodlett:** None. **R.J. Roper:** None.

Poster

PSTR382. Down Syndrome

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Program #/Poster #: PSTR382.07/A19

Topic: A.07. Developmental Disorders

Support: São Paulo Research Foundation Grant 2018/15371–8
São Paulo Research Foundation Grant 2019/04859–2
Fondation Jérôme Lejeune

Title: Increased NMDA-receptor-mediated calcium influx in iPSC-derived neurons from individuals with Down syndrome

Authors: J. DA SILVA FAUSTO[1], T. GLASER[2], A. HENNING ULRICH[2], A. C. S. COSTA[3], ***B. L. ZAMPIERI**[1];
[1]Hosp. Israelita Albert Einstein, São Paulo, Brazil; [2]Dept. of Biochemistry, Chem. Inst., Univ. of São Paulo, São Paulo, Brazil; [3]Dept. of Psychiatry, Case Western Reserve Univ., Cleveland, OH

Abstract: Down syndrome (DS, or trisomy 21, T21), is the most common genetic cause of intellectual disability. Individuals show a spectrum of cognitive and neurobehavioral impairments with deficits in memory and learning. Calcium (Ca^{2+}) signaling is an essential mechanism for neuronal function, transducing electrical activity into intracellular molecular signals. N-Methyl-D-aspartate receptors (NMDARs) are glutamate-gated channels with high Ca^{2+} permeability, thus central for learning and information processing in the brain. Dysfunction and hyperactivity of NMDARs may lead to Ca^{2+} overload, synaptic noise, and neuronal death. We aim to investigate NMDAR-mediated Ca^{2+} influx in induced pluripotent stem cells (iPSCs)-derived neurons from individuals with DS. iPSCs were established from urine-derived cells isolated from 6 individuals with DS (T21-iPSCs) and 4 controls. Differentiation into cortical neurons was performed using the dual SMAD inhibition strategy and confirmed by immunoreactivity for neuron-specific markers and the obligate GluN1 subunit of the NMDAR. To investigate Ca^{2+} influx, iPSC-derived neurons (DIV 75+) were loaded with Fluo 4-AM (5 μM) for 40 min at 37°C. Imaging and washes were performed with artificial cerebrospinal fluid. Imaging was done using an ECLIPSE-TiS microscope with a 10X objective at 2 frame/s for 10 min. Baseline recordings were done for 30s after which 1 μM glycine was added and then 1 min later 500 μM NMDA. In a different set of neurons NMDAR blocker memantine (10 μM) was added 2 min before the addition of NMDA. Data were analyzed using NIS-Elements Advanced Research software. Fluorescence traces were normalized to the initial fluorescence intensity and mean peak amplitude values (DF/F0) were calculated from specified regions of interest manually selected. Area under the curve (AUC) was calculated with the integration function. Results show that the influx of Ca^{2+} and AUC were significantly higher in untreated T21-iPSC-derived neurons compared to untreated controls ($p=0.0003$ and $p<0.0001$, respectively). Blocking the NMDA receptors with 10 μM memantine had no detectable effect on the influx of Ca^{2+} in T21-neurons, but it significantly reduced the AUC in treated T21-neurons compared to untreated T21-neurons ($p=0.030$). The effect of memantine on the Ca^{2+} influx is being further investigated in our laboratory. T21-iPSCs-derived neurons revealed alteration in NMDA receptor-mediated

calcium influx, leading to increased Ca^{2+} activity. These findings provide supporting to the hypothesis of excessive NMDAR signaling based on previous observations in mouse models of DS and that NMDAR should be further investigated in DS.

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Poster

PSTR382. Down Syndrome

Location: WCC Halls A-C

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Program #/Poster #: PSTR382.08/A20

Topic: A.07. Developmental Disorders

Support: NIH (1R15NS098389)
Jerome Lejeune Foundation (Grant #1920)
SC INBRE NIGMS (P20GM103499)

Title: Altered adhesions and local translation in Down syndrome hiPSC-derived cortical neurons

Authors: *K. RYGEL[1], M. AGRAWAL[2], N. KIRKISE[1], S. BAILEY[2], G. GOTBERG[1], P. CASSIDY[2], S. KELEMEN[2], T. BUMBLEDARE[2], L. KERSHNER[2], A. MONTAZZOLI[2], C. NEIFERT[2], K. WELSHHANS[1];
[1]Biol. Sci., Univ. of South Carolina, Columbia, SC; [2]Biol. Sci., Kent State Univ., Kent, OH

Abstract: Down syndrome is a neurodevelopmental disorder resulting from the triplication of human chromosome 21. One ubiquitous phenotype of Down syndrome is intellectual disability, which results, in part, from altered neuronal connectivity. During development, neuronal growth cones sense and respond to guidance cues to reach their appropriate synaptic targets and form this neuronal connectivity. Two critical mechanisms that regulate this process are local translation and adhesion. Prior work suggests that local translation may occur at adhesion sites, thus connecting these two processes. To better understand how neuronal connectivity is altered in Down syndrome, here we used both human fibroblasts and human induced pluripotent stem cell (hiPSC)-derived cortical neurons from apparently healthy individuals and individuals with Down syndrome and examined changes in local translation and adhesion. Fibroblasts are a valuable model for discovering potential cellular and molecular processes underlying axon growth and guidance during neuronal development because these two cell types use similar migration mechanisms. We first used a transwell migration assay and found a decrease in cell migration in Down syndrome fibroblasts. Using a puromycin assay, we also found that local translation in the leading edge of Down syndrome fibroblasts is altered. Multiple proteins in the adhesion complex, including paxillin, vinculin, talin, and RACK1, are increased in Down syndrome fibroblasts. Using hiPSC-derived cortical neurons, we performed a Dunn chamber turning assay with the attractive guidance cue, netrin-1, and found that growth cone turning is lost in hiPSC-derived neurons from individuals with Down syndrome. Additionally, both talin

and RACK1 are significantly increased, and local translation is decreased in growth cones of Down syndrome hiPSC-derived neurons at basal conditions. We are currently examining whether adhesion proteins, local translation, and growth cone turning are altered following stimulation with the attractive guidance cue brain-derived neurotrophic factor (BDNF). Taken together, this work shows that dysregulation of local translation and adhesion-based mechanisms during development may contribute to the neuronal connectivity changes that occur in Down syndrome.

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Poster

PSTR382. Down Syndrome

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR382.09/A21

Topic: A.07. Developmental Disorders

Support: NIH Grant R01HD104640-01A1

Title: Feeding ability and intrinsic tongue maturation at weaning in the Ts65Dn mouse model of Down syndrome

Authors: *T. J. GLASS[1], R. E. BRUTTO[2], B. A. CHATWIN[2];
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Abstract: Down syndrome (DS) is associated with developmental delays and difficulties related to feeding during infancy and childhood. Successful weaning, or transitioning from suckling to independent deglutition, requires dramatic developmental change in movements of the tongue. However, little is known about whether maturation of the intrinsic tongue is impacted in DS. This study tests the hypothesis that DS causes delays in maturation of the tongue muscle system at weaning. Methods: The Ts65Dn mouse model of DS and euploid sibling controls were evaluated at 21 days of age to quantify measures related to eating and drinking during a 24-hour period immediately after weaning. Mice were also evaluated at 35 days of age, which is a timepoint associated with mature mastication and drinking behaviors (n = 14-16 male mice and 8-14 female mice per group). After behavioral assays, intrinsic tongue muscles were sectioned and stained to permit quantification of myofiber size and percentages of myofibers expressing Myosin Heavy Chain isoform (MyHC) 2a and MyHC 2b. Separate data analysis was performed for anterior, middle, and posterior regions of intrinsic tongue muscles (n=5-6 male mice and 5-6 female mice per group). Data were analyzed by 2-way ANOVA and mixed effects analysis as appropriate. Results: At 21 days of age, Ts65Dn lost a significant amount of weight compared to euploid controls during the 24 hours immediately after weaning (p=.0004). Ts65Dn consumed significantly less food (p=.009) and significantly less water (p=.025) during this time period.

However, there were no genotype-specific differences in weight change or feeding measures over a 24-hour period at 35 days of age. Preliminary results of intrinsic tongue analysis at 21-22 days of age indicate significant differences as a function of anatomical region within the intrinsic tongue muscle system in myofiber size (p=.001) and MyHC isoforms (p=.005), but not as a function of sex or genotype. Conclusions: Ts65Dn show significant differences in feeding efficacy at weaning, but do not show these differences two weeks later. Weaning may be a process that is particularly susceptible to oromotor delays in DS, and the Ts65Dn mouse model is useful for studying these delays. At weaning, the intrinsic tongue muscle system has dramatic anterior-to-posterior specificity in the properties of myofibers. While Ts65Dn and controls broadly share major organizational principles of the tongue muscle system at weaning, work is ongoing to increase sample sizes in tongue muscle analysis to permit detection of genotype-specific and sex-specific differences with smaller effect sizes, if present.

Disclosures: T.J. Glass: None. **R.E. Brutto:** None. **B.A. Chatwin:** None.

Poster

PSTR382. Down Syndrome

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR382.10/A22

Topic: A.07. Developmental Disorders

Title: Neocortical development in the novel TcMAC21 Down syndrome mouse model

Authors: *Z. ATAK[1,3], S. GANDHI[4,2], K. A. PASION[1], J. WILLIAMS[1,5], T. F. HAYDAR[1,6,3]; [1]Ctr. For Neurosci. Res., [2]Ctr. for Neurosci. Res., Children's Natl. Med. Ctr., Washington, DC; [3]Neurosci. and Cognitive Sci., Univ. of Maryland, College Park, MD; [4]Sch. of Neurosci., Virginia Tech., Virginia, VA; [5]Biol., Howard Univ., Washington, DC; [6]Sch. of Med. and Hlth. Sci., George WashingtonUniversity, Washington, DC

Abstract: The cerebral cortex is a six-layered structure that develops from a simple epithelial sheet during early embryonic development. The neocortex subserves cognition, social behavior, speech, and motor skills, behaviors known to be detrimentally impacted by trisomy 21/Down's syndrome (DS). Prior research using mouse models of DS has revealed that cortical neuronal precursor proliferation and fate specification are faulty in DS and may underpin adult behavioral alterations. Here we utilized a novel mouse model of DS, TcMAC21, in which each cell contains an extra copy of the human long arm of chromosome 21 (HSA21q), harboring 93% of the protein-coding genes present on HSA21q. How triplication of HSA21q leads to the structural and behavioral manifestations of DS remains unclear. Our goal in this study is deeply characterize prenatal neurogenesis in the TcMAC21 brain. In particular, we will assess whether lineage-specific neurogenesis, a key mechanism of cortical neuron diversification, is altered in this novel DS mouse model. We utilized acute EdU labeling to capture cycling cortical neural precursor cells in the S phase of the cell cycle in TcMAC21 trisomic and control brains to determine precursor proliferative capacity and positioning in the embryonic neocortex. Furthermore, we

performed in utero electroporation (IUE) surgeries to fate-map neurons born through direct and indirect neurogenesis. To determine the contribution of different precursor lineages to cortical development in TcMAC21, brains were collected and analyzed 24 hours after IUE. Neural precursor cell marker and morphology data were obtained by immunohistochemical staining of electroporated tissue slices. Thus far, we have not detected significant differences in mediolateral and rostrocaudal cortical measurements between euploid and trisomic brains, either prenatally or postnatally. This finding is very different from previous DS mouse models. At the microstructural level, we found evidence of altered cortical plate thickness and lamination patterns in the trisomic brain. Our experiments so far revealed no overall differences in levels of proliferation between euploid and trisomic cortices. However, we detected altered positioning of proliferative precursors in trisomic cortices. Moreover, our preliminary lineage analysis in TcMAC21 mouse embryos has demonstrated a decrease in the number of intermediate progenitor cells and consequently in the neurons that they normally produce. Focused analysis of TcMAC21 brain development may improve our understanding of humans developing with trisomy 21.

Disclosures: **Z. Atak:** A. Employment/Salary (full or part-time):; Children's National Medical Center, University of Maryland. **S. Gandhi:** A. Employment/Salary (full or part-time):; Virginia Tech. **K.A. Pasion:** None. **J. Williams:** A. Employment/Salary (full or part-time):; Howard University, Children's National Medical Center. **T.F. Haydar:** A. Employment/Salary (full or part-time):; George Washington University, University of Maryland, Children's National Medical Center.

Poster

PSTR382. Down Syndrome

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR382.11/Web Only

Topic: A.07. Developmental Disorders

Support: NIH Grant AG056850
NIH Fellowship AG00096

Title: Microglia morphological and functional differences in the developing Down syndrome brain

Authors: *I. RIVERA[1,2,3], M. LIOUDYNO[2,3], B. HSU[4], T. XIAO[4], A. MISHRA[4], J. BUSCIGLIO[1,2,3,4];
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Abstract: Lifespan studies investigating the development of Alzheimer's disease (AD) in individuals with Down syndrome (DS) has provided insight into various contributing factors,

including mitochondrial dysfunction, oxidative stress, neuroinflammation, microglial alteration, and accelerated amyloid beta accumulation. While microglia morphological changes and neuroinflammation have been observed in DS as early as during childhood, the precise timing of the emergence of chronic inflammation and microglial alterations in DS, which arises as a genetic disorder at conception, remains unclear. Here, we explore microglia morphology and function in the Dp(16)1Yey/+ (Dp16) mouse model of DS at early stages of development. Immunofluorescence analysis using the microglial marker IBA1 at postnatal day (P)9, corresponding to an infantile stage of development, showed increased surface area in cortical microglia, and increased cell body area, branch number and branch length in hippocampal microglia. Building upon our lab's previous findings demonstrating the therapeutic potential of mitochondrial catalase (mCAT) in reducing oxidative damage and reversing mitochondrial dysfunction in Dp16 cells in vitro; we investigated whether overexpression of mCAT could reverse the microglia alterations in infantile Dp16 mice by generating a mCAT:Dp16 transgenic mouse model. Preliminary results indicate that overexpression of mCAT in Dp16 mice reverses the above-described alterations in microglia morphology. Thus, the abnormal microglia phenotype observed in trisomic mice is associated with oxidative stress and mitochondrial dysfunction in infant trisomic mice. Analysis of both normal and Down syndrome human prenatal cortical tissue showed similar microglial alterations. Further we evaluated microglial function, by establishing microglia cultures from euploid and trisomic prenatal cortical tissue suspensions. Phagocytic function was tested using fluorescent bioparticles and IBA1 immunostaining. Preliminary results indicate that trisomic microglia exhibited an increase in particle engulfment per cell compared to euploid cultures. Ongoing analysis of cell-specific gene expression profiles and functional assays will further characterize the structural and functional differences in trisomic microglia. These results suggest that early interventions during development may be crucial to prevent and/or reduce chronic inflammation and microglial structural and functional abnormalities in DS.

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Poster

PSTR382. Down Syndrome

Location: WCC Halls A-C

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Program #/Poster #: PSTR382.12/A23

Topic: A.07. Developmental Disorders

Support: Funded by RegenXbio company

Title: Anti-nkcc1 gene therapy rescues cognitive deficits and alleviates neuroinflammation in the ts65dn mouse model of down syndrome

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Abstract: A common feature of diverse brain disorders is the alteration of GABA-mediated inhibition due to aberrant intracellular chloride homeostasis induced by changes in the expression and/or function of chloride transporters. Notably, pharmacological inhibition of the chloride importer NKCC1 is able to rescue brain-related core deficits in animal models of these pathologies, and in some clinical studies in patients. In this study, we tested the potential therapeutic applications of NKCC1 gene knock-down by intraparenchymal injection of three different doses of adeno-associated virus type 9 (AAV9) expressing neuron-specific artificial microRNA (amiR) targeting NKCC1 in adult animals of the Ts65Dn mouse model of Down syndrome (DS). We found that NKCC1 silencing rescued cognitive impairment of Ts65Dn mice toward their WT littermate levels. Moreover, we showed that basal microglia over-activation, a characteristic feature of DS phenotype, was also rescued by reducing neuronal NKCC1 expression in Ts65Dn mice. Notably, AAV9 vector expressing neuron-specific NKCC1 amiR also prevented astrogliosis, microglial activation and NF-kB upregulation induced by control AAV9 injection in both wildtype and Ts65Dn mice. Our results provide compelling evidence that NKCC1 is implicated in cognitive dysfunctions in neuroinflammation in DS adult mice, thereby firmly supporting the potential breakthrough of NKCC1 knockdown gene therapy in DS.

Disclosures: F. Ghandour: None. **A. Contestabile:** None. **L. Cancedda:** Other; co-founder of IAMA Therapeutics.

Poster

PSTR382. Down Syndrome

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR382.13/B1

Topic: A.07. Developmental Disorders

Support: NIH Grant R01HD100607-01

Title: Augmented GIRK2 channel signaling in neonatal Ts65Dn mice: Implications for aberrant formation of developing neural circuits in Down syndrome

Authors: J. JIN, D. CHAN, B. LE, A. TAN, ***A. KLESCHEVNIKOV**;
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Abstract: Down syndrome (DS) is a genetic disorder resulting from the triplication of chromosome 21 (HSA21) and is characterized by cognitive impairment. Neuronal and synaptic abnormalities most profoundly accumulate during the initial period of brain development. We hypothesized that triplication of *Kcnj6*, which encodes Girk2 subunits of G-protein-coupled potassium channels, contributes significantly to the development of faulty neural circuits in early brain development in DS. To assess the feasibility of this hypothesis, we compared neuronal and molecular properties of *Kcnj6*/Girk2 in neonatal Ts65Dn mice, a genetic model of DS. Consistent with previous observations in adult animals, Western blot analysis showed that Girk2 protein levels were approximately 50% higher in the hippocampus of Ts65Dn *vs*. 2N neonatal

(p5-p8) mice. The efficiency of Girk2 channel signaling was also considerably increased in the hippocampus of neonatal Ts65Dn mice. Thus, bath applications of the GABAB agonist baclofen (40 μM for 5 minutes) to acute hippocampal slices evoked greater whole-cell currents and produced a more substantial reduction in input resistance in granule cells of Ts65Dn compared to 2N pups. Furthermore, neuronal excitability, which depends on the functional activity of Girk2 channels, was significantly reduced in regular Ts65Dn mice, but not in Ts65Dn mice with only 2 copies of *Kcnj6* (Ts65Dn/*Kcnj6*++- mice). Indeed, the threshold currents required for neuronal excitation were in 2N 25.5±2.9 pA, in Ts65Dn 45.7 ± 6.2 pA ($p = 0.01$), and in Ts65Dn/*Kcnj6*++-: 21.0 ± 1.1 pA ($p = 0.3$, n.s.). Finally, the spontaneous activity of neurons, which depends on neuronal excitability, was lower in Ts65Dn *vs*. 2N cells. Thus, in support of our hypothesis, this data suggests that the triplication of *Kcnj6* and subsequent increased expression of Girk2 result in enhanced Girk2 channel signaling, leading to reduced neuronal excitability and spontaneous neuronal activity during the period of intensive synaptogenesis. These alterations may contribute to the formation of faulty neural circuits in the hippocampus of mouse models of Down syndrome.

Disclosures: J. Jin: None. **D. Chan:** None. **B. Le:** None. **A. Tan:** None. **A. Kleschevnikov:** None.

Poster

PSTR382. Down Syndrome

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Program #/Poster #: PSTR382.14/B2

Topic: A.07. Developmental Disorders

Support: FPI-SO Grant PRE2018- 084504
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Agencia Estatal de Investigación Grant: PID2019-110755RB-I00/AEI/ 10.13039/501100011033

Title: Defective engram allocation contributes to impaired fear memory performance in Down syndrome

Authors: *Á. FERNÁNDEZ BLANCO, A. ZAMORA-MORATALLA, M. SABARIEGO NAVARRO, C. SIERRA NOGUERA, M. DIERSSEN;
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Abstract: Down syndrome (DS) is the most common genetic form of intellectual disability and is caused by the triplication of the human chromosome 21. Memory deficits are a hallmark feature of DS, but the underlying cellular mechanisms are not completely understood. Our laboratory and others demonstrated alterations in key mechanisms that are fundamental for engram formation and reactivation in DS mouse models. These previous results led us to propose that DS is a model of defective engrams. Thus, we investigated engram-specific alterations using

engram tagging techniques and chemogenetic intervention in Ts65Dn, a DS mouse model. We detected a sparser neuronal activation in Ts65Dn mice after both the acquisition and recall of a contextual fear conditioning memory in the dentate gyrus, compared to WT mice. Memory impairment was not recovered by artificially reactivating the trisomic engram cells using clozapine N-oxide (CNO) in Ts65Dn mice. While WT engram cells showed an enhanced number of dendritic spines compared to non-engram cells, trisomic engram cells did not, indicating a lack of structural plasticity that is required for a proper memory consolidation. Moreover, we also found that trisomic engram cells did not enhance their excitability immediately after memory recall, which has been reported to be important for context discrimination. Our results indicate that the trisomic engram is not properly formed, thus preventing memory to be completely recalled in the future. Hence, focusing on strategies capable of restoring the process of engram formation, such as manipulating neuronal excitability, might be promising to rescue memory deficits in trisomic mice.

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Poster

PSTR382. Down Syndrome

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR382.15/B3

Topic: A.07. Developmental Disorders

Support: CIHR

Title: Spatiotemporal gene expression profiling in Down Syndrome reveals perturbed microglia-neural cell interactions in the subventricular niche

Authors: *A. FENG, B. KUKREJA, N. TAHMASIAN, B. RUSU, T. CAO, B. KALISH; Neurosci. and Mental Hlth., The Hosp. For Sick Children, Toronto, ON, Canada

Abstract: Down syndrome (DS) is the most common genetic cause of intellectual disability. However, our current understanding of the early-life molecular alterations contributing to DS pathogenesis remains limited. Here, we employed a multi-modal spatial and single-cell gene expression analysis of mouse and human fetal brain tissue to identify transcriptomic changes during both early life and aging in DS. Using Multiplex Error-Robust Fluorescent In-Situ Hybridization (MERFISH), we curated a panel of 500 genes and performed cross-sectional comparisons between euploid (n=3) and Ts65Dn mouse model of DS (n=3) brain samples at postnatal day 0 (P0) and 6 months (6mo). Simultaneously, we employed single-nucleus RNA sequencing (snRNA-seq) on human fetal euploid (n=5) and DS (n=5) brain tissues collected during gestational weeks 13-19. Over 1 million mouse cells were profiled by MERFISH, while over 107,000 human cells were profiled by snRNA-seq. Focusing on the subventricular zone (SVZ) in P0 mice, our spatially-resolved differential gene expression analyses revealed a

downregulation of the cell surface ligand CD47, responsible for the “don’t eat me” signal, in several neuronal lineages, including transit-amplifying progenitors, neuroblasts, and mature neurons. Concurrently, our snRNA-seq results demonstrated a similar downregulation of CD47 and CD24 across neuronal cells of the fetal DS brain. CD33, a cell surface ligand known to reduce microglia phagocytosis upon activation, was downregulated in P0 Ts65Dn SVZ microglia. These findings indicate the downregulation of receptors implicated in the "don't eat me" signaling pathway accompanied by a concomitant increase in phagocytic microglia expression in the DS brain. In addition, spatially-aware ligand-receptor analysis of the P0 SVZ revealed differentially expressed cell-cell interactions involving chemokine, interferon, and sonic hedgehog ligand and receptors between immune and cell types along the neuronal lineage. Moreover, pathway analyses revealed significant alterations in signaling pathways known to impact microglial activity, including the Wnt, JAK/Stat, Notch, cytokine, and interleukin pathways. Based on these collective findings, we propose that microglial dysregulation leads to negative reprogramming of NSCs within the neurogenic niche in DS, thus contributing to impaired neurogenesis, altered neuronal morphology, and cortical hypocellularity in DS.

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Poster

PSTR382. Down Syndrome

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR382.16/B4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA U01 AG051412
NIA U19 AG068054

Title: Hippocampal subiculum volume is associated with memory performance in older adults with Down syndrome

Authors: ***J. LORITSCH**[1], N. QUEDER[3], J. ADAMS[4], J. NGUYEN[3], N. DIPROSPERO[4], N. TUSTISON[5], D. KEATOR[2], L. MCMILLAN[2], M. T. SATHISHKUMAR[2], L. TAYLOR[2], E. DORAN[2], D. NGUYEN[2], C. HOM[2], J. C. PRICE[6], H. D. ROSAS, 02129[7], A. M. BRICKMAN[8], N. SCHUPF[8], W. SILVERMAN[2], I. LOTT[2], E. HEAD[2], M. MAPSTONE[2], M. YASSA[2]; [1]Neurobio. and Behavior, [2]Univ. of California Irvine, Irvine, CA; [3]Univ. of California, Irvine, Irvine, CA; [4]UC Irvine, Irvine, CA; [5]Univ. of Virgina, Charlottesville, VA; [6]Radiology, Massachusetts Gen. Hosp., Boston, MA; [7]Martinos Ctr. for Biomed. Imaging, Charlestown, MA; [8]Columbia Univ., New York, NY

Abstract: Individuals with Down syndrome (DS) are at a high risk of developing Alzheimer’s disease (AD), which is thought to be due to the overexpression of the amyloid precursor protein on chromosome 21 and associated early age of onset of beta-amyloid plaque accumulation. AD

progression in the neurotypical population is associated with hippocampal volume loss and a decline in memory. Additionally, there is a relationship between decreases in hippocampal subfield volumes, particularly the cornu ammonis 1 (CA1) and subiculum, and cognitive decline. Here, we assess the relationship between CA1 and subiculum volumes and memory performance in older adults with DS who progressed clinically to mild cognitive impairment (MCI) and dementia compared to those who remained cognitively stable (CS). We used 1mm3 T1-weighted MRI scans from 74 cognitively stable individuals with DS (mean age 48 +/- 6.18, 45% female) enrolled in the Alzheimer's Disease in Down Syndrome (ADDS) study that included 3 sites. Hippocampal subfields were segmented using Deep Fusion Labeling for Automated Segmentations for the Hippocampus (DeepFLASH) software, and were normalized by total intracranial volume. Memory performance was assessed with the modified cued recall test (mCRT). Participants who converted to dementia or MCI in the follow-up visit, approximately 18 months later, were deemed as converters (n=11 or 15% of sample). We assessed the relationship between regions of interest and memory using linear regression analysis and evaluated the differences between sub-groups using linear regression analysis. Sex and site were used as covariates. Lower memory scores were associated with reduced subiculum volume across all participants (R2=.084, p=.032). No association between memory performance and volume was found in the CA1 (R2=.007, p=.329). Additionally, we found no differences in subiculum and CA1 volumes between the converter and non-converter groups. Our results suggest that decreased subiculum volume is associated with worse memory scores in older adults with DS who converted from CS to MCI or dementia. Future analysis will investigate the effect of structural changes in the hippocampus on longitudinal memory decline in this cohort and in younger adults with DS.

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Poster

PSTR382. Down Syndrome

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR382.17/B5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA U01 AG051412

Title: Characterizing the Relationship Between Hippocampal Subfield Volumes, Plasma Neurofilament Light Chain, and Memory in Older Adults with Down Syndrome

Authors: ***J. NGUYEN**[1,2], N. DIPROSPERO[2], J. ADAMS[2], J. LORITSCH[2], N. QUEDER[2], N. TUSTISON[3], D. B. KEATOR[4], L. MCMILLAN[2], M. T. SATHISHKUMAR[2], L. TAYLOR[2], E.

DORAN, 92617[5], C. HOM[4], J. C. PRICE[8], H. D. ROSAS[8], A. M. BRICKMAN[9], N. SCHUPF[10], W. SILVERMAN[5], I. T. LOTT[5], E. HEAD[6], M. MAPSTONE[7], M. YASSA[11];
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Abstract: Down syndrome (DS) is associated with a heightened risk of developing Alzheimer's disease (AD), with an estimated 70% of individuals manifesting AD by age 60. Neurofilament light chain (NfL) is a protein that is a marker for axonal injury and is elevated in cerebrospinal fluid and blood with neurodegeneration. Plasma NfL levels increase with age and with AD progression in individuals with DS. This study investigated the association between plasma NfL, hippocampal subfield volumes, and memory function in DS. The analyses included 109 participants with DS from 3 sites (age 49.3 ± 6.6 years, 47 female, 78 cognitively stable, 21 mild cognitive impairment, 10 possible dementia). We collected T1-weighted 1mm3 magnetic resonance imaging (MRI) scans to assess subfield volumes, modified Cued Recall Test (mCRT) scores to measure episodic memory performance, and plasma to measure NfL. MRI data were processed with DeepFLASH, a recently-developed deep learning-based tool for automatic segmentation of hippocampal subfields. We hypothesized a positive association between hippocampal subfield volumes and memory function and a negative association with plasma NfL.
After adjusting for covariates (age, sex, site) and intracranial volume, we found significant positive associations between volumes of hippocampal subfields (dentate gyrus/CA3, CA1, and subiculum) and mCRT scores. Our findings also revealed that higher plasma NfL levels, which indicate more neurodegeneration, were associated with decreased subfield volumes in the hippocampus and poorer memory performance on the mCRT.
While the memory-volume correlations showed no difference across clinical groups, the correlations between hippocampal subfield volumes and plasma NfL were present only in individuals with MCI and dementia.
These results highlight the vulnerability of all hippocampal subfields to neurodegeneration in individuals with DS. Increases in plasma NfL may reflect clinically-relevant hippocampal atrophy, demonstrating its potential use as a biomarker for neurodegeneration in this population.

Disclosures: J. Nguyen: None. **N. DiProspero:** None. **J. Adams:** None. **J. Loritsch:** None. **N. Queder:** None. **N. Tustison:** None. **D.B. Keator:** None. **L. McMillan:** None. **M.T. Sathishkumar:** None. **L. Taylor:** None. **E. Doran:** None. **C. Hom:** None. **J.C. Price:** None. **H.D. Rosas:** None. **A.M. Brickman:** None. **N. Schupf:** None. **W. Silverman:** None. **I.T. Lott:** None. **E. Head:** None. **M. Mapstone:** None. **M. Yassa:** None.

Poster

PSTR383. Molecular Mechanisms of Neurodevelopmental Disorders II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR383.01/B6

Topic: A.07. Developmental Disorders

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Jerome Lejune Foundation -Role of neuropeptides on BBB formation and maintenance in a 22q11.2DS mouse mode

Title: Oxytocin promotes blood brain barrier formation and maintenance in brain microvascular endothelial cells in early postanatal developmental stages

Authors: *M. BUSNELLI[1], C. PAOLINI[1], A. BENEDETTI[2], W. BARCIK[2], G. CASTELLANI[2], G. TRIGILIO[2], F. RE[3], F. PAPALEO[2], B. CHINI[1];
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Abstract: Blood brain barrier (BBB) alterations have detrimental consequences on brain development, resulting in cognitive and behavioral deficits. In a 22q11.2 deletion syndrome mouse model, also known as Di George syndrome, an impairment of the BBB has been recently described (Crocket et al., 2021). This BBB defect was confirmed in the LgDel/$^{+}$ mouse model of the syndrome, extensively characterized at the neurobiological and behavioral level (see Castellani G et al. abstract n. 2023-S-9935-SfN). In this animal model, a perinatal OXT treatment was found to rescue developmental trajectories and behavioral deficits in adolescent and adult mice. These effects were linked to an increase of the tight-junction protein claudin-5 and restoration of BBB integrity (see Castellani G et al. abstract n. 2023-S-9935-SfN) suggesting a new, direct effect of OXT on brain microvascular endothelial cells.
Here, we thus investigated at the cellular and molecular level the effects of OXT on brain microvascular endothelial cells, focusing on early postnatal developmental stages. To check the formation and integrity of the barrier, we prepared brain microvascular endothelial cells (BVEC) from wild type (WT) and LgDel/$^{+}$ mice at postnatal day 3-5 and we measured transepithelial electrical resistance (TEER), small solutes permeability and claudin-5 levels. Cells were treated for up to 14 days with OXT or vehicle. We observed that chronic OXT treatment in LgDel/+ BVEC i) significantly increased TEER; ii) restored permeability for the small tracer Lucifer yellow; iii) normalized claudin-5 levels. Similar effects on TEER, permeability and claudin-5 levels were also observed in WT BVEC cells and confirmed in two well established brain endothelial cell lines, BEND-3 and hCMEC/D3 cells, routinely used to study BBB properties *in vitro*. OXT effects on proliferation, migration and tube formation are in progress. As these results indicate a key role of OXT in regulating brain microvascular endothelial cells, we investigated the molecular targets of OXT. We found that BVEC, hCMEC/D3 and BEND-3 express OXT receptors (OXTR) and V1a vasopressin receptors (V1aR), both of which are pharmacological targets of the neuropeptide. We are currently testing a series of selective OXT and vasopressin agonists and antagonists to dissect the intracellular pathways that contribute to barrier properties of brain microvascular endothelial cells.

-References: Crockett AM, et al. Disruption of the blood-brain barrier in 22q11.2 deletion syndrome. Brain. (2021)

Disclosures: M. Busnelli: None. **C. Paolini:** None. **A. Benedetti:** None. **W. Barcik:** None. **G. Castellani:** None. **G. Trigilio:** None. **F. Re:** None. **F. Papaleo:** None. **B. Chini:** None.

Poster

PSTR383. Molecular Mechanisms of Neurodevelopmental Disorders II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR383.02/B7

Topic: A.07. Developmental Disorders

Support: NIH Grant 5R01MH112860
NIH Grant UG3
2022 NARSAD Young Investigator Grant

Title: Unveiling the molecular mechanisms of neurodevelopmental alteration in a SETD1A-deficient forebrain organoid model

Authors: *Z. SUN[1], H. ZHU[1], Y. SUN[1], J. FAN[1], B. XU[1], J. A. GOGOS[1,2,3,4];
[1]Psychiatry, Columbia Univ., new york, NY; [2]Mortimer B. Zuckerman Mind Brain and Behavior Inst., [3]Dept. of Physiol. and Cell. Biophysics, [4]Dept. of Neurosci., Columbia Univ., New york, NY

Abstract: Genetic disruption of *SETD1A*, a lysine methyltransferase best known for its role in mediating methylation of the lysine 4 on the histone H3 protein, is robustly associated with schizophrenia (SCZ) ($p = 1x10^{-12}$) and confers substantial risk for SCZ (odds ratio = 20). *SETD1A* mutations appear to increase neurodevelopmental vulnerability but the detailed mechanisms, including the genomic binding properties and targets of SETD1A in the developing human brain, as well as the cell type-specific alterations resulting from *SETD1A* mutations remain largely unknown. To address these questions, we generated forebrain organoid models of the developing human cerebral cortex derived from wild type and mutant isogenic human induced pluripotent stem cells (hiPSCs) carrying *SETD1A* SCZ risk mutations introduced by CRISPR/Cas9 genome editing. Chromatin profiling using CUT&Tag assays combined with RNA sequencing on sorted cortical neurons led to the identification of high-confident target genes, which are significantly enriched in neuropsychiatric genetic liability. Using single-cell RNA sequencing and follow-up experimental validation, we find that *SETD1A* disruption leads to the changes in the expression of lineage driver genes and altered development of cortical excitatory neurons. The aberrant transcription program underlying impaired development contains molecular signatures of key regulatory factors known to modulate neurogenesis. Our ongoing experiments are investigating the morphological and functional alterations caused by *SETD1A* deficiency. Our study advances our understanding of SETD1A genomic binding

properties, uncovers molecular and neurodevelopmental alterations due to *SETD1A* mutations and may facilitate efforts for therapeutic interventions.

Disclosures: **Z. Sun:** None. **H. Zhu:** None. **Y. Sun:** None. **J. Fan:** None. **B. Xu:** None. **J.A. Gogos:** None.

Poster

PSTR383. Molecular Mechanisms of Neurodevelopmental Disorders II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR383.03/B8

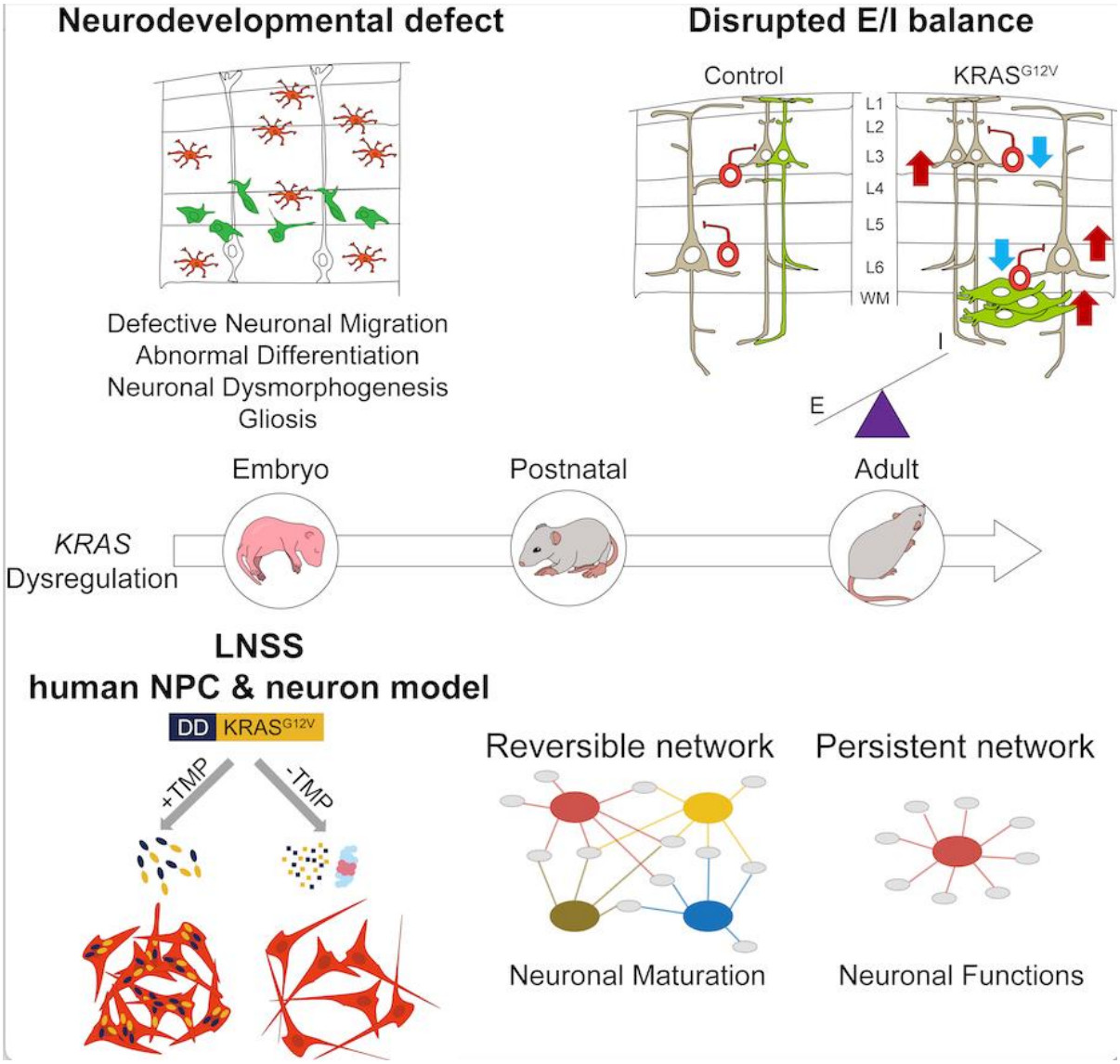
Topic: A.07. Developmental Disorders

Support: NRF-2022R1A2C1002925
NRF-2017R1A5A1015366

Title: Unraveling the Pathological Mechanisms and Reversibility of Neurodevelopmental Defects in Linear Nevus Sebaceous Syndrome caused by dysregulation of KRAS

Authors: Y. KIM, *H.-E. LEE, S. BAEK;
POSTECH, Pohang-si, Korea, Republic of

Abstract: Linear nevus sebaceous syndrome (LNSS) is a neurocutaneous disorder arising from somatic gain-of-function mutations in *KRAS* or *HRAS*. LNSS brains exhibit neurodevelopmental defects, such as cerebral defects and epilepsy, yet the underlying pathological mechanisms and potential treatment strategies remain largely unclear. In this study, we demonstrate that the introduction of $KRAS^{G12V}$ during mouse cortex development induces subcortical nodular heterotopia and heightened excitability, effectively recapitulating major pathological manifestations observed in LNSS. Additionally, we uncover that the reduced firing frequency of inhibitory neurons, independent of $KRAS^{G12V}$ expression, disrupts the delicate balance between excitation and inhibition. Leveraging transcriptional profiling following destabilization domain-mediated clearance of $KRAS^{G12V}$ in human neural progenitors and differentiating neurons, we identify reversible functional networks that contribute to LNSS. Neurons expressing $KRAS^{G12V}$ exhibit molecular changes associated with delayed neuronal maturation, the majority of which can be restored upon $KRAS^{G12V}$ clearance. These findings provide valuable insights into the molecular networks involved in the reversibility of some neuropathologies observed in LNSS, arising from dysregulation of the RAS pathway.



Disclosures: **Y. Kim:** None. **H. Lee:** None. **S. Baek:** None.

Poster

PSTR383. Molecular Mechanisms of Neurodevelopmental Disorders II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR383.04/B9

Topic: A.07. Developmental Disorders

Support: NIH R01 DA053743
NIH R01 DA054714
NIH R01 NS114086

NIH T32 HG008961
Civitan International Research Center Emerging Scholar Award

Title: Transcription factor 4 coordinates striatal neuron specification, maturation, and dopamine transmission

Authors: *N. ROBINSON[1], J. HINDS[1], L. JAMES[3], S. THOMPSON[1], J. TUSCHER[1], L. IANOV[2], B. PHILPOT[3], J. DAY[1];
[1]Neurobio., [2]Civitan Intl. Res. Ctr., Univ. of Alabama at Birmingham, Birmingham, AL; [3]Cell Biol. and Physiol., Univ. of North Carolina, Chapel Hill, NC

Abstract: The striatum subserves myriad roles in motor learning, executive function, and motivated behaviors. At a molecular level, striatal development is governed by spatiotemporally precise gene expression programs that are orchestrated by master transcription factors (TFs) operating within progenitor populations of the ganglionic eminences (GEs). Notably, a host of neurodevelopmental disorders are caused by mutations in these TFs and are characterized by delayed motor development coupled with cognitive and behavioral dysfunction, thus implicating the striatum in the pathophysiology of these disorders. Pitt-Hopkins syndrome (PTHS), which is caused by loss-of-function mutations in the neuronal master TF, transcription factor 4 (TCF4), manifests as a constellation of motor and behavioral symptoms. However, the extent to which striatal neurodevelopment is impaired in PTHS remains poorly understood. Using gene expression profiling across diverse paradigms of rodent and human striatal neurodevelopment, we have uncovered a previously unknown function for TCF4 in regulating gene networks governing neurogenesis, cell type specification, and neuroplasticity in medium spiny neurons (MSNs) and their GE progenitors. Notably, these transcriptional networks are disrupted in the striatum of *Tcf4*$^{+/-}$ mice, which serve as a robust preclinical model of PTHS. Mechanistically, TCF4 choreographs the temporal dynamics of MSN fate specification, and TCF4 deficiency results in persistent progenitor marker expression in the mature striatum. Moreover, TCF4 controls the distribution of D1- *versus* D2-MSNs, which mediate the motor and behavioral outputs of the striatum through their coordinated responses to dopamine. In line with this, modulation of *Tcf4* expression using an innovative dual CRISPR activation/interference strategy alters the transcriptional and electrophysiological responses of MSNs to dopamine. Our results reveal TCF4 as an essential regulator of the molecular events that define MSN development and neuromodulation. These findings expand our fundamental understanding of neurodevelopment and provide a foundation for examining the contributions of TCF4-driven transcriptional programs in the striatum to neurodevelopmental disease.

Disclosures: **N. Robinson:** None. **J. Hinds:** None. **L. James:** None. **S. Thompson:** None. **J. Tuscher:** None. **L. Ianov:** None. **B. Philpot:** None. **J. Day:** None.

Poster

PSTR383. Molecular Mechanisms of Neurodevelopmental Disorders II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR383.05/B10

Topic: A.07. Developmental Disorders

Support: NIH Grant 1R01AA023508-01A1

Title: Maternal Environment Shapes Mouse Embryonic Hindbrain Vulnerability to Prenatal Alcohol Exposure: Insights from Allele-Specific Expression Analysis

Authors: N. ELAZZABI[1], J. RICHARD ALBERT[1], P. PAVLIDIS[2], *K. HAMRE[3], D. GOLDOWITZ[1];
[1]Med. Genet., [2]Psychiatry, Univ. of British Columbia, Vancouver, BC, Canada; [3]Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Fetal alcohol spectrum disorders (FASD) pose a global challenge, encompassing lifelong neurocognitive and growth deficiencies resulting from prenatal alcohol exposure (PAE). Previous studies on monozygotic and dizygotic twins, as well as investigations using selectively bred and inbred rodents, have implicated the role of both maternal and fetal genetics as risk factors for FASD. Notably, certain mouse inbred lines (e.g, C57BL/6) exhibit susceptibility to PAE-induced morphological malformations, while others (eg, DBA/2J) demonstrate relative resistance. However, the primary determinants of FASD susceptibility, whether it is the maternal environment, the fetal genome, or both, remains poorly understood. In the present study, we employed a mouse model of PAE to generate inbred and hybrid F1 embryos using female and male C57BL/6J (B6) and DBA/2J (D2). We implemented four mating schemes: B6xB6, B6xDBA, DBAxB6, DBAxDBA (maternal strain X paternal strain). On embryonic day 9, around the time of neural tube closure, pregnant female mice were randomly assigned to a two-dose gavage of either an iso-caloric control (maltose dextrin) or an alcohol (a total dose of 5.8g/kg) treatment group. Seven hours following the administration of the first dose, we collected the E9.5 embryos, dissected the hindbrains, and performed RNA extraction. Employing a multi-pronged approach utilizing both allele-specific and allelic agnostic RNA-seq differential analysis, our findings demonstrate: (1) a lack of evidence supporting PAE-induced differential allelic expression, including genomic imprinting and strain-biased expression AND (2) embryos with identical genomic compositions but developing in mothers of different inbred lines exhibit contrasting responses to ethanol exposure. PAE significantly alters gene expression in hybrid embryos developing in B6 mothers, with a comparable significant increase and decrease in gene expression observed. In contrast, minimal changes in gene expression are seen in hybrid embryos developing in D2 mothers. Specifically, the genes with significant increases in expression are associated with immune effector processes such as leukocyte-mediated immunity. Conversely, the genes with significant decreases in expression are involved in histone modifications, particularly peptidyl-lysine acetylation. Based on our findings, we conclude that the maternal environment has a significant role in determining the vulnerability of the embryonic hindbrain to ethanol-induced transcriptome changes. These findings provide molecular insights into the mechanisms underlying FASD vulnerability.

Disclosures: N. Elazzabi: None. **J. Richard Albert:** None. **P. Pavlidis:** None. **K. Hamre:** None. **D. Goldowitz:** None.

Poster

PSTR383. Molecular Mechanisms of Neurodevelopmental Disorders II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR383.06/B11

Topic: A.07. Developmental Disorders

Support: Eureka! Scholarship for Biological Majors

Title: Effects of Oxidative Stress During Cortical Development

Authors: *A. ACHANTA[1], B. C. CAMPBELL[1], M. CAMPBELL[2];
[1]UCSD, La Jolla, CA; [2]UCSD Dept. of Neurosciences, La Jolla, CA

Abstract: The balance of oxidation and reduction (redox) reactions are critical for normal cellular function. An excess of reactive oxygen species (ROS) creates a redox imbalance, leading to oxidative stress. Oxidative stress can damage cellular components and disrupt normal cellular mechanisms, making it a critical cellular phenomenon to understand. To determine the natural redox state of developing cortical neurons, we electroporated mouse embryos with Grx1-roGFP2 at E13 and performed both live and modified fixed imaging of the embryo brains at E15. For the modified fixed imaging, brain slices were first alkylated by NEM treatment before fixation to lock the sensor's status. On the other hand, sequential detection of oxidized and reduced Grx-roGFP2 during live confocal imaging allowed the determination of neuronal redox states. After establishing the baseline redox states, we exposed the pregnant mice to different concentrations of known oxidants and reductants (diamide and DTT) to create a gradient of the redox state at those concentrations of the oxidants and reductants. We next sought to determine the developmental impact of the most commonly prescribed analgesic during pregnancy, acetaminophen. Acetaminophen, when metabolized, generates toxic byproducts which deplete glutathione, one of the most abundant and critical antioxidants in the brain, increasing neuronal susceptibility to oxidative stress. We exposed pregnant mice to acetaminophen and compared the neuronal redox state to that after exposure to known oxidants and reductants to assess the impact of the acetaminophen. Our data shows that assessing the susceptibility of developing neurons to changes in the redox system is critical for understanding environmental stressors and their impact on neurodevelopment. This knowledge can further elucidate the mechanisms of many neurodevelopmental disorders as well as the potential environmental causes.

Disclosures: A. Achanta: None. **B.C. Campbell:** None. **M. Campbell:** None.

Poster

PSTR383. Molecular Mechanisms of Neurodevelopmental Disorders II

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Program #/Poster #: PSTR383.07/B12

Topic: A.07. Developmental Disorders

Support: NIH Grant 3R01NS123163-01A1S1
NIH Grant 1R01NS123163-01A1

Title: Confirming a gain of function (GoF) pathogenic mechanism related to PASC1 Syndrome through treatment with antisense oligonucleotides (ASOs)

Authors: *M. FAIRBANKS SANTANA[1], A. D. GUEMEZ-GAMBOA[2];
[1]Dept. of Neurosci., [2]Physiol., Northwestern Univ., Chicago, IL

Abstract: Confirming a gain of function (GoF) pathogenic mechanism related to PACS1 Syndrome through treatment with antisense oligonucleotides (ASOs).Authors *M. Fairbanks Santana, A. D. Guemez-Gamboa; Neuroscience, Northwestern University, Chicago, IL.**DisclosuresM. Fairbanks Santana:** None**. A. D. Guemez-Gamboa:** None.**Abstract**PACS1 Syndrome is a neurodevelopmental disorder (NDD) caused by a single missense R203W substitution in the Phosphofurin Acidic Cluster Sorting 1 (PACS1) protein. PACS1 is known for its roles in the endosomal pathway and, more recently, it is being studied for its functions in the cell nucleus. Over 200 patients have been identified with PACS1 Syndrome, all presenting the same variant, and all display convergent phenotypes of Autism Spectrum Disorder (ASD) and intellectual disability (ID). Analysis conducted in our lab has shown that mature neurons are strongly enriched for risk genes of NDDs with overlapping phenotypes of PACS1. Moving forward, I am investigating whether ASO-mediated changes in gene expression from PACS1 R203W derived neuronal cell models are consistent with a GoF mechanism, additionally researching the potential of ASOs as a treatment. First, I tested the effect of four different non-allele specific PACS1 ASO candidates, provided by IONIS Pharmaceuticals, in induced pluripotent stem-cell (iPSC) derived neurons (iNeurons). Differentiation was induced by overexpression of transcription factor neurogenin-2 (NGN2). iNeurons were then matured for 3 weeks prior to a 72-hour treatment. Control ASOs were used at a concentration of 20 µM, whilst the PACS1 candidates were studied at concentrations of 1, 10 and 20 µM. The knock-out effect was then evaluated through Western blotting and candidate PACS1 ASO 0001 was identified as the most effective option to move forward. I obtained neurons for three different isogenic pairs: two patient lines and their respective CRISPR/Cas9 corrected cell lines, as well as wildtype line and its corresponding R203W edited variant. I treated two separate differentiations for all cells with both positive and negative control ASOs as well as PACS1 ASO 0001. Running this experiment in parallel with three different CRISPR edited PACS1 full knock-out lines, my objective is to discern ASO efficiency. Extracted RNA after treatment was then submitted for Bulk RNA Sequencing. I expect to assess whether changes in the transcriptomic profile due to a lack of PACS1 as caused by ASO treatment are a result of a GoF mechanism, as well as examining if genotype specific DEGs are restored and confirm therapeutical viability.

Disclosures: M. Fairbanks Santana: None. **A.D. Guemez-Gamboa:** None.

Poster

PSTR383. Molecular Mechanisms of Neurodevelopmental Disorders II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR383.08/B13

Topic: A.07. Developmental Disorders

Support: R01NS117434
T32NS115656-01A1

Title: Understanding mechanisms underlying white matter regeneration after rodent and piglet perinatal hypoxia

Authors: *L. ROSKO, K. BRANCH, V. GALLO;
Neurosci., Children's Natl. Hosp., Washington, DC

Abstract: Diffuse white matter injury (DWMI) is a highly prevalent neonatal brain injury leading to life-long neurological and motor impairments such as those seen in cerebral palsy. Perinatal hypoxia (HX) is a major contributor to DWMI with rodent HX models recapitulating many hallmarks of neurological damage occurring in DWMI patients. Since DWMI is a modifiable risk for adverse neurodevelopmental outcomes, understanding the cellular and molecular mechanisms underlying HX could lead to new therapies that harness endogenous regenerative mechanisms. Previous work from our lab shows that endothelin-1, an important signaling molecule present in radial glial cells of the subventricular zone (SVZ), is essential for maintaining rodent progenitor proliferation in development and after white matter demyelination. In addition to rodents, in this study, we utilized a piglet model which is gyrencephalic and highly translational to humans. We hypothesized that endothelin-1 signaling may regulate progenitor proliferation in SVZ after HX in both rodents and piglets. To investigate this hypothesis, we utilized a rodent HX model where male and female mice were exposed to 10.5% oxygen from postnatal (P) day P3 to P11 and then sacrificed at varying timepoints to investigate cellular expression of endothelin-1 and its receptors A (EndrA) and B (EndrB). We show that about one week post HX, endothelin-1 (p=0.009, n=4) and EndrB mRNA (p=0.03, n=3) are upregulated in the SVZ with little change in expression of EndrA mRNA (p=0.4, ns, n=4). The rodent SVZ displays increased proliferation of $Olig2^{+}$ oligodendrocyte lineage cells (OLC) one week following HX (p=0.048, n=3), but this proliferative response is ablated when endothelin-1 is removed from radial glial cells using a *nestin-cre^{ER}* model (p=0.025, n=3). Furthermore, the removal of endothelin-1 from radial glia led to fewer OLC in the white matter 3 weeks following HX (p=0.019, n=3). This suggests that endothelin-1 is essential for the proliferation of OLC in the rodent SVZ following HX. We also investigated endothelin-1 signaling in the piglet brain after HX using a model where male and female neonatal piglets were exposed to 10.5% oxygen from P3 to P14. In normoxic piglets, endothelin-1 is highly expressed in $sox2^{+}$ radial glia (100%, n=2) and expressed in about ten percent of oligodendrocyte precursor cells (10% +/- 1.2, n=2). Preliminary data suggest that endothelin-1 expression increases in the piglet SVZ following HX. These data suggest that endothelin-1 signaling could play a role in progenitor regeneration after hypoxia with a potential therapeutic avenue for developing therapeutics for infants with DWMI.

Disclosures: L. Rosko: None. **K. Branch:** None. **V. Gallo:** None.

Poster

PSTR383. Molecular Mechanisms of Neurodevelopmental Disorders II

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Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR383.09/B14

Topic: A.07. Developmental Disorders

Support: American SIDS Institute

Title: Cerebellar Spatial Transcriptomic Landscape of Neonates with Sudden Infant Death Syndrome (SIDS)

Authors: J. GHAEMMAGHAMI, A. VALENZUELA, T. DEAN, V. GALLO, *P. KRATIMENOS;
Children's Natl. Hospital, George Washington Univ. Sch. of Med. and Hlth. Sci., Washington, DC

Abstract: Approximately 3500 infants die annually in the United States from sudden infant death syndrome (SIDS). The cerebellum makes direct connections with vital centers of brainstem nuclei, indicating a potential neurological link between abnormal cerebellar development and SIDS.We hypothesized that cerebellar cytoarchitectural and molecular abnormalities in the human neonate result in neural circuitry disruption, leading to cardiorespiratory suppression and SIDS.We analyzed postmortem human cerebellum from term infants with SIDS and compared them with matched non-SIDS subjects to delineate mechanisms of SIDS pathogenesis. We performed neuropathological analysis combined with bulk, single-cell, and spatial transcriptomics. We compared postmortem human cerebellum of infants with SIDS with matched controls, and we found disrupted cytoarchitecture, including 1) Purkinje cell (PC) and Granule cell (GC) heterotopias. Furthermore, significant changes in the morphology of PCs were also observed, together with a thicker external GC layer (EGL), indicating arrested migration of the GCs from the EGL to the Internal granule cell layer (IGL). To investigate the molecular mechanisms underlying cerebellar abnormalities in SIDS pathogenesis, we defined the transcriptome of early postnatal human cerebellum using RNA-seq. We found a significant number (904) of differentially expressed genes (DEGs) between SIDS and matched controls, indicating complex changes in specific gene networks that regulate cerebellar functions in infants with SIDS. Using spatial and single-cell transcriptomics, we identified maturational signature differences in infants with SIDS compared to matched non-SIDS subjects.The current studies provide a comprehensive analysis of cerebellar abnormalities that contribute to dysfunctional cerebellar-brainstem circuitry in infants with SIDS. Our study may contribute to developing novel biomarkers for primary or secondary preventative strategies of SIDS.

Disclosures: J. Ghaemmaghami: None. **A. Valenzuela:** None. **T. Dean:** None. **V. Gallo:** None. **P. Kratimenos:** None.

Poster

PSTR383. Molecular Mechanisms of Neurodevelopmental Disorders II

Location: WCC Halls A-C

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Program #/Poster #: PSTR383.10/B15

Topic: A.07. Developmental Disorders

Support: JSPS KAKENHI Grant JP19H03629
JSPS KAKENHI Grant JP19K07059
JSPS KAKENHI Grant JP20K21589

Title: *MED13L* and its pathogenic variants influence the dendritic development of cerebral cortical neurons in mouse brain

Authors: *K.-I. NAGATA, N. HAMADA;
Inst. Dev. Res., Aichi Dev. Disability Ctr., Kasugai, Japan

Abstract: The Mediator complex comprises multiple subcellular subunits that collectively function as a molecular interface between RNA polymerase II and gene-specific transcription factors. Recently, genetic variants to one subunit of the complex, known as MED13L, has been implicated in syndromic intellectual disability and distinct facial features, frequently accompanied by heart defects. We investigated the impact of 5 disease-associated MED13L variants on the subcellular localization and biochemical stability of MED13L protein in vitro as well as in vivo. In overexpression assays using cortical neurons from embryonic mouse cerebral cortices transduced by in utero electroporation-mediated gene transfer, we found that mouse orthologues of human MED13L-P866L and -T2162M missense variants accumulated in the nucleus, while the S2163L and S2177Y variants were diffusely distributed in the cytoplasm. In contrast, we found that the Q1922-term truncation variant barely detectable in transduced cells, a phenotype reminiscent of this variant that results in MED13L haploinsufficiency in humans. Next we analyzed these variants for their effects on neuronal migration, dendritic growth, spine morphology, and axon elongation of cortical neurons in vivo. There, we found that overexpression of P866L variant resulted in reduced number and length of dendrites of cortical layer II/III pyramidal neurons. Furthermore, we show that mMED13L-knockdown abrogated dendritic growth in vivo, and this effect was significantly rescued by co-expression of an RNAi-resistant mMED13L, but weakly by the T2162M variant, and not at all by the S2163L variant. However, overexpression of the S2163L variant inhibited mature dendritic spine formation in vivo. Expression of each of the 5 variants did not affect neuronal cell migration and callosal axon elongation in vivo. Taken together, our results demonstrate that MED13L expression is relevant to corticogenesis and influences the dendritic branching characteristics of cortical excitatory neurons. Our study also suggests that disease-associated MED13L variants may directly cause morphological and functional defects in cortical neurons in different ways.

Disclosures: K. Nagata: None. **N. Hamada:** None.

Poster

PSTR383. Molecular Mechanisms of Neurodevelopmental Disorders II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR383.11/B16

Topic: A.07. Developmental Disorders

Title: Epigenetic dysregulation in developing neocortical neurons associated with neurodevelopmental disorders

Authors: *Y. KIM[1], T. GOTO[1], T. WATANABE[2], S. FUJINO[2], Y. SUGAYA[2], M. KANO[2,3], D. KAWAGUCHI[1], Y. GOTOH[1,3];
[1]Grad. Sch. of Pharmaceut. Sciences, The Univ. of Tokyo, Bunkyo-ku, Tokyo, Japan; [2]Grad. Sch. of Medicine, The Univ. of Tokyo, Bunkyo-ku, Tokyo, Japan; [3]Intl. Res. Ctr. for Neurointelligence (WPI-IRCN), The Univ. of Tokyo, Bunkyo-ku, Tokyo, Japan

Abstract: Epigenetic regulation plays a critical role in gene expression dynamics during brain development, and its dysregulation has been implicated in neurodevelopmental disorders. KDM6B (also known as JMJD3), an H3K27me3 specific demethylase, is a high-risk autism spectrum disorder (ASD) gene; mutations at the *Kdm6b* locus have also been found in schizophrenia (SCZ) and intellectual disability (ID) patients. However, it remains largely unknown whether deletion of *Kdm6b* during development results in phenotypes associated with neurodevelopmental disorders. We therefore generated *Kdm6b* conditional knockout (cKO) mice using a *Nex*-Cre line, in which Cre recombinase was expressed in postmitotic excitatory neurons in the developing neocortex and hippocampus. Results showed that adult *Kdm6b* cKO mice exhibited hyperactivity in open field tests and reduced sociability in three-chamber tests. In addition, *Kdm6b* cKO pups at postnatal day 7 emitted less calls than control pups after being separated from their dams in an ultrasonic vocalization test. These results indicate that dysfunction of *Kdm6b* in cortical and hippocampal excitatory neurons leads to behavioral abnormalities associated with neurodevelopmental disorders. Remarkably, we also found that *Kdm6b* cKO results in abnormal neocortical layer structures such as a significant reduction of cortical expression of the layer 4 marker RORb (also a high-risk gene for ASD) and mislocalization of SATB2-positive callosal neurons to layer 1. Furthermore, a reduction in the size of rostral areas containing the prefrontal cortex (PFC) was observed in the early postnatal stages. In addition to these morphological abnormalities, a physiological excitation/inhibition (E/I) imbalance within the medial PFC was observed, which may underlie behavioral deficits associated with neurodevelopmental disorders. RNA-seq analysis revealed dynamic molecular changes in *Kdm6b* cKO mice, and the differentially expressed genes were highly enriched in psychiatric disease-related genes. Taken together, our data indicate that KDM6B in postmitotic excitatory neurons plays a pivotal role in cortical layer and neural circuit formation, which may be necessary for suppressing neurodevelopmental disorders, through the regulation of gene expression dynamics during development.

Disclosures: Y. Kim: None. **T. Goto:** None. **T. Watanabe:** None. **S. Fujino:** None. **Y. Sugaya:** None. **M. Kano:** None. **D. Kawaguchi:** None. **Y. Gotoh:** None.

Poster

PSTR383. Molecular Mechanisms of Neurodevelopmental Disorders II

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Program #/Poster #: PSTR383.12/B17

Topic: A.07. Developmental Disorders

Support: NIEHS T32 training grant T32ES007254
NIEHS R01 grant R01ES031823
NIEHS P30 grant P30ES030285

Title: Brain derived extracellular vesicle biogenesis and memory-related synaptic plasticity are altered by early-life exposure to Deltamethrin

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Abstract: During development, the blood brain barrier is vulnerable to the lipophilic properties of Deltamethrin (DM) a type of commonly used pesticide. With the popularity of DM and other related pesticides in both agricultural and general public use, there is growing concern of links between developmental pyrethroid exposure and the incidence of neurodevelopmental disorders. Brain derived extracellular vesicles (BDEVs) are phospholipid nanovesicles involved in communication between cells that have been linked to the pathophysiology of neurological diseases. Here, we hypothesized DM exposure during development alters the role of the biogenesis of BDEVs, which may lead to dysfunctional synaptic plasticity in the hippocampus. Using a developmental exposure model, pregnant dams were exposed to 3 mg/kg/72 hours, equivalent to 1mg/kg/day, DM or vehicle through pregnancy and lactation. At post-natal day 30 BDEVs were extracted from both control and DM-exposed males and analyzed by transmission electron microscopy (TEM) to determine size and validated by western blot analysis. BDEVs were marked with a green-fluorescent membrane marker to locate them in the brain after ICV injections. Male WT mice received either control or DM-exposed exosomes and processed for hippocampal slice electrophysiological field recordings. BDEV presence in the hippocampus was confirmed in all slices used for recording fEPSP in the CA1 region. To study the effects of BDEVs (control vs. DM) on synaptic plasticity at Schaffer collateral - CA1 pyramidal cell synapses, a high-frequency stimulation was used to induce long-term potentiation (LTP) paradigm. We found that WT mice treated with BDEVs from DM-exposed animals showed significantly impaired LTP, with no changes in basal synaptic transmission (input-output curves and pair-pulse ratio). These results indicate that BDEVs play a key role in DM-induced neurotoxicity in the brain.

Disclosures: L. Koff: None. **J. Di Re:** None. **Y. Avchalumov:** None. **M. Marosi:** None. **S. Chand:** None. **L. hallberg:** None. **B. Ameredes:** None. **T. Green:** None. **S. Yelamanchili:** None. **G. Pendyala:** None. **F. Laezza:** None.

Poster

PSTR383. Molecular Mechanisms of Neurodevelopmental Disorders II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR383.13/B18

Topic: A.07. Developmental Disorders

Support: NIMH R01_MH125528

Title: Uncovering the cellular mechanism of Schizophrenia associated gene SETD1A heterozygous mutation in a human neural model

Authors: *X. SU[1,2], Y. HONG[3], H. ZHANG[4], L. WANG[2], S. ZHANG[4], Q. YANG[3], H. SONG[3], J. DUAN[4], G.-L. MING[3], Z. P. PANG[2];
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Abstract: Rare mutations in SETD1A are strongly associated with schizophrenia (SZ), a debilitating mental disorder affecting 1% of the population, and other severe neurodevelopmental disorders. SETD1A encodes a component of the histone methyltransferase complex producing mono-, di, and trimethylated histone H3 at Lysine 4 (H3K4). H3K4 trimethylation (H3K4me3) and H3K4me1 are epigenomic marks of active gene transcriptional promoters and enhancers, respectively. Interestingly, histone methylation has also been suggested as one of the most enriched gene pathways in common variant-based genome-wide associations studies (GWAS) of major psychiatric disorders. However, the detailed molecular mechanism by which it causes neuronal dysfunction is still unclear. Recent advances in stem cell biology have allowed the efficient conversion of human stem cells into defined neuron subtypes allows to address this question. Using CRISPR/Cas9 gene editing, we have generated isogenic hiPSC lines carrying heterozygous mutations of SETD1A on different genetic backgrounds. We are able to generate neuronal subtypes including excitatory, inhibitory and dopaminergic neurons to study cell-type specific impact of SETD1A heterozygous mutations. Preliminary morphological and electrophysiological analyses of induced excitatory neurons carrying SETD1A heterozygous mutations showed defective synaptic transmission. The transcriptomic result reveals dysregulated synaptic gene expressions in induced excitatory neurons carrying SETD1A heterozygous mutations, which suggests SETD1A heterozygous mutations may lead to synaptic dysfunction in excitatory neurons. Ongoing experiments are evaluating SETD1A heterozygous mutations with functional, morphological, biochemical and genomic parameters in other neuronal subtypes to understand the cellular mechanisms that how SETD1A heterozygous mutations contributes to the pathogenesis of SZ. The study enables us to perform a well-controlled assessment of the impact of SETD1A heterozygous mutations on the molecular and cellular mechanisms underlying deficits in early neurodevelopment and synaptic properties.

Disclosures: **X. Su:** None. **Y. Hong:** None. **H. Zhang:** None. **L. Wang:** None. **S. Zhang:** None. **Q. Yang:** None. **H. Song:** None. **J. Duan:** None. **G. Ming:** None. **Z.P. Pang:** None.

Poster

PSTR383. Molecular Mechanisms of Neurodevelopmental Disorders II

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Topic: A.07. Developmental Disorders

Support: NIH Grant R21-D097561
NIH Grant R01-HD104609
Doft Family postdoctoral Innovator Award

Title: Mutations in the autism risk gene DDX3X affect morphogenesis and synaptogenesis

Authors: *J. LUKIN[1,2,3,4,5], A. MOSSA[2,3,4,5], C. FIORENZANI[6], Y. PARK[2,3,4,5], Z. AKPINAR[2,3,4,5], S. DE RUBEIS[2,3,4,5];
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Abstract: Mutations in the X-linked RNA helicase DDX3X cause DDX3X syndrome, a neurodevelopmental condition frequently associated with autism spectrum disorder. DDX3X escapes X chromosome inactivation in human and mice, resulting in higher expression in female brains when compared with male brains. Further, mutations in the *DDX3X* gene affect primarily females. DDX3X regulates mRNA translation, but the mechanisms of action in neurons, the impact of clinical mutations, and the influence of sex have not been studied yet.Using a *Ddx3x*^{flox} mouse line generated at the Seaver Autism Center, we developed a cellular model to examine the impact of *Ddx3x* mutations in both male and female neurons. Primary cortical neurons were cultured from male embryos (*Ddx3x*$^{+/y}$ or *Ddx3x*$^{flox/y}$) or female embryos (*Ddx3x*$^{+/+}$, or *Ddx3x*$^{flox/+}$, or *Ddx3x*$^{flox/flox}$). Transfections with constructs carrying Cre and mCherry allowed us to model male control (*Ddx3x*$^{+/y}$) or null (*Ddx3x*$^{-/y}$) neurons, as well as female control (*Ddx3x*$^{+/+}$), haploinsufficient (*Ddx3x*$^{+/-}$) or null (*Ddx3x*$^{-/-}$) neurons. We also introduced *DDX3X* pathogenic mutations identified in male and female patients. We then examined dendritic arborizations and synapse number and morphology and compared sexes and genotypes. Additionally, we conducted immunoprecipitations and unbiased quantitative proteomics to gain insight into the molecular mechanisms underlying the effects of DDX3X mutations.Our findings demonstrate the critical involvement of Ddx3x in dendritogenesis and synaptogenesis, with its effects being influenced by both sex and gene dosage. Changes in neuronal morphogenesis were accompanied by alterations in the neuronal proteome, affecting the expression of proteins crucial for neuronal migration, neurite outgrowth, and those encoded by risk genes for neurodevelopmental disorders.

We also found that mutations identified in female patients are more deleterious than mutations identified in male patients.Our findings support a sex-specific role of *Ddx3x* in neuronal development and lay the bases to understand the sex biases in the prevalence and severity of DDX3X syndrome. These findings also contribute to a better understanding of the molecular mechanisms underlying DDX3X syndrome and may inform future research and therapeutic approaches.

Disclosures: J. Lukin: None. **A. Mossa:** None. **C. Fiorenzani:** None. **Y. Park:** None. **Z. Akpinar:** None. **S. De Rubeis:** None.

Poster

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Program #/Poster #: PSTR383.15/B20

Topic: A.07. Developmental Disorders

Support: NIH Grant R01NS131620

Title: Toward understanding the molecular and cellular basis of RNA exosome-linked pontocerebellar hypoplasia type 1b: a cerebellar organoid model

Authors: *N. A. BARR, J. L. BURFORD, R. E. KANG, J. GADA, L. A. HIGGINSON, A. SETH, D. J. MORTON;
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Abstract: Pontocerebellar Hypoplasia Type 1b (PCH1b) is a devastating autosomal recessive neurodevelopmental disorder characterized by significant atrophy of the cerebellum and pons. The cerebellum and pons integrate information from sensory systems, the spinal cord, and other parts of the brain to regulate breathing, motor movements, and learning motor behavior. Currently, there is no cure for PCH1b and treatment is purely palliative. Mutations that cause PCH1b occur in the *EXOSC3* gene, encoding a structural cap subunit of an essential RNA processing complex, the RNA exosome. The RNA exosome complex is an evolutionary conserved and ubiquitously expressed ribonuclease composed of structural and catalytic subunits that play a critical role in the post-transcriptional regulation of RNA. Although the RNA exosome is expressed in all cells and tissues, alterations in RNA exosome function disproportionately result in significant neurological impairments, thus underscoring the enhanced reliance of the nervous system on post-transcriptional regulation of gene expression. Cerebellar pathology in RNA exosome-linked PCH1b is challenging to understand based on current models of PCH1b. The goal of this study is to begin to define requirements for RNA exosome subunit EXOSC3 during cerebellar development through the study of *EXOSC3*-linked PCH1b mutations. To determine requirements for the RNA exosome during cerebellar development, we developed a 3D human induced pluripotent stem cell (hiPSC)-derived cerebellar organoid system to recapitulate neuronal dysfunction and degeneration known to characterize PCH1b. We have

engineered an allelic series of missense mutations encoding PCH1b-linked amino acid changes (EXOSC3-G31A and EXOSC3-G191C) of differing severity in hiPSCs via CRISPR/Cas9 technology to generate a cerebellar organoid model of PCH1b. We are combining single-cell transcriptomics and novel molecular tools to investigate the transcriptomic dynamics of generated cells during cerebellar differentiation as well as defining key post-transcriptional regulatory events mediated by the RNA exosome. My preliminary data show reduced organoid size as well as molecular and cellular defects that alter the differentiation of cerebellar cell lineages in organoids modeling EXOSC3-G31A, a severe genotype in individuals with PCH1b. Results from this work will provide a framework to study how the RNA exosome functions in early cerebellar development and has the potential to transform our understanding of PCH1b pathogenesis.

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Poster

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Program #/Poster #: PSTR383.16

Topic: A.07. Developmental Disorders

Support: NIH Grant F99NS125819

Title: The role of the Vsx2 super-enhancer during human retinal organoid development

Authors: *V. HONNELL, S. SWEENEY, M. DYER;
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Abstract: Prior studies have identified thousands of enhancers with developmental stage-specific and cell-type specific activity in retinal neurons. However, the major challenge in the field is determining which enhancer(s) regulates each gene in a cell-type and developmental stage-specific manner. We recently identified a super-enhancer upstream of the *Vsx2* gene that is necessary and sufficient for the complex pattern of *Vsx2* expression during development. *Vsx2* is expressed in retinal progenitor cells and maintained in differentiated bipolar neurons and Müller glia. We found that this developmental stage-specific and cell-type specific expression pattern is achieved by distinct modules within the mouse super-enhancer. One module (Region 0) is responsible for retinal progenitor cell proliferation. Deletion of this module leads to microphthalmia. A separate module (Region 3) regulates bipolar cell genesis. Deletion of this module leads to a complete loss of a single cell type, the bipolar cells. Bipolar neurons are present in the Region 0 deletion retina and the eye is normal size in the Region 3 deletion retina demonstrating that the *Vsx2* super-enhancer is modular. We hypothesize that this pattern of expression will be recapitulated in a model of human retinal development. To test this hypothesis, we generated retinal organoids from human embryonic stem cells (hESCs)

containing the region deletions previously examined in mice. We examined cell type presence and retinal progenitor cell proliferation using RNA-sequencing, immunofluorescence, EdU labeling, and scRNA-seq across multiple stages of retinal organoid development. We found that Region 0 deletion organoids are small compared to wildtype controls, and that they recapitulate the phenotype observed in microphthalmic Region 0 deletion mice. These datasets will offer insights into the enhancer regions regulating *Vsx2* gene expression in humans and could identify previously unknown enhancer candidates contributing to microphthalmia.

Disclosures: V. Honnell: None. **S. Sweeney:** None. **M. Dyer:** None.

Poster

PSTR383. Molecular Mechanisms of Neurodevelopmental Disorders II

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Program #/Poster #: PSTR383.17/B21

Topic: A.07. Developmental Disorders

Title: Uncovering the Influence of Maternal Immune Inflammation and IL-17A on Autism Spectrum Disorder Pathways

Authors: *M. JHANJI[1,2], P. GHATE[4,5], J. M. VACHARASIN[4,7], F. D. RITCHIE[6], E. CHUKWURAH[4], S. MATHEW[8], S. LIZARRAGA[3,2];
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Abstract: Autism spectrum disorder (ASD) encompasses a spectrum of neurodevelopmental conditions characterized by impairments in social interaction, communication, and repetitive behaviors. Emerging evidence suggests that maternal immune inflammation during pregnancy, triggered by severe viral infections, may contribute to the development of some forms of ASD. Our study aims to elucidate the role of maternal immune activation (MIA) and the cytokine IL-17A in ASD pathogenesis. Animal studies have demonstrated that MIA leads to elevated levels of IL-17A in the developing offspring, resulting in ASD-like phenotypes. IL-17A, primarily produced by Th17 lymphocytes, has been detected in immune cells and plasma of children with ASD. However, the precise mechanisms through which IL-17A exposure impacts the development of early human neuronal circuitry and contributes to ASD pathogenesis remain poorly understood. To address this knowledge gap, we have developed human cellular models of neuronal development by leveraging the use of human embryonic stem cells (ESCs). Our preliminary findings indicate that exposure to IL-17A induces alterations in the expression of known ASD-associated genes and cytokine genes. Notably, these changes in gene expression are accompanied by a decrease in synaptic activity in IL-17A-exposed ESC-derived neurons. We

propose that IL-17A serves as a crucial mediator in ASD pathogenesis by modulating the regulation of both ASD-related and cytokine genes, as well as synaptic activity. Understanding the interplay between maternal immune inflammation, IL-17A, and ASD pathobiology holds promise for the development of targeted therapeutic strategies for individuals with ASD.

Disclosures: M. Jhanji: None. **P. Ghate:** None. **J.M. Vacharasin:** None. **F.D. Ritchie:** None. **E. Chukwurah:** None. **S. Mathew:** None. **S. lizarraga:** None.

Poster

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Program #/Poster #: PSTR383.18/B22

Topic: A.07. Developmental Disorders

Support: NIH Grant HD104558

Title: Molecular mechanisms of translational control during human neuronal development in Fragile X Syndrome

Authors: *V. G. SHANKAR[1], I. TISNOVSKY[2], J. ZHANG[1], N. RAJ[4], O. KATSARA[5], M. KALINOWSKA[1], R. SCHNEIDER[5], G. J. BASSELL[4], E. KLANN[1,3];
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Abstract: Dysregulation of translation forms the basis of the molecular pathogenesis of Fragile X Syndrome (FXS), one of the most characterized, X-inherited neurodevelopmental disorders, with individuals presenting intellectual disabilities and autistic spectrum disorder (ASD). FXS is caused by loss of the protein product of the *FMR1* (Fragile X Messenger Ribonucleoprotein 1) gene, which results in alterations in synaptic plasticity, neuronal development and growth, and an overall increase in global *de novo* translation and proteostasis. An RNA binding protein, FMRP (Fragile X Messenger Ribonucleoprotein) has a wide array of roles and appears to regulate the localization and the translation status of specific mRNAs, and it is required for proper differentiation and development of neurons. Although FMRP is known to regulate translation by affecting elongation, perhaps binding to and stalling ribosomes, the precise molecular mechanisms that are responsible for phenotypes in FXS have been elusive. Here, we explore the role of FMRP in regulating translation, particularly translation initiation, using excitatory neurons differentiated from patient-derived induced pluripotent stem cells (iPSCs). We observed changes in levels of various translation initiation factors (IFs) over the course of neuronal development in both FXS patients and normal individuals. Studies have shown that human neuronal development requires various stages of translational programming, with differentiation leading to progressively less actively translating cells. This reduced translational load appears to be via a switch to alternate, non-canonical translation initiation machinery, evident in changes in

levels of various translation IFs over the course of neuronal development. In FXS however, levels of certain non-cannonical IFs, such as eIF4G2, eIF4G1 and eIF3d continue to remain elevated through development, as do S6K and 4E-BP, effectors of the mTORC1 pathway. FXS patient-derived neurons are more sensitive to knock down of eIF4G2 (DAP5), but less sensitive to 4EGI-1 based inhibition of cap-dependent translation, hinting at a compensatory mechanism in place that necessitates eIF4G2 upon loss of FMRP. Regardless, proper translation control is lost in FXS over neuronal development, and growth of neurons is slower. We predict that neuronal development requires utilizing cap-dependent but eIF4E-independent methods of translation instead of the canonical eIF4-based translation initiation, a shift that is mediated directly or indirectly by FMRP.

Disclosures: **V.G. Shankar:** None. **I. Tisnovsky:** None. **J. Zhang:** None. **N. Raj:** None. **O. Katsara:** None. **M. Kalinowska:** None. **R. Schneider:** None. **G.J. Bassell:** None. **E. Klann:** None.

Poster

PSTR383. Molecular Mechanisms of Neurodevelopmental Disorders II

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Title: The regulation of mRNA stability during cortical development

Authors: *L. D. SERDAR[1], J. EGOL[1], G. HU[2], D. L. SILVER[3];
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Abstract: The cerebral cortex is a region of the brain responsible for higher cognitive function. Defects in cortical development are associated with neurodevelopmental disorders including autism and intellectual disability. Mechanisms controlling the dynamic regulation of gene expression in the developing brain are incompletely understood. While transcription has long been recognized as an important regulator of gene expression during corticogenesis, the contribution of mRNA decay is understudied. Here, we employ mouse models to investigate the mRNA stability landscape of the developing brain and reveal the consequences of misregulating mRNA stability in the cortex. We use SLAM-seq to measure mRNA decay rates transcriptome-wide across multiple stages of development. mRNA stability profiles are compared across each stage, and between mRNAs enriched in individual cortical cell types to generate an expansive

view of mRNA stability in the developing brain. We further examined the cis-acting factors underlying stability regulation and identified codon content as a major predictor of mRNA half-lives. To demonstrate the impact of mRNA decay on cortical development, we conditionally knocked out *Cnot3*, an essential component of the CCR4-NOT deadenylase complex, in neural progenitors. We find that loss of *Cnot3* leads to severe microcephaly and widespread p53-dependent apoptosis. These defects are accompanied by a reduction in progenitor density, prolonged cell cycle duration, and disorganization of cortical neurons. To assess the contribution of apoptosis to these phenotypes we generated *Cnot3;Trp53* double cKO mice, and discovered that defects in corticogenesis arise from both p53-dependent and independent mechanisms. Overall, our data reveal critical new insights into the mRNA stability landscape of the developing brain and underscore the important role of mRNA turnover during development.

Disclosures: **L.D. Serdar:** None. **J. Egol:** None. **G. Hu:** None. **D.L. Silver:** None.

Poster

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Topic: A.07. Developmental Disorders

Support: NINDS Grant R01NS123163-01

Title: Identifying the molecular mechanism of PACS1 Syndrome pathogenesis

Authors: *A. L. SCHRODER, A. D. GUEMEZ-GAMBOA;
Neurosci., Northwestern Univ., Chicago, IL

Abstract: PACS1 Syndrome is a NDD hallmarked by craniofacial abnormalities and intellectual disability.Patients with PACS1 Syndrome have a single *de novo* missense mutation at c.607C>T of the Phosphofurin Acidic Cluster Sorting 1 (PACS1) protein, which causes an Arginine to Tryptophan substitution (R203W). PACS1 is a multifunctional sorting protein, with key roles in regulating trafficking of target proteins to and from the trans-Golgi Network (tGN). We have previously shown that PACS1 (+/R203W) forebrain organoids develop mature glutamatergic neurons with impaired expression of synaptic signaling genes when compared to isogenic controls.Additionally, PACS1 (+/R203W) neurons have prolonged network bursts, which has implications for circuit formation. While these results highlight the impact that the R203W variant of PACS1may play in the broader context of neuronal development, it remains unknown how the R203W variant alters the function of PACS1 in the cell. Using a combination of biochemistry and live cell imaging, we are now identifying how the R203W variant impacts the function of PACS1. TheR203W variant is in the Furin Binding Region (FBR) of PACS1, which is the region responsible for cargo protein interactions dependent upon acidic-cluster motifs. We hypothesize that theR203W variant will impact the trafficking dynamics of PACS1-associated cargo in between the tGN and plasma membrane through its interactions with membrane specific

adaptor proteins for localization. Our data indicates there is no difference between the localization of PACS1 WT and R203W. This, however, does not provide any information regarding the dynamics ofPACS1-associated trafficking, thus we are currently performing live imaging of PACS1 and its associated cargo. Given the localization of the R203W variant to the FBR, we are also performing BioID to identify how the R203W variant impacts PACS1 interactome, with the hypothesis that multiple binding interactions will be disrupted and/or gained as a consequence of this R203W substitution.

Disclosures: A.L. Schroder: None. **A.D. Guemez-Gamboa:** None.

Poster

PSTR383. Molecular Mechanisms of Neurodevelopmental Disorders II

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Program #/Poster #: PSTR383.21/B25

Topic: A.07. Developmental Disorders

Support: Research Training Program stipend
The National Health and Medical Research Council

Title: The role of medial pulvinar in the maturation of Parvalbumin interneurons; implications in cognitive neurodevelopmental disorders

Authors: *N. SEYED HOSSEINI FIN[1], J. SCOTT[2], L. TEO[1], J. HOMMAN-LUDIYE[1], J. A. BOURNE[2];
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Abstract: Background Cognitive neurodevelopmental disorders (CNDs) are associated with abnormal maturation of parvalbumin (PV+) interneurons in the prefrontal cortex (PFC). However, the mechanisms underlying this impairment remain unclear. The medial pulvinar (PM), a primate-specific thalamic subdivision, establishes widespread connectivity with the PFC. PV+ cells are particularly a major recipient of thalamic inputs. Mounting evidence indicates consistent PM disruption accompanied by decreased PV expression in CNDs. Thus, we investigated the time course of PV+ cell maturation in the PFC and examined the consequence of PM-PFC dysconnectivity during early postnatal life on PV+ cell maturation in adulthood.**Experimental design** Non-human primates (NHPs) recapitulate PFC subdivisions (i.e. dorsolateral PFC) as well as PM entity, similar to humans. Hence, an NHP model, the marmoset monkey, was selected for this research. We mapped the maturation trajectory of PV+ cells in the PFC in ages ranging from neonatal (postnatal day 7), infancy (postnatal month(pm)1), juvenile (pm3), and adolescence (pm9 and pm12) to adulthood (>pm19) (n=2). In the second experiment, we investigated the role of PM in the PV+ cell maturation by PM ablation through magnetic resonance imaging-mediated N-methyl-D-aspartate (NMDA) injection in the PM of neonates. NMDA injection leads to the excitotoxicity of PM neurones. The effects of early-life PM ablation on PV+ cell maturation were characterised in adulthood

(n=3).**Results** The density of PV+ interneurons (PV+ NeuN+ cells) peaked from neonatal to juvenile (0.01 vs. 0.09, p=0.005), while accumulation of perineuronal nets (PNNs) around PV+ cells (WFA+ PV+ cells) occurred between infancy and mid-adolescence (0.12 vs. 0.69, p<0.0001). PNN accumulation stabilises PV+ cell connectivity associated with the last stage of PV+ cell maturation. Notably, early-life PM lesion reduced PV+ cell density (0.13 vs. 0.06, p<0.0001), impaired PV+ inputs onto pyramidal neurones through quantifying perisomatic PV+VGAT+ puncta (0.24 vs. 0.09, p=0.002) and diminished the accumulation of PNNs around the PV+ cells (0.73 vs. 0.52, p=0.001) in the PM lesion compared to the control group. For statistics, the non-parametric Kruskal-Wallis test was accompanied by Dunn's multiple comparisons.**Conclusions** Our study underscores that PV+ cell maturation is protracted until mid-adolescence, and PM-PFC dysconnectivity alters this process leading to alterations of local neuronal networks, suggesting this subtype is a key determinant of normal PFC function. Further, the data support the PM hypo/dysconnectivity as a potential early indicator for CNDs diagnosis.

Disclosures: **N. Seyed Hosseini Fin:** None. **J. Scott:** None. **L. Teo:** None. **J. Homman-ludiye:** None. **J.A. Bourne:** None.

Poster

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Program #/Poster #: PSTR383.22/B26

Topic: A.07. Developmental Disorders

Support: NIH Grant R15NS124008-01

Title: Automated Processing of Neuronal Axon Initial Segment Morphology

Authors: *E. A. AQUINO, A. CONTRERAS, D. J. HINES, R. M. HINES;
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Abstract: The axon initial segment (AIS) is a highly specialized compartment of neuronal cells that controls cell firing. The AIS is an elaborate subcellular compartment of neurons with intracellular and extracellular components that concentrate and arrange voltage-gated ion channels and associated proteins critical for generating the action potential. A variety of homeostatically regulated factors can influence the probability of action potential generation at the AIS, including distance from the soma, AIS length, and changes in AIS diameter. Our understanding of the details of AIS morphology throughout development and pathology is somewhat limited due to the time-intensive analysis required to assess thousands of neuronal AIS at multiple time points. More autonomous, unbiased methods for analyzing large datasets are needed to expand our knowledge of AIS morphology throughout development and in nervous system pathology. In this project, we developed a new Python program to quickly and efficiently analyze immunohistochemically stained AISs in confocal images from different developmental

stages and animal models of human neurodevelopmental syndromes. It can individually label AISs, and provide morphology, proximity, and protein periodicity readouts for each AIS. This program will prove to be a powerful tool for experimenters interested in AIS analysis by improving the efficiency and fidelity of AIS analysis. Additionally, the program can be quickly altered to analyze the characteristics of other subcellular compartments. This research will aid in our understanding of AIS development, its progression, and how it is modified in neurodevelopmental disorders.

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Poster

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Topic: A.07. Developmental Disorders

Support: NS100007

Title: Conditional ablation of Miro1 from cortical excitatory neurons causes agitative- and anxiety-like behavior in mice

Authors: N. KERN[1], M. SAKHEIM[1], M. CASE[1], L. JARRAHY[1], L. BASEL[1], C. CHAPMAN[1], R. SHIN[1], *A. K. MYERS[1], J. A. GOLDEN[2];
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Abstract: Cognitive disorders are a diverse group of diseases with complex etiologies, many posited to arise during development. Autism spectrum disorder, schizophrenia, and bipolar disorder are a cluster of cognitive disorders with associated behavioral features that manifest early in life and have a wide range of symptoms including repetitive behavior, agitation, and anxiety. While the etiology of these disorders is not completely understood, recent studies have implicated mitochondrial dysfunction playing a role. Mitochondria are double membraned organelles that produce ATP and buffer intracellular calcium. To perform their cellular functions, mitochondria use intracellular transport. Failure to move to metabolically active regions of the cell results in vital energy deficits and reduced buffering capacity. Mitochondrial Rho-GTPase 1 (Miro1) is a mitochondrial outer membrane protein known to participate in microtubule-based mitochondrial motility and homeostasis in neurons. Previous research suggests that abrogation of MIRO1 can contribute to the onset of neurodegenerative diseases including Amyotrophic Lateral Sclerosis, Alzheimer's Disease, and Parkinson's Disease. In this study, we ablated *Miro1* from excitatory neurons using an *Emx1-cre* and examined the behavior of the young mice. *Miro1* conditional mutant animals exhibited agitative-like behaviors including decreased nesting behavior, repetitive behaviors, and decreased interactions with littermates. They also spent less time in the center of an open space and showed increased freezing behavior during the open field test, both considered anxiety-like behaviors. Daily cage activity, social behavior, and brain cFos

levels were atypical in the *Miro1* conditional mutant mice. Our data associate MIRO1 with the pathogenesis of some developmental neurocognitive disorders and implicate mitochondrial localization in anxiety-like behaviors.

Disclosures: **N. Kern:** None. **M. Sakheim:** None. **M. Case:** None. **L. Jarrahy:** None. **L. Basel:** None. **C. Chapman:** None. **R. Shin:** None. **A.K. Myers:** None. **J.A. Golden:** None.

Poster

PSTR383. Molecular Mechanisms of Neurodevelopmental Disorders II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR383.24/B28

Topic: A.07. Developmental Disorders

Support: Lundbeckfonden grant no. R361-2020-2654

Title: Exploring the Roles of Reticular Thalamic Nucleus in Brain Function and Dysfunction

Authors: *D. KIM;
Biomedicine, Aarhus Univ., Aarhus, Denmark

Abstract: Autism spectrum disorder (ASD) is a highly heritable (70-90%) neurodevelopmental disorder. Individuals with ASD show sleep dysregulation and defects in sensory gating, which is one of the key hypotheses put forward to explain ASD-associated sleep disruptions. Both preclinical and clinical studies highlight that the zona incerta (ZI) and reticular thalamic nucleus (TRN) are key brain regions that gate both sensory inputs and regulate sleep. Thus, some aspects of ASD features could be contributed to these two brain regions. In addition, ASD-associated genes identified in large human genetic studies are highly enriched in the ZI and TRN. Despite the importance of the ZI and TRN in ASD, however, relatively little is known about the development of these regions, as well as the potential roles of ASD-associated genes in the process.ASD and other neuropsychiatric disorders are linked to the aberrant development of cortical GABAergic neurons. ASD-like features are shown with disruptions in the specification of cortical GABAergic neurons in animal models. Developing ZI and TRN GABAergic neurons express many transcription factors that control the development of cortical GABAergic neurons, some of which are linked to ASD. Using molecular and genetic approaches, we 1) characterize the molecular mechanisms controlling the development of the ZI and TRN, and 2) identify the roles of ASD-associated genes on the ZI and TRN development.Our data find that loss-of-function of these ASD-associated genes leads to a loss of GABAergic neurons and alters the E/I balance in the ZI and TRN. The dysfunction in ZI/TRN GABAergic neuronal development could potentially contribute to ASD features, and I am to improve our understanding of two brain regions that are closely linked with ASD pathology.

Disclosures: **D. Kim:** None.

Poster

PSTR383. Molecular Mechanisms of Neurodevelopmental Disorders II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR383.25/B29

Topic: A.07. Developmental Disorders

Support: Teva bioinnovators program

Title: Sleep and temperature deficits in a Dravet syndrome mouse model

Authors: *S. FADILA[1], B. BREUCHER[2], I. GONZÁLEZ DOPESO-REYES[3], E. KREMER[3], M. RUBINSTEIN[4];
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Abstract: Rationale:Dravet syndrome (Dravet) is a developmental and epileptic encephalopathy (DEE) caused in most cases by de novo loss of function mutations in *SCN1A*. Sleep disturbances are very common in Dravet, and include problems initiating and maintaining sleep and daytime drowsiness. Problems in thermoregulation are also prominent in Dravet patients with reports of a tendency to overheat and decreased sweating. Thermoregulation is highly associated with mechanisms of sleep and sleep quality. Thus, we set to test if problems in temperature regulation and sleep deficits are connected in Dravet. **Methods**: To explore the neural link between thermoregulation and sleep, we performed body temperature, electrocorticography (ECoG), and depth recordings in the ventrolateral preoptic area (vLPO), with focus on the changes in body temperature in epochs of wake and NREM sleep. **Results**: Dravet mice exhibited lower baseline temperature, with reduced ability to maintain and control their body temperature in changing temperatures. Moreover, ECoG-temperature recordings revealed a failure to reduce their body temperature during wake-NREM transition. The vLPO is a sleep-promoting region involved in regulating body temperature. Recordings of the vLPO showed that an increase in the ambient temperature (from 25°C to 36°C) was associated with an increase in delta frequency (0.5-4Hz) in WT but not in Dravet mice. Delivery of *SCN1A* coding sequence using canine adenovirus 2 (CAV2-SCN1A) lead to an improved delta modulation in Dravet mice. Furthermore, chemogenetic-mediated increased activation of the vLPO also restored the temperature-dependent increase in delta activity. **Conclusions**: Together, our data indicate a connection between sleep and temperature deficits in Dravet and demonstrate an involvement of the vLPO and related regions.

Disclosures: S. Fadila: None. **B. Breucher:** None. **I. González Dopeso-Reyes:** None. **E. Kremer:** None. **M. Rubinstein:** None.

Poster

PSTR383. Molecular Mechanisms of Neurodevelopmental Disorders II

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Program #/Poster #: PSTR383.26/B30

Topic: A.07. Developmental Disorders

Support: DC011284
1F32DC020659

Title: Developmental hearing loss-induced perceptual deficits are rescued by genetic restoration of pre- and postsynaptic cortical inhibitory mechanisms

Authors: *S. MASRI[1], R. FAIR[3], T. MOWERY[4], D. SANES[2];
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Abstract: Even a transient period of developmental hearing loss during the developmental critical period can induce long-lasting deficits in temporal and spectral perception, which correlate with speech perception in humans. In gerbils, these hearing loss-induced perceptual deficits are correlated with a reduction of both ionotropic GABAA and metabotropic GABAB receptor-mediated synaptic inhibition. Therefore, we developed viral vectors to express proteins that would upregulate gerbil postsynaptic inhibitory receptor subunits (GABAA , Gabra1; GABAB, Gabrb1b) in pyramidal neurons, and an enzyme that mediates GABA synthesis (GAD65) presynaptically in parvalbumin-expressing interneurons. A transient period of developmental hearing loss from P11 to P23 during the auditory cortex critical period significantly impaired perceptual performance of the mongolian gerbil (Meriones unguiculatus, 52 normal hearing and 47 transient hearing loss animals of both sexes) on two auditory tasks: amplitude modulation depth detection and spectral modulation depth detection. We then tested the capacity of bilateral auditory cortex injections of each vector to restore perceptual performance on each task, relative to controls injected with GFP and to normal hearing animals. While both GABA receptor vectors increased the amplitude of cortical inhibitory postsynaptic potentials, only viral expression of postsynaptic GABAB receptors improved perceptual thresholds to those observed in normal hearing animals. Furthermore, presynaptic GAD65 expression in parvalbumin-expressing interneurons alone improved perceptual performance on spectral modulation detection. These findings suggest that recovering performance on auditory perceptual tasks depends on GABAB receptor-dependent transmission at the auditory cortex, especially at parvalbumin interneuron to pyramidal neuron synapses, and point to potential therapeutic targets for developmental sensory disorders.

Disclosures: S. Masri: None. **R. Fair:** None. **T. Mowery:** None. **D. Sanes:** None.

Poster

PSTR384. Development of Sensory Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR384.01/B31

Topic: A.08. Development of Neural Systems

Title: Developmental expression of P2X3 receptor in the mouse trigeminal ganglion

Authors: *M. ISHIKAWA[1], T. SATO[2], T. YAJIMA[2], K. MIZUTA[1], H. ICHIKAWA[2,3]; [1]Div. of Dento-oral Anesthesiol., [2]Div. of Oral and Craniofacial Anatomy,, Tohoku Univ., Sendai, Japan; [3]Sch. Organization Shokuryo Gakuin, Tokyo Shokuryo Dietitian Acad., Tokyo, Japan

Abstract: P2X3 receptor is activated by extracellular ATP. The purinergic receptor channel is associated with nociceptive transmission in the sensory ganglion. In this study, expression of P2X3 receptor was examined in trigeminal ganglion (TG) and facial tissues during development by immunohistochemistry. An avidin-biotin complex method was performed on the mouse TG and vibrrisal pad at postnatal day 0-21. At postnatal day 0 (P0), virtually all TG neurons were immunoreactive for P2X3 receptor. At P3-14, the proportion of P2X3-positive TG neurons gradually decreased (80-90%). And, approximately 60% of TG neurons in P21 mice contained P2X3 receptor-immunoreactivity. Only 40% of sensory neurons in the adult TG were immunoreactive for P2X3 receptor. In P21 and adult TGs, P2X3-containing neurons had mainly small to medium-sized cell bodies. At P0-7, numerous nerve fibers contained P2X3 receptor-immunoreactivity in the vibrissal pad. These nerve fibers showed intense immunoreaction and formed nerve bundles beneath vibrissae. They ascended their thick and thin branches toward vibrissae. Many P2X3 receptor-containing nerve fibers surrounded and entered vibrissal follicles. Within vibrissal follicles, P2X3 receptor-containing nerve fibers ramified repeatedly and made nerve terminals close to hair shafts. In the epithelium of the vibrissal pad, numerous free nerve endings also showed P2X3 receptor-immunoreactivity. At 14 and 21, P2X3 receptor-containing nerve fibers dramatically decreased in the vibrissal pad. As a result, some nerve terminal showed weak immunoreaction within hair follicles. However, many P2X3 receptor-containing nerve fibers within the epithelium appeared to remain unchanged. In the adult vibrissal pad, P2X3 receptor-containing nerve fibers were very infrequent. The present study suggests that P2X3 receptor and ATP play an important role in TG neurons during postnatal development.

Disclosures: M. Ishikawa: None. **T. Sato:** None. **T. Yajima:** None. **K. Mizuta:** None. **H. Ichikawa:** None.

Poster

PSTR384. Development of Sensory Systems

Location: WCC Halls A-C

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Topic: A.08. Development of Neural Systems

Support: Brain Convergence Research Programs of the National Research Foundation (NRF2019M3E5D2A01058328)

National Research Foundation of Korea(NRF), under 2023 Project BK21 Four

Title: Development of a biomimetic spiking neural network model for multidimensional tactile perception

Authors: *J. KIM, S.-P. KIM;
Ulsan Natl. Inst. of Sci. & Technol., Eonyang-eup, Ulju-gun, Korea, Republic of

Abstract: Implementing an artificial tactile system that approaches the human perception level has proven to be a formidable challenge. This work proposes a biomimetic tactile perception model based on a Spiking Neural Network (SNN) to addresses this challenge. Designed to mimic the tactile pathway from cutaneous mechanoreceptors to S1 cortex, the proposed model utilizes a multi-layered SNN architecture, with each layer representing a distinct component in the tactile pathway. The first layer comprises Slowly Adapting-1 (SA-1) and Rapidly Adapting-1 (RA-1) afferent neurons, which receive tactile stimuli from mechanoreceptors stochastically. The second layer processes the information derived from SA-1 and RA-1 afferents in separate streams, simulating the functions of the cuneate nucleus. This layer follows the neuronal circuit mechanisms intrinsic to the cuneate nucleus, particularly the dynamic interaction between excitatory and inhibitory neurons that facilitates lateral inhibition for minimizing noise accumulation and maintaining the fidelity of spatial information. The final layer, wherein inputs from SA-1 and RA-1 afferents converge, is constructed to emulate the S1 cortex. This layer utilizes a diverse combination of excitatory and inhibitory fields for the encoding of various stimulus properties. The layered organization of the proposed SNN is capable of the processing of multidimensional tactile features, thereby enhancing the efficiency of information processing. In the static perceptual dimension, the proposed SNN could distinguish two separate points of pressure stimulation at 3mm or greater, which is on par with human performance. It also accurately identified a tactile stimulus orientation, ranging from 0 - 170 degrees in 5 degrees increments. This was achieved using only four types of final layer neurons oriented to 0, 45, 90, and 180 degrees by decoding their activity into the stimulus orientation with 90.1% classification accuracy. In the dynamic perceptual dimension, the proposed SNN demonstrated the ability to rapidly detect the slip of an object within 5 ms. Furthermore, it could estimate the speed of the object with a resolution of 1 mm/s and discriminate vibration frequency of a stimulus in the range from 1 to 100 Hz. These results not only reinforce the proposed model's competency in mimicking biological tactile systems but also provide substantial implications for enhancements in the architecture and functions of tactile intelligence systems.

Disclosures: J. Kim: None. **S. Kim:** None.

Poster

PSTR384. Development of Sensory Systems

Location: WCC Halls A-C

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Program #/Poster #: PSTR384.03/B33

Topic: A.08. Development of Neural Systems

Support: EU Grant - MSCA 953327
MRC grant MR/T033320/1

Title: Neurophysiological consequences of developmental serotonin transporter disruption

Authors: *G. OCANA-SANTERO, H. WARMING, V. MUNDAY, C. GIBEILY, C. HEMINGWAY, H. A. MACKAY, A. SAHA, G. PARAMESWARAN, A. M. PACKER, T. SHARP, S. J. B. BUTT;
Univ. of Oxford, Oxford, United Kingdom

Abstract: The impact of serotonin on developing neural activity and consequences for emergent higher order cognition are poorly understood. Altered function of the serotonin transporter (SERT), arising from polymorphisms in the SERT promoter or embryonic exposure to selective serotonin re-uptake inhibitors (commonly prescribed antidepressants), is thought to be a risk factor in a range of neurodevelopmental psychiatric disorders but the mechanism is unknown. To examine this further, we performed longitudinal multiphoton imaging of transgenic mice to better understand how variation in SERT might affect serotonin dynamics and subsequently lead to pathological phenotypes during early postnatal life. Specifically, we imaged either neuronal activity using the GCaMP6s calcium indicator or serotonin dynamics using the fluorescent GRAB(5-HT3.0) biosensor in the somatosensory cortex of postnatal transgenic mice with specific neuron populations conditionally labelled with tdTomato. To perturb serotonin, we used either a SERT knock-out line or we dosed wildtype pups with the SERT inhibitor fluoxetine. The behaviour of head-fixed mice was simultaneously recorded with an infrared camera allowing us to assign neural activity and serotonin fluctuations to sleep-wake state. Experiments revealed that developmental serotonin dynamics in postnatal supragranular layers of S1 are defined by sensory stimuli and sleep-wake behavioural state. Reciprocally, SERT disruptions altered sleep architecture. We followed this up by assessing the impact of altered SERT on *in vivo* activity of both pyramidal projection neurons and GABAergic interneurons during a critical phase in development - the transition from synchronous to desynchronised activity mid-way through the second postnatal week. Finally, we investigated the long-term consequences of altered serotonin - both acute (SSRIs) and chronic (SERT KO) on sensory representation in the adult cortex of the same animals. Our data suggest that early postnatal life is a critical juncture in emergent cognition when SERT activity acts to sculpt and constrain emergent circuits. Thus, altered serotonin signalling likely leads to an imbalance in early sensory representation that manifests in a life-long manner.

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Poster

PSTR384. Development of Sensory Systems

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Topic: A.08. Development of Neural Systems

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Clarendon scholarship
Corpus Christi College

Title: Gabaergic circuits reflect different requirements for emergent perception in postnatal mouse neocortex

Authors: F. GHEZZI[1], L. J. BARUCHIN[2], ***I. LAZARTE**[1], L. GEYER[3], N. HA[1], M. SHAH-OSTROWSKI[1], A. G. CIANCONE CHAMA[4], J. STACEY[1], J. HJERLING LEFFLER[3], S. J. B. BUTT[1];
[1]Univ. of Oxford, Oxford, United Kingdom; [2]Univ. of Sussex, Brighton, United Kingdom; [3]Karolinska Institutet, Stockholm, Sweden; [4]Univ. de Montréal, Montreal, QC, Canada

Abstract: Information transfer in the mammalian cerebral cortex is dependent on locally-projecting GABAergic interneuron circuits that are widely assumed to be uniform across neocortical areas. We demonstrate that this does not hold true during the highly dynamic period of postnatal life prior to the onset of active sensory exploration at the end of the second postnatal week. We developed an optotagging approach in neonates to determine the contribution of GABAergic interneuron to neonatal activity, including formative beta-frequency spindle burst oscillations, on the millisecond time scale. During the neonatal period, we find that a subset of interneuron defined by expression of the neuropeptide somatostatin (SST) differentially contribute to sensory-evoked activity in primary somatosensory (S1BF) and visual (V1) cortices. This functional divergence between the two areas is explained by differences in the transient circuits formed by these cells. In S1BF, layer (L)5 SST interneurons form transient, translaminar feed-forward circuits to directly regulate early sensory activity. Whereas in V1, SST interneurons do not receive direct thalamic input but are rather engaged in local feedback networks. We further performed Patch-seq analysis to distinguish if this divergence in function at the circuit and systems level arises due to either different SST subtypes across both areas or the same subtype fulfilling different roles in neonatal cortex in an area-dependent manner. The sum of these data suggests that SST interneurons in neonatal S1BF are specialized to regulate neonatal touch information in a manner not required in V1. Such area-dependent differences point to additional complexity in early GABAergic circuits, variations that further complicating our endeavors to understand the etiology of developmental psychiatric disorders.

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Poster

PSTR384. Development of Sensory Systems

Location: WCC Halls A-C

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Program #/Poster #: PSTR384.05/B35

Topic: A.08. Development of Neural Systems

Support: R01EY022730
R01NS106244

Title: Nmda receptors maintain network diversity by suppressing parasitic correlations during development

Authors: *R. A. TIKIDJI-HAMBURYAN[1], M. T. COLONNESE[2,3];
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Abstract: In early development, cortical and subcortical networks exhibit several hallmark features, such as slow neuron dynamics, the dominance of NMDA synaptic receptors, and broad and imprecise connections. The developing visual system uses these features to respond appropriately to spontaneous retinal activity and refine thalamocortical connections (Colonnese and Phillips, 2018).
Recently we used large-scale network modeling to suggest that the level of NMDA receptor current regulates the precision of spike correlations in the developing dorsal lateral geniculate nucleus during the retinal waves (Tikidji-Hamburyan et al. 2023). We showed that NMDARs actively prevent the correlation of thalamic relay neurons on the millisecond timescale. Such temporally precise correlations dramatically reduce spatial information in the firing of thalamocortical neurons and are therefore expected to be detrimental to network refinement. We called such correlations parasitic, because they mask information essential for network formation. However, how parasitic correlations affect intracortical network formation is unclear. Here we use a theoretical approach (Gjorgjieva et al., 2009) and show that, if learning rules and dynamics of the adult cortical network are similar, parasitic correlations are expected to favor the formation of a single dominant group of excitatory neurons in the cortex (a clique) rather than the diverse connectivity required for vision. A phenomenological model of the visual cortex (Wu et al., 2020) confirmed these predictions, and indicated that a quarter of the excitatory neurons completely lose connections to postsynaptic pairs when the network is driven by input with parasitic correlations. Furthermore, inhibitory synaptic conductances are profoundly strengthened in models driven by parasitic correlations compared to normal activity. Such an increase in inhibitory drive would be expected to maintain the dominance of the excitatory clique.
Overall, our results suggest that visual thalamic networks developing in the absence of NMDA receptors are expected to produce parasitic correlations that reduce neural response diversity in the cortex, likely degrading visual responses and perception. This work suggests a novel role for NMDA receptors beyond their regulation of synaptic plasticity and coincidence detection.

Disclosures: R.A. Tikidji-Hamburyan: None. **M.T. Colonnese:** None.

Poster

PSTR384. Development of Sensory Systems

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Program #/Poster #: PSTR384.06/B36

Topic: A.08. Development of Neural Systems

Support: NIH Grant EY022730
NIH Grant NS106244

Title: Corticothalamic excitatory feedback generates spindle-burst oscillations and amplifies the transmission of retinal waves during development

Authors: *M. T. COLONNESE[1], R. HERRERA-MOLINA[2];
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Abstract: During the developmental period of thalamic axon ingrowth and circuit formation within the neocortex, the thalamocortical network produces powerful oscillations that synchronize activity throughout the cortex and associated thalamus. In the visual system, spontaneous retinal waves critical for receptive field development, drive ‘spindle-burst’ oscillations that arise in the lateral geniculate nucleus (dLGN) and synchronize activity in the visual cortex (VC). Here we use cre-dependent viral expression of excitatory channelrhodopsins and inhibitory DREADDs to determine the role cortiothalamic thalamic (CT) feedback during retinal waves. Using multielectrode recordings in headfixed unanesthetized neonatal mice, we show direct drive of the dLGN at P5 and P10 by NTSR1-cre neurons when retinal waves are present . NeuroPixel recordings show that CT feedback neurons oscillate in phase with spindle-burst oscillations in P5-6 mice. High-density multishank (NeuroNexus) recordings from dLGN reveal that relay neurons make eye-specific correlations that drop off with distance as expected if the dLGN is primarily driven by retinal waves. This nearest-neighbor synchronization depends on activity in VC as inhibition of cortical activity using DREADD expression in Emx1-cre mice eliminates eye-specific correlations and reduces total correlation among dLGN neurons. Reducing CT neuron activity also degrades the coherence of spindle-burst oscillations. Together our results show that corticothalamic feedback is critical for the normal transmission of retinal waves. CT feedback generates spindle-burst oscillations through a feedback excitatory loop that synchronizes and amplifies local retinal inputs during the period of retinal waves.

Disclosures: M.T. Colonnese: None. **R. Herrera-Molina:** None.

Poster

PSTR384. Development of Sensory Systems

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Topic: A.08. Development of Neural Systems

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NIH Grant R01NS106244
GWU Luther Rice Undergraduate Research Fellowship
GWU Sigelman Undergraduate Research Enhancement (SURE) Award

Title: Regional heterogeneity in the developmental maturation of thalamic reticular nucleus neurons

Authors: *P. PANG[1], M. T. COLONNESE[2];
[1]George Washington Univ., Washington, DC; [2]The George Washington Univ. Sch. of Med., The George Washington Univ. Inst. For Biomed. Sci., Washington, DC

Abstract: The thalamic reticular nucleus (TRN) provides the bulk of the inhibition to thalamocortical relay neurons in thalamus through closed recurrent loops with each thalamic nucleus. The TRN plays a critical role in the generation of sleep spindles and other oscillatory brain activity as well as the regulation of sensory attention, and cognition. Adult TRN neurons all express parvalbumin (PV)-a calcium binding protein that contributes to the bursting properties of TRN cells-and perineuronal nets (PNNs)-an extracellular matrix associated with the opening and closing of critical periods. How this expression is regulated during development and how it relates to function in thalamocortex is poorly understood. We examined the expression of PV and PNNs in visual and somatosensory TRN, two systems known to differ in the timing of their development. We quantified the number of neurons expressing PV and the normalized intensity of this staining on post-natal day (P)4, 9, and 15 using combined immunohistochemistry for PV and NeuroTrace in both visual (v)TRN and somatosensory (s)TRN. We find that at every age more neurons expressed PV in sTRN than vTRN, that in both regions the proportion of PV expressing neurons increased between P4 and P9 but was stable thereafter (sTRN: 56.9% ± 7.6%, n=4 for P4; 98.8% ± 0.4%, n=6 for P9; 94.4% ± 1.2%, n=6 for P15; vTRN: 12.3% ± 4.6%, n=4 for P4; 85.1% ± 8.9%, n=6 for P9; 87.6% ± 1.9%, n=6 for P15). The mean intensity of PV staining was more dynamic, increasing dramatically between P4 and P9 and again by P15 in vTRN but was relatively stable in sTRN (sTRN: 0.30 ± 0.04, n=4 for P4; 0.43 ± 0.01, n=6 for P9; 0.46 ± 0.02, n=6 for P15; vTRN: 0.10 ± 0.01, n=4 for P4; 0.16 ± 0.01, n=6 for P9; 0.25 ± 0.02, n=6 for P15). Qualitative evaluation of the developmental expression of PNNs suggest they follow PV maturation in both regions, with the sTRN first showing expression at P11 which increased at P14, and the vTRN first showing expression at P14 which increased at P21. Viral mediated tract tracing using conditional expression of EGFP in PV neurons reveals that dense axonal projections of the vTRN to the lateral geniculate nucleus and sTRN to the ventral posteromedial nucleus are robust even before full expression of PV. In summary, we find that while GABAergic TRN neurons, unlike cortical interneurons, express PV early in development, developmental upregulation of PV and accumulation of PNNs likely reflects and may influence maturation of activity in thalamus and cortex.

Disclosures: P. Pang: None. **M.T. Colonnese:** None.

Poster

PSTR384. Development of Sensory Systems

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Program #/Poster #: PSTR384.08/B38

Topic: A.08. Development of Neural Systems

Support: NIH Grant EY022730
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Title: A corticoreticular inhibitory feedback loop generates spindle-burst oscillations and synchronizes dLGN during retinal waves

Authors: *R. HERRERA-MOLINA[1,2], P. PANG[3], R. A. TIKIDJI-HAMBURYAN[3], M. T. COLONNESE[3];
[1]Leibniz Inst. for Neurobio., Magdeburg, Germany; [2]Cibqa, Univ. Bernardo O'Higgins, Santaigo, Chile; [3]Pharmacol. and Physiol., The George Washington Univ., Washington, DC

Abstract: During brain circuit formation, the developing thalamocortical network produces powerful oscillations that synchronize activity throughout the cortex and thalamus. In the visual system, spontaneous retinal waves, critical for receptive field development, drive 'spindle-burst' oscillations (～10Hz) that arise in the lateral geniculate nucleus (dLGN) and synchronize activity in the visual cortex (VC). Here we use cre-dependent viral expressions of excitatory channelrhodopsins and inhibitory DREADDs to determine functional connectivity and the role of inhibition from the thalamic reticular nucleus (vTRN) to dLGN during retinal waves. Using multi-shank high-density probes (NeuroNexus) to perform simultaneous recordings of dLGN and vTRN in head-fixed, unanesthetized P5 and P10 mice, we show that vTRN fires during retinal waves and participates in spindle-burst oscillations with zero phase offset to dLGN. Optogenetic stimulation of VC drives vTRN and dLGN with similar latency. Tract tracing with conditional expression of EGFP shows dense axonal arbors of both parvalbumin and somatostatin expressing TRN neurons to dLGN. Optogenetic stimulation of parvalbumin-expressing TRN neurons inhibits dLGN and 10 Hz stimulation of vTRN synchronizes activity in dLGN and VC at this frequency. These results, together with data showing that corticothalamic feedback neurons are required for spindle-bursts, and computational modeling of thalamo-cortico-reticular synaptic delays using modified spike-rate modeling approaches, suggest that spindle-bursts are generated by intact thalamo-cortico-reticular loop that results in recurrent excitation and long-latency inhibition in the relay thalamus.

Disclosures: R. Herrera-Molina: None. **P. Pang:** None. **R.A. Tikidji-Hamburyan:** None. **M.T. Colonnese:** None.

Poster

PSTR384. Development of Sensory Systems

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Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR384.09/B39

Topic: A.08. Development of Neural Systems

Support: NSF-IOS-2029980

Title: Refinement and maintenance of receptive field size in V1 neurons of mice occurs despite the absence of visual experience.

Authors: *P. FERNANDEZ, K. SUDANA, S. L. PALLAS;
Univ. of Massachusetts, Amherst, Amherst, MA

Abstract: The role of visual experience in the refinement of visual receptive fields has been studied for years using models with well-developed vision (e.g., carnivores, primates) but more recently in rodents exhibiting less elaborate visual processing circuitry. In nocturnal rodents, such as hamsters and mice, receptive fields refine completely before adulthood. However, hamsters dark reared from birth exhibit a reversion to large receptive fields by adulthood if kept in the dark. We demonstrated previously that in normally reared hamsters, the progressive decrease in receptive field (RF) size of visual neurons in the primary visual cortex (V1) and superior colliculus is achieved before adulthood. Dark reared hamsters also refine their RFs to adult size by adulthood (~60 postnatal days (pnd)), but then exhibit a gradual increase in RF size if kept in the dark after 60 pnd, suggesting a role of visual experience in the maintenance but not in the refinement of the RF sizes. We have now examined whether this difference from carnivores and primates is common to other nocturnal rodents. Abnormally large V1 RF sizes have been reported in dark reared mice around puberty, however, a more detailed study reporting the progression of RF size across different ages is still lacking. We investigated the changes in RF size of single unit V1 neurons (layer 2/3) in anesthetized mice at 30, 60 and 90 pnd. Our preliminary data show progressive refinement of V1 RF sizes between 30 and 60 pnd in normally reared mice (Normal RF areas at 30 pnd: 145 ± 16.8 deg^2, n=35; Normal RF areas at 60 pnd= 116± 16.8 deg^2, *n*= 37). Dark-reared mice exhibit a similar trend, showing increasingly refined RFs from P30 to P60 (DR RF areas at 30 pnd: 147± 24.1 deg^2, *n*=31; DR RF areas at 60 pnd: 121± 7.6 deg^2, *n*=73). Unlike hamsters, however, dark and normally reared mice retain refined RFs long into adulthood (Normal RF areas at 90 pnd: 96 ± 18.3 deg^2, *n*= 15; DR RF areas at 90 pnd: 83 ± 7.8 deg^2, *n*=70). Within each age group, no significant differences were observed between dark and normally reared individuals. These results suggest that in mice, both refinement and maintenance of RFs in V1 neurons are independent of visual experience. Our study is an important step in developing a better understanding of the role of visual experience in the development of visual processing circuitry in a commonly studied species.

Disclosures: **P. Fernandez:** None. **K. Sudana:** None. **S.L. Pallas:** None.

Poster

PSTR384. Development of Sensory Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR384.10/B40

Topic: A.08. Development of Neural Systems

Title: Microglia are Dispensable for Experience-Dependent Plasticity and the Maturation of Visual Circuitry

Authors: *T. BROWN, A. MCGEE;
Univ. of Louisville, Louisville, KY

Abstract: Microglia are proposed to be critical for the refinement of developing neural circuitry. However, evidence identifying specific roles for microglia is limited and often indirect. Here we examined whether microglia are required for the experience-dependent refinement of visual circuitry and visual function during development. We ablated microglia by chronically administering an inhibitor colony-stimulating factor 1 receptor and then examined the consequences for receptive field tuning of neurons in primary visual cortex (V1) and experience-dependent visual plasticity. Mice raised on chow containing PLX5662 from P14, the age of natural eye opening, lacked microglia by P18, prior to the opening of the critical period for experience-dependent visual plasticity. Despite the loss of microglia, the receptive field tuning properties of neurons in V1 were normal at P32, as was ocular dominance plasticity in response to brief monocular deprivation. Thus, none of these principal measurements of visual function differed in the absence of microglia. These findings contradict with the proposed critical role of microglia in refining neural circuitry. We conclude microglia are dispensable for experience-dependent plasticity and maturation of visual circuitry and function.

Disclosures: T. Brown: None. **A. McGee:** None.

Poster

PSTR384. Development of Sensory Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR384.11/B41

Topic: A.08. Development of Neural Systems

Support: NIH Grant 5T32 EY007143-27
NIH Grant 1R01 EY032095-01

Title: The role of Robo1/Slit2 signaling in the formation of the mammalian direction-selective circuit

Authors: *J. KIRALY, A. L. KOLODKIN;
Johns Hopkins Med. Institutions, Baltimore, MD

Abstract: A major goal of modern neuroscience is to understand how complex neural circuits develop, function, and elicit behavioral responses. The direction-selective (DS) circuit in the vertebrate visual system is an excellent model for studying circuit assembly mechanisms since

DS circuits rely on proper execution of neural development programs to produce robust behavioral responses. Within the Accessory Optic System (AOS), DS circuits mediate the stabilization of slow object motion across the retina. This stabilization is primarily driven by a select set of retinal ganglion cells known as On direction-selective ganglion cells (oDSGCs). Individual oDSGCs responses to directional object motion are in part mediated by asymmetric inhibitory connections between starburst amacrine cells (SACs) and oDSGCs. In the AOS, oDSGCs preferentially respond to slow object motion in a single direction, leading to four major subtypes of oDSGCs tuned to respond to forward, backward, upward, and downward motion. Although direction-selectivity depends critically upon SAC-DSGC connections, the cellular mechanisms that establish such precise directional tuning remain unknown. Through single cell transcriptomics, we have identified a candidate gene that regulates vertical tuning in the mouse: *roundabout-1* (*Robo1*). Disruption of *Robo1* loss-of-function leads to a striking enhancement of vertical slow motion tracking, particularly in the downward response. Additionally, *Robo1* mutants exhibit significant and specific axonal projection defects to the medial terminal nucleus (MTN), a key AOS brainstem target that regulates this vertical tracking. Preliminary data from a SAC-specific conditional knockout of *Slit2*, a canonical *Robo1* ligand, reveal a similar behavioral phenotype, with increased downward tracking. These data suggest that *Robo1*/*Slit2* interactions within the retina are essential for the establishment of SAC-DSGC synaptic interactions and proper visual tracking of slow object motion. Furthermore, *Robo1* may play additional targeting roles for the refinement of downstream oDSGC axon targeting. Ongoing investigation of *Robo1/Slit2* signaling events will provide insight into how DS circuits are established and function.

Disclosures: **J. Kiraly:** None. **A.L. Kolodkin:** None.

Poster

PSTR384. Development of Sensory Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR384.12/B42

Topic: A.08. Development of Neural Systems

Support: 1R01 EY032095-01

Title: Investigating the role of R2B Receptor Protein Tyrosine Phosphatases (RPTPs) in mammalian direction-selective circuit assembly

Authors: *T.-H. LIN[1], T. AL-KHINDI[1], S. HARRIS[2], A. BALRAJ[4], F. A. DUNN[3], A. L. KOLODKIN[1];
[1]Johns Hopkins Univ., Baltimore, MD; [2]Ophthalmology, UCSF, San Francisco, CA; [3]UCSF, San Fransisco, CA; [4]Univ. of California San Francisco, San Francisco, CA

Abstract: The vertebrate retina harbors a complex array of neural circuits dedicated to detecting visual features. Among these circuits, the direction-selective (DS) circuit within the accessory

optic system (AOS) plays a crucial role in detecting slow motion and orchestrating the optokinetic reflex (OKR), a behavioral response that coordinates eye movements and allows image stabilization. There are four distinct types of direction-selective ganglion cells (DSGCs)—upward (up-oDSGCs), downward (down-oDSGCs), nasal-to-temporal and temporal-to-nasal (hoizontal-oDSGCs)—within the DS circuit of the AOS. However, the mechanisms underlying the acquisition of distinct directional tuning properties in these DSGCs remain unclear. To shed light on the development of the DS circuit, we have undertaken a comprehensive investigation using high-depth single-cell RNA sequencing and have successfully identified three R2B receptor protein tyrosine phosphatases (RPTPs) with selective expression patterns: Ptprk and Ptprm preferentially expressed in vertically tuned oDSGCs and Ptprt potentially expressed in horizontally tuned oDSGCs. Our preliminary findings indicate that the loss of Ptprk disrupts the upward OKR, alters upward oDSGC dendritic morphology, and impairs electrophysiological properties. Moreover, Ptprm mutants exhibit defects in vertical directional tuning, suggesting a potential interaction between Ptprm and Ptprk in AOS circuit functionality. Our ongoing research aims to unravel the mechanisms by which Ptprk tunes up-oDSGCs to detect upward motion and whether Ptprm and Ptprt function similarly to modulate the tuning of vertical and horizontal oDSGCs, respectively. This work may not only unveil a novel molecular code governing neural circuit assembly but also has the potential to provide valuable insights into the attainment of asymmetric computations within other neural circuits.

Disclosures: **T. Lin:** None. **T. Al-Khindi:** None. **S. Harris:** None. **A. Balraj:** None. **F.A. Dunn:** None. **A.L. Kolodkin:** None.

Poster

PSTR384. Development of Sensory Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR384.13/B43

Topic: A.08. Development of Neural Systems

Support: EY022122

Title: Visual experience through a single, prematurely opened eye drives aberrant development of receptive field properties in visual cortex

Authors: *S. GRISWOLD[1], S. VAN HOOSER[2];
[1]Brandeis Univ., Boston, MA; [2]Brandeis Univ., Waltham, MA

Abstract: Cortical/cerebral visual impairment, or CVI, visual impairment occurring in the presence of a normal eye exam, or out of proportion with observed ocular pathology, is the most common cause of visual impairment in children in the developed world. This disorder of vision is more common in very preterm infants than their full term counterparts. Due to advances in neonatal medicine, very preterm infants are surviving with increasing frequency, causing a concomitant increase in CVI

Given that preterm infants may have up to 8 weeks of premature visual experience, and that premature vision is capable of aberrantly driving receptive field development in visual cortex, we propose that early exposure to visual stimuli may increase the susceptibility of preterm infants to CVI. Here we hypothesize that prematurely opening the eyes of ferrets will aberrantly impact the development of visual acuity in visual cortex (VC). We have tested this hypothesis by prematurely opening one or both eyes of ferrets at 25 days post parturition and subsequently examining the impact of premature eye opening on contrast sensitivity, direction tuning, orientation tuning, and temporal frequency tuning between P55-65. These receptive field properties were examined by recording from ipsilateral and contralateral monocular cortex while showing anesthetized animals drifting grating stimuli in which direction, spatial frequency, and contrast were covaried. We find that in ferrets that had one eye prematurely opened, cells in monocular cortex corresponding to the prematurely opened eye demonstrate reduced direction tuning and temporal frequency preferences in comparison with monocular cortex corresponding to the developmentally normal eye. Additionally, these cells had substantially altered temporal frequency preferences and a reduction in orientation angle preference for cardinal angles. These findings suggest that different amounts of visually evoked activity between the eyes can drive aberrant development, and that experience-dependent interactions between the inputs from the two eyes shape cortical circuitry before the canonical critical period for ocular dominance plasticity, and even prior to eye opening.

Disclosures: **S. Griswold:** None. **S. Van Hooser:** None.

Poster

PSTR384. Development of Sensory Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR384.14/B44

Topic: A.08. Development of Neural Systems

Support: European Union Horizon 2020 Grant Agreement 813457
La Ligue Contre le Cancer

Title: Lrrn2 and Lrrn3a cell adhesion molecules specify precise retino-tectal connections in the zebrafish visual system

Authors: ***E. PUTTI**, F. EGGELER, G. FAINI, S. ALBADRI, F. DEL BENE, Seniot;
Inst. de la Vision, Paris, France

Abstract: Leucine-rich repeat proteins (LRR) play critical roles in various aspects of neuronal circuit development, from synapse formation to axon guidance. Within the LRR protein family, the leucine-rich repeat neuronal (LRRN) subfamily comprises distinct adhesion molecules, including Lrrn2 and Lrrn3a. Remarkably, their expression patterns are conserved across species, ranging from Drosophila to humans. However, the functional significance of Lrrn2 and Lrrn3a in the context of visual system formation and axonal targeting remains elusive. In Drosophila, the

orthologous protein Capricious governs the synaptic targeting of specific photoreceptors to precise laminae in the fly brain. To determine if similar mechanisms are at play in vertebrates, we examined the expression patterns of *lrrn2* and *lrrn3a* in the retina of zebrafish larvae. Our findings reveal sparse expression of these adhesion molecules in the developing retinal ganglion cells (RGCs). The absence of Lrrn2 and Lrrn3a lead to mistargeting defects in a specific subset of RGCs. These RGCs fail to reach their designated laminae in the retino-recipient optic tectum, impairing the formation of precise synaptic connections. Moreover, behavioral analyses reveal compromised hunting abilities in *lrrn2* and *lrrn3a* mutants. Together, our findings highlight a crucial role for Lrrn2/3a in the specification and function of precise retino-tectal circuits in the vertebrate visual system.

Disclosures: E. Putti: None. **F. Eggeler:** None. **G. Faini:** None. **S. Albadri:** None. **F. Del Bene:** None.

Poster

PSTR384. Development of Sensory Systems

Location: WCC Halls A-C

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Program #/Poster #: PSTR384.15/B45

Topic: A.08. Development of Neural Systems

Support: NIH R01 EY033835
NIH R21 EY030588

Title: Semi-automated quantification of cytoarchitecture reveals more changes in neural populations than glial in the developing postnatal human visual cortex

Authors: *A. REZAI[1,2,4], A. DALIRI[2,4], H. LAMBING[4], B. DALIRI[4], L. C. VILLALOBOS[2], K. NAKAMURA[2,4], M. PAREDES[4,5,6], K. GRILL-SPECTOR[2,3];
[1]Emory Univ., Atlanta, GA; [2]Dept. of Psychology, [3]Wu Tsai Neurosciences Inst., Stanford Univ., Stanford, CA; [4]Dept. of Neurol., [5]Eli and Edythe Broad Ctr. of Regeneration Med. and Stem Cell Res., [6]Neurosci. Grad. Div., Univ. of California, San Francisco, San Francisco, CA

Abstract: Visual cortex undergoes considerable physical and morphological growth, especially during the first year of life. However, the development of the neural and glial cytoarchitecture across different visual areas remains unknown. Here, we used immunohistochemistry (IHC) in 7 ex vivo human brain samples, aged 34 gestational weeks (gw) to 9 years old (yo), to determine cytoarchitectural development in primary visual cortex (V1, calcarine) and higher-level place- (collateral sulcus) and face-selective (lateral fusiform gyrus) regions. IHC markers – neuronal nuclear protein (NeuN), aldehyde dehydrogenase 1 family member L1 (ALDH1L1), oligodendrocyte transcription factor 2 (Olig2) – and a 4′,6-diamidino-2-phenylindole (DAPI) counterstain identify neurons, astrocytes, oligodendrocyte lineage cells, and all cell nuclei, respectively. We developed a semi-automated pipeline to subsample confocal tilescan images over a total of 6,724 fields of view (FOVs; mean ± SD nuclei per FOV: 10.2 ± 5.9) across

cortical depths, brain regions and samples. For each FOV, automated nuclear segmentations, generated with a Statistical Region Merging algorithm (Nock & Nielsen, 2004), are manually corrected, and the overlapping IHC marker expression is confirmed to quantify the density and proportion of cells of each cell type. We observe that the total cell (DAPI) and neuron (NeuN) densities decrease significantly only from 34 gw to 3 months old (mo). In contrast, there is no significant age-related change in the density of astrocytes or oligodendrocytes. Furthermore, the development of the overall cell and neuron densities is similar across primary and higher-level visual areas. While the overall astrocyte density does not vary between cortical areas, the density of the oligodendrocyte lineage population is lowest in V1 regardless of age. We observe complementary changes in the relative cellular composition of visual cortex: the proportion of cells that are neurons decreases from 34gw to 3mo in all 3 visual areas, while the proportion that are oligodendrocytes only increases in the lateral fusiform gyrus. Together, these data suggest that there are changes to the neuron cytoarchitecture across all visual areas. Furthermore, the development of V1 versus higher-level visual cortex differs primarily in the oligodendrocyte lineage population. Characterizing cytoarchitectural changes in visual cortex provides insight into shared and unique developmental features across the human visual system and has important implications for functional development.

Disclosures: **A. Rezai:** None. **A. Daliri:** None. **H. Lambing:** None. **B. Daliri:** None. **L.C. Villalobos:** None. **K. Nakamura:** None. **M. Paredes:** None. **K. Grill-Spector:** None.

Poster

PSTR384. Development of Sensory Systems

Location: WCC Halls A-C

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Program #/Poster #: PSTR384.16/B46

Topic: A.08. Development of Neural Systems

Support: NSF Grant 2212591
NIGMS Grant P20GM103432

Title: Distinct developmental programs displayed by the Xenopus tadpole retinotectal and retinotegmental projections

Authors: *U. UDOH[1], K. ZHENG[2], K. G. PRATT[2];
[2]Zoology and Physiol., [1]Univ. of Wyoming, Laramie, WY

Abstract: The Xenopus tadpole retinotectal projection – the synapse between the retinal ganglion cells (RGCs) in the eye and the midbrain optic tectum – has served as a popular model to study how neurons self-assemble into circuits. But the retinotectal projection is only one of several components of the tadpole visual system. Using anatomical and electrophysiological approaches, we previously identified a functional retinotegmental projection, a direct projection from the RGCs to the ventral midbrain tegmental neurons. To compare these ventral and dorsal visual projections, we activated RGC axons by placing a bipolar stimulation electrode on the

optic chiasm and recorded RGC-evoked responses using an isolated brain preparation that allows access to both tectal and tegmental neurons. Recordings were carried out at three key stages of retinotectal development: stage 42 (approximately 5 days post-fertilization; dpf), 44-46 (7-9 dpf), and stage 48/49 (12-21 dpf). We found that the maximum strength of RGC input to tectal neurons peaked during stage 44-46, then steeply declined by stage 48/49. The addition of the N-methyl-D-aspartate receptor (NMDAR) blocker APV (2-amino-5-phosphonovaleric acid) at development stage 45 abolished the observed decline in retinotectal synaptic transmission, indicating an NMDAR-dependent mechanism. In contrast, the strength of RGC input onto tegmental neurons did not display a transient peak, remained constant across the 3 developmental stages, and was unaltered by NMDAR blockade. Paired pulse recordings indicate that, across the three stages of development studied, RGC input onto tegmental neurons display a higher probability of transmitter release compared to the input onto tectal neurons, suggesting that different populations of RGCs may project to the different visual centers. To begin to study tegmental outputs, a bipolar stimulation electrode was placed on the tegmentum and evoked responses recorded in different brain regions. We found monosynaptic evoked responses in the midbrain tectum and hindbrain, indicating that tegmental neurons send direct projections to those areas. Furthermore, stimulation of tegmental neurons resulted in contraction of extraocular eye muscles and eye movement, showing that the tegmentum may be involved in the optokinetic response. Overall, these results suggest that these two visual projections are built differently and carry out different functions as part of the visual system.

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

Poster

PSTR384. Development of Sensory Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR384.17/B47

Topic: A.08. Development of Neural Systems

Support: 2021YFA1101804
XDB32060100
2018SHZDZX05
SSMU-ZLCX20180601

Title: Genetic Interactions between Insm1 and Ikzf2 in Cochlear Outer Hair Cell Development

Authors: *S. LI[1,2], S. HE[1], Y. LU[1], Z. LIU[1,3];
[1]Inst. of Neuroscience, CAS Ctr. for Excellence in Brain Sci. and Intelligence Technol., Chinese Acad. of Sci., Shanghai, China; [2]Univ. of Chinese Acad. of Sci., Beijing, China; [3]Shanghai Ctr. for Brain Sci. and Brain-Inspired Intelligence Technol., Shanghai, China

Abstract: The development of cochlear outer hair cells (OHCs) and inner hair cells (IHCs) in the mammalian inner ear is orchestrated by a complex genetic program. In Insm1 mutants,

approximately half of the OHCs undergo transdifferentiation into converted IHCs (cIHC), while OHCs in Ikzf2-deficient mice exhibit dysfunctional characteristics and maintain partial IHC gene expression. Although recent HC multi-omics sequencing studies suggest the repressive activity of Insm1, there are pronounced similarities between the phenotypes of Insm1 and Ikzf2. We hypothesize that Insm1 determines OHC fate and activates the expression of Ikzf2, which is crucial for OHC maturation. However, direct evidence supporting this hypothesis is currently lacking. In this study, we employed multiple genetic tools to test this hypothesis. First, we generated *Ikzf2*$^{3*V5\text{-}P2A\text{-}tdTomato}$ (*Ikzf2*^{V5}) and *Rosa26-loxp-stop-loxp-tdToamto* (*Rosa26*^{Insm1}) mouse models and characterized Ikzf2 expression in *Atoh1*$^{Cre/+}$*; Rosa26*$^{Insm1/+}$*; Ikzf2*$^{V5/+}$ mice at postnatal day 7 (P7) (n=3). Our results demonstrate that ectopic Insm1 induces the expression of both Prestin (an OHC marker) and Ikzf2 in 94.7% ± 1.8% and 22.2% ± 4.4% of IHCs, respectively. Second, we analyzed *Atoh1*$^{P2A\text{-}Cre/+}$*; Insm1*$^{flox1/flox}$*; Ikzf2*$^{V5/+}$ mice at P5 and P30 (n=3). Immunostaining assays revealed that the expression of Ikzf2 in OHCs is dependent on normal Insm1 expression. OHCs lacking Insm1 downregulated Ikzf2 as early as P5, leading to upregulated expression of vGlut3 (an IHC marker) and downregulated expression of Prestin. Third, we overexpressed Ikzf2 in the absence of Insm1 using *Atoh1*$^{Cre/+}$*; Insm1*$^{flox/flox}$*; Rosa26*$^{Ikzf2/+}$ mice at P21 (n=3). Quantification revealed that the percentage of vGlut3+ cIHCs (6.9% ± 1.0%) in the *Atoh1*$^{Cre/+}$*; Insm1*$^{flox/flox}$*; Rosa26*$^{Ikzf2/+}$ model was significantly lower ($p<0.05$) than that (16.6% ± 2.3%) in *Atoh1*$^{Cre/+}$*; Insm1*$^{flox/flox}$ mice. Finally, double knockout of Insm1 and Ikzf2 resulted in similar OHC defects as observed with Insm1 ablation alone. Collectively, our findings elucidate an indirect transcriptional cascade from Insm1 to Ikzf2, providing valuable insights for future investigations into the molecular mechanisms underlying OHC development and regeneration.

Disclosures: **S. Li:** None. **S. He:** None. **Y. Lu:** None. **Z. Liu:** None.

Poster

PSTR384. Development of Sensory Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR384.18/B48

Topic: A.08. Development of Neural Systems

Support: NIH Grant 5R01DC016595-05

Title: Sema5a regulates spontaneous neural activity in developing cochlea.

Authors: *S. GHIMIRE, R. CHERELSTEIN, T. COATE;
Georgetown Univ., Washington, DC

Abstract: In the pre-hearing cochlea, spiral ganglion neurons (SGN) fire spontaneously in the absence of external stimuli following the glutamate release from the hair cell. The pattern of spontaneous firing by SGNs becomes more refined from infrequent and uncoordinated during embryonic stages, to more frequent and coordinated by early postnatal stages. The underlying

molecular mechanism pertaining to the generation and refinement of spontaneous activity is not well known. Our data show that SEMA5A acts as an inhibitor of spontaneous activity in the developing cochlea. SEMA5A belongs to a large family of proteins called semaphorins, which signal through neuropilin and/or plexin receptors and play important role in wiring of neural circuits in the brain. SEMA5A, is also associated with Autism Spectrum Disorder in humans. In the brain, loss of *Sema5a* is reported to have increased neuronal excitation in mice.
In-situ hybridization shows a wider expression of *Sema5a* in SGNs and mesenchyme of developing cochlea. To evaluate the effect of SEMA5A on spontaneous activity of SGNs, we utilized *Snap25-GCaMP6s*$^{+}$ cochlear explants and recorded calcium transients before and after the application of recombinant SEMA5A-Fc protein. We found that the application of SEMA5A-Fc protein resulted in reduced area of activity, frequency and coordinated events compared to baseline data. Analyses of cochlear explants from *Sema5a*$^{-/-}$;*Snap25-GCaMP6s*$^{+}$ mice showed increased fluorescence levels of calcium compared to littermate controls. We also observed increased phosphorylated CREB in SGNs, cochlear epithelium and mesenchymal cells in *Sema5a*$^{-/-}$ samples as an indicator of elevated activity. Supporting these findings, we also observed reduced terminal branches of type I SGNs in *Sema5a*$^{-/-}$ cochleae suggesting a homeostatic mechanism. It is possible that SEMA5A interacts with one or more ionic channels, which directly regulate SGN excitability. Interestingly, SEMA5A contains a motif that shares partial analogy with spider venom, hanatoxin, which acts as an inhibitor of potassium and calcium channels. We have found that the hanatoxin analog heteropodatoxin (HpTx2) inhibits calcium transient in SGN, similar to the effects of SEMA5A-Fc.
We are currently examining the effects of hanatoxin-like domain (HD) of SEMA5A by generating mutated protein in the context of SGN spontaneous activity and protein pull-down experiments designed to discover SEMA5A binding partners.

Disclosures: **S. Ghimire:** None. **R. Cherelstein:** None. **T. Coate:** None.

Poster

PSTR384. Development of Sensory Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR384.19/B49

Topic: A.08. Development of Neural Systems

Support: NIH NIDCD R01DC016346

Title: Cortical Reorganization and Cognitive Outcomes in Young Adults with Cochlear Implants

Authors: *G. KARTHEISER[1], D. BELL-SOUDER[2], M. W. DYE[3], A. SHARMA[4];
[1]Sensory, Perceptual, and Cognitive Ecology Res. Ctr., Rochester Inst. of Technol., Rochester, NY; [2]Univ. of Colorado, Boulder, Boulder, CO; [3]Sensory, Perceptual, and Cognitive Ecology Res. Ctr., Rochester Insitute of Technol., Rochester, NY; [4]Univ. Colorado, Boulder, Boulder, CO

Abstract: As of 2012, approximately 38,000 American children had received a cochlear implant (CI) with mixed spoken language and cognitive outcomes (Barnard, 2015). There is some debate about the factors that contribute to variability in outcomes, with some studies suggesting that exposing deaf children to a sign language prior to implantation is disadvantageous (Geers, 2017), and yet others showing evidence that children with CI who are exposed to a sign language outperform CI children who were not exposed to a sign language on a variety of spoken language assessments (Hassandazeh, 2012). Furthermore, there are also studies that show children's auditory systems develop differently based on the timing of implantation (e.g. Kral & Sharma, 2012). Here, we examine the effect that the timing of CI has on cortical reorganization of the central auditory and visual systems in young adults with CI and how this, alongside the timing of language exposure, impacts linguistic and cognitive development.
In this study we compare the outcomes of early and late implanted groups of young (aged 18-32) adult CI users (n=17). The early implanted group had received their CI by the age of 3.5 years of age, which has previously been shown to be within the critical period for developing typical auditory cortical development, with late implanted individuals implanted after this age. All were given the cognitive fluidity battery from the NIH Toolbox. All were administered the American Sign Language Comprehension Test and Test of Silent Contextualized Reading Fluency. Cortical development was measured using electroencephalography (EEG) using two paradigms: cortical auditory evoked potentials (CAEP) to examine auditory cortex maturation, and cortical visual evoked potentials (CVEP) to measure cross-modal recruitment of the auditory cortex.
The findings did not show a difference between these two groups in cognition, English language exposure, and English reading fluency. There was a significant difference in ASL comprehension ($t(15) = 5.25$, $p < 0.001$), with the late CI group having significantly higher ASL comprehension. Cortical auditory development, as measured by CAEP P1 latencies and estimated sources, was found to be delayed in the late CI group. In the late CI group, there was some evidence of cross-modal recruitment from the visual system as well as persistent changes in EEG spectral power that could be consistent with constant use of attentional control networks in the brain to listen. Overall, these results suggest that some compensation is possible in the brain that enables typical cognitive ability even in the face of different trajectories of sensory and central auditory development.

Disclosures: G. Kartheiser: None. **D. Bell-Souder:** None. **M.W. Dye:** None. **A. Sharma:** None.

Poster

PSTR384. Development of Sensory Systems

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Program #/Poster #: PSTR384.20/B50

Topic: A.08. Development of Neural Systems

Support: Elizabeth H. Solomon Center for Neurodevelopmental Research
Behavioral and Neural Science Program Center for Molecular and Behavioral Neuroscience Rutgers University- Newark, NJ

Title: Differences in high frequency power spectral density in pre-linguistic infants due to exposure to an interactive auditory experience.

Authors: *B. MAHMOOD, K. WOLFERT, A. A. BENASICH;
Rutgers Univ. Behavioral and Neural Sci., Newark, NJ

Abstract: Spontaneous brain activity has recently garnered much attention for its posited utility as an early biomarker of atypical neural development. Previous studies have shown that spontaneous electroencephalographic (sEEG) data from infants that are at higher risk for developmental delay demonstrate differences in spectral power composition when compared to infants who are at lower risk, and that these differences may correspond to later cognitive deficits. However, no study to date has examined whether spectral power composition can be impacted by early behavioral intervention. In the present study, we examine longitudinal dense-array sEEG data collected from infants at 7, 9, 12 and 18 months. This study consisted of three participant groups: (1) infants in the Active group were presented with an interactive acoustic experience (IAE) between 4-7 months of age, (2) infants in the Passive group were exposed to the acoustic stimuli but no infant response or overt attention was required and (3) a Naïve group which consisted of age-matched infants who had no previous exposure to the sounds. We hypothesized that spectral power in high frequency, such as gamma, would differ by group. Absolute Power Spectral Density (PSD) values were calculated for each infant for alpha, beta, theta, and gamma frequency ranges and the three groups were compared across age. Consistent with previous literature, our results showed that overall Frontal PSD values in the higher frequency ranges increased across age. Group differences were seen in the higher frequencies at 12 and 18 months of age between the Active and Naïve groups and the Active and Passive groups. Interestingly, the Passive and Naïve groups did not show significant differences across age. Additionally, group differences were seen in standardized cognitive assessment scores between the Passive and Active groups. These findings begin to identify important features in early auditory experiences that can impact learning and how these experiences support the development of auditory networks in a manner that can enhance later cognitive and language ability.

Disclosures: B. Mahmood: None. **K. Wolfert:** None. **A.A. Benasich:** None.

Poster

PSTR384. Development of Sensory Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR384.21/B51

Topic: A.07. Developmental Disorders

Support: Funded by FRAXA Research Foundation

Title: Acute administration of a biased serotonin 1A receptor agonist NLX-101 improves auditory temporal processing in a mouse model of fragile X syndrome during early development

Authors: *X. TAO[1], K. CROOM[1], A. NEWMAN-TANCREDI[2], M. A. VARNEY[2], K. RAZAK[1];
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Abstract: Fragile X syndrome (FXS) is a neurodevelopmental disorder caused by the loss of fragile X messenger ribonucleoprotein (FMRP). Humans with FXS show altered sensory processing, which is also seen in the *Fmr1*KO mouse model of FXS. Our previous findings showed that activating serotonin 1A receptors (5-HT1ARs) acutely with NLX-101, a biased agonist that preferentially targets post-synaptic 5-HT1ARs, strongly protected *Fmr1* KO mice from audiogenic seizures (Tao et. al. 2023). Here we tested whether activating 5-HT1ARs acutely with NLX-101 is beneficial in improving translation-relevant electroencephalographic (EEG) phenotypes in *Fmr1* KO mice. The EEG deficits found in humans with FXS and *Fmr1* KO mice include deficits in temporal processing and hypersensitive responses to sounds as measured using inter-trial phase clustering (ITPC), event-related potentials (ERP) and background single-trial power (STP). We recorded epidural EEG signals from auditory and frontal cortex of FVB *Fmr1* WT and KO mice (both males and females) at two age points: postnatal (p)21 and p30. Saline or NLX-101 fumarate salt (at 1.8 mg/kg) was given through intraperitoneal (i.p.) injection immediately before EEG recording. Narrow band noise and 40 Hz gap-in-noise auditory steady state response (gap-ASSR) stimuli were used to evoke ERP and ASSR. Gap-ASSR inserts “gaps” in the continuous background noise at 40Hz to induce resonant auditory cortical steady-state oscillations. Gap-ASSR tests the ability of cortex to respond to narrow gaps in sounds, a measure commonly used to test auditory temporal acuity. We measured ERP peak amplitudes (P1, N1 and P2), STP in ERP and ITPC in gap-ASSR. Acute administration of NLX-101 significantly rescued the ITPC deficit in response to gap-ASSR in *Fmr1* KO mice at both p21 and p30, suggesting improved temporal processing. NLX-101 also significantly reduced gamma band STP in *Fmr1* KO mice at p30, but not at p21. NLX-101 did not correct elevated N1 amplitudes in *Fmr1* KO mice at either age point. Taken together, the results indicate that 5-HT1AR activation regulates auditory temporal processing and background noise, but not ERP amplitudes, in developing mice. Given the relationship between temporal processing and speech recognition, these data suggest that activation of 5-HT1AR may be a promising therapeutic avenue to enhance language function in humans with FXS.

Disclosures: **X. Tao:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Neurolixis. **K. Croom:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Neurolixis. **A. Newman-Tancredi:** A. Employment/Salary (full or part-time):; Neurolixis. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurolixis. **M.A. Varney:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurolixis. **K. Razak:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Neurolixis.

Poster

PSTR384. Development of Sensory Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR384.22/B52

Topic: A.08. Development of Neural Systems

Support: Canadian Institutes of Health Research
BCCHRI: Brain, Behaviour and Development Theme

Title: Upstream to Atoh1, the Msx genes are new candidates to play critical roles in establishing cell lineage in the developing cerebellum

Authors: I. GUPTA[1], J. YEUNG[2], M. RAHIMI-BALAEI[2], S.-R. WU[3], ***D. GOLDOWITZ**[2]; [2]Ctr. for Mol. Med. and Therapeut., [1]Univ. of British Columbia, Vancouver, BC, Canada; [3]Neurosci., Baylor Col. of Med., Houston, TX

Abstract: The understanding of cerebellar development, particularly the spatio-molecular signatures that define the GABAergic (Ptf1a+) and Glutamatergic (Atoh1+) lineages that colonize the cerebellum, has evolved dramatically in the last 20+ years. Our examination of the transcriptome in the Pax6-null cerebellum has provided a new view of the molecular players in the cells that arise from the rhombic lip (RL)/Glutamatergic lineage (PMID: 27581449, 25209290). While Pax6 is required for the development of cells committed to the glutamatergic lineages, within the RL it is repressed mutually by Wls expression. Wls expression demarcates the interior face of RL (iRL), and this region is populated by cells that are yet to committed to glutamatergic lineages (i.e. Atoh1-negative). These findings have recently been confirmed in the early development of the human cerebellum (PMID: 31624095).Our current work examined the homeobox containing Msx gene family in the cerebellum. The three genes in the Msx family in mouse are candidates for mediating BMP signaling in cerebellar development and known transcriptional repressors. They are predominately expressed in the germinal zones in the early developing cerebellum. Msx1 and Msx2 are expressed in the RL region, while Msx3 expression is found in the ventricular zone (VZ). We found that Msx1 is co-expressed with Wls in the iRL and do not overlap with Atoh1 expression in the RL. In contrast, Msx2 expression overlaps with Atoh1 in the RL; forming an interesting Msx1-Msx2-Msx1 banding in the RL. We showed that Msx1 and Msx2 expression is independent of Atoh1 as their expression persisted in the Atoh1-null cerebellum. There is, however, an altered banding formed by Msx1 and 2 expressions in the Atoh1-null cerebellum, such that Msx1 expression is expanded into the presumptive Atoh1-positive region in the RL.In the ventricular zone, Msx3 expression largely overlaps with Ptf1a, the marker of VZ. Msx3 expression, however, extends beyond the posterior limit of Ptf1a and shares the VZ/RL border with Atoh1 expression. Thus, between the germinal zones, there is a region between Ptf1a and Atoh1 expressions, that is positive for Msx3, exclusively. In the Atoh1 null, the expression of Msx3 expands to the RL into the Atoh1-positive region. Despite the overlapping expansion of Msx1+ and Msx3+ cells, this does not result in cells that co-express Msx1 and Msx3.The current study highlights the molecularly distinct compartments that are demarcated by the interaction between cells that express Msx genes, Atoh1 and Ptf1a; and places Msx1 (and 2) upstream to Atoh1 in the developmental genetics of the Glutamatergic lineage.

Disclosures: I. Gupta: None. **J. Yeung:** None. **M. Rahimi-Balaei:** None. **S. Wu:** None. **D. Goldowitz:** None.

Poster

PSTR385. Comparative Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR385.01/B53

Topic: A.10. Development and Evolution

Support: Wellcome Trust PhD Programme in Neural Dynamics 108899/Z/15
NSF IOS 2128543, NSF GRFP
NIH DA10981, MH14602, R56MH121918, R21DA058215, DA10981, MH129112-01A1, R01NS117899, NIMH R01 600670, R21 MH-093904, R01MH126351, R01MH133066, R01MH085802, NIH R01MH112267, R01NS119813, R21MH125107, R01-DC012557, U19-NS107616, R01MH119250, R01 NS1015
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Ministerio de Ciencia e Innovacion PDC2022-133987-I00, Ministerio de Salud-Instituto de Salud Carlos III PI18/01691, CIBERSAM: CIBER-Consorcio Centro de Investigacion Biomedica en Red (CB07/09/0033)
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Simons Foundation Autism Research Iniative
Helsinki Institute of Life Science
NSERC postgraduate doctoral scholarship
NIDCD Predoctoral Fellowship F30DC017351
National Alliance for Research on Schizophrenia and Depression Young Investigator Grant
U01AA020911-0851, R01DA049261, 5T32NS96050-24 1F31AG069502-01 T32AA007573,

Title: Locus coeruleus activity differs as a function of species, sex, age, and genetic manipulation

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Abstract: Locus coeruleus (LC) neurons are intimately connected with diverse brain functions, most prominently attention, arousal, stress responses, and cognition. Published studies of LC activity cover many traits (species, sex, etc.) but rely on small sample sizes (usually tens of neurons), which has precluded a systematic and robust assessment of how these features affect LC activity. Here, we leverage a pooled dataset of 1,855 single units from 20 laboratories to comprehensively compare LC activity. Verified LC activity was recorded from deafferenated brain slices and intact animals during wakefulness or under different anesthetics. Samples included male non-human primates and rats and mice of both sexes. In some cases, LC activity was recorded from disease models and cell-type selective genetic modifications. We used a negative binomial regression model to identify the individual and combined effects of various attributes on firing rate. This powerful analysis revealed important species-, sex-, age-, and disease model-specific activity, as well as significant effects in Cre-expressing lines. Lastly, in contrast to the foundational concept of two-mode (tonic-phasic) LC activity, we discovered multiple bursting sub-modes and complex second order spike train patterns associated with different LC preparations. In sum, inherent differences in LC activity result from species-, age- and sex-dependent factors, as well as genetic modification. Our findings offer insight into why LC-dependent behavioral and cognitive functions depend on sex and age and may help explain the known association of sex and age with psychiatric disorders.

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Poster

PSTR385. Comparative Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR385.02/B54

Topic: A.10. Development and Evolution

Support: Supported by the University of Kentucky Office of Undergraduate Research (OUR)

Title: Effects of zinc on physiological processes in Drosophila and crawfish: cardiac, neural, synaptic transmission, and behavioral assays

Authors: *E. ELLIOTT[1], S. L. SPEED[1], D. M. CRAWFORD[1], M. S. DATTA[1], J. T. HIRTLE[1], A. B. LEACH[1], R. D. MCINTOSH[1], K. A. ROEMER[1], L. C. SOTINGEANU[1], A. C. TAUL[1], B. D. VESSELS[1], K. E. BROCK[1], S. ALTENBURG[2], S. M. BIERBOWER[2], J. NADOLSKI[3], R. L. COOPER[1];
[1]Univ. of Kentucky, Lexington, KY; [2]Dept. of Chem. & Life Sci., United States Military Acad., West Point, NY; [3]Mathematical and Computat. Sci., Benedictine Univ., Lisle, IL

Abstract: Zinc (Zn^{2+}) is an essential element that affects proper organ function, cell growth, and immune function. However, it can also be present in too great a quantity, with zinc toxicity resulting in both minor and major physiological effects. This study examined the effects of $ZnCl_2$ on both nervous and cardiac function through a number of different experimental methods, using crawfish and *Drosophila* as model organisms. Cardiac function, activity at the neuromuscular junction, and survival were all assessed following the organisms' exposure to zinc. Function of the crawfish sensory muscle receptor organ (a proprioceptive organ analogous to the mammalian muscle spindle) was also examined. Dose-response was used to examine any changes in *Drosophila* larval crawling rates, mouth-hook movement rates, and touch sensitivity. Increased exposure to zinc generally led to decreased survival rate, in both crawfish and *Drosophila*; similarly zinc exposure led to depressed or even eliminated heart rates in both models. The neuromuscular junctions in both organisms saw varying effects: high concentrations (1mM, 10mM; n=6, $p>0.05$) eliminated synaptic transmission without affecting spontaneous quantal events, while lower concentrations (0.1mM, 0.5mM) merely depressed that transmission. At the crawfish MRO, high concentrations of $ZnCl_2$ (that is, 5mM; n=6, $p>0.05$) depressed neural activity (but this returned during washout), while lower concentrations (0.1mM and 1mM) saw MRO activity increase. No consistent effect was yet observed in *Drosophila* behavior with differing zinc concentrations.

Disclosures: E. Elliott: None. **S.L. Speed:** None. **D.M. Crawford:** None. **M.S. Datta:** None. **J.T. Hirtle:** None. **A.B. Leach:** None. **R.D. McIntosh:** None. **K.A. Roemer:** None. **L.C. Sotingeanu:** None. **A.C. Taul:** None. **B.D. Vessels:** None. **K.E. Brock:** None. **S. Altenburg:** None. **S.M. Bierbower:** None. **J. Nadolski:** None. **R.L. Cooper:** None.

Poster

PSTR385. Comparative Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR385.03/B55

Topic: A.10. Development and Evolution

Support: CAS, No.YJKYYQ20190052
Grant No. STI2030-2021ZD0200100

Title: Single-cell spatial transcriptomic atlas of the macaque claustrum

Authors: Y. LEI[1], Y. LIU[2], L. DING[1], N. YUAN[3], M. WANG[3], Z. ZHU[1], C. LI[3], Z. WU[4], C. LI[3], M.-M. POO[5], J. YAO[4], Z. LIU[3], L. LIU[1], W. WEI[2], X. XU[1], ***Z. SHEN**[3];
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Abstract: The claustrum is extensively connected with the whole cerebral cortex, yet its function remains to be clarified. A comprehensive and quantitative characterization of the gene expression in various cell types, and their spatial distributions could help to elucidate the connectivity and function of various claustrum neurons. Using single-nucleus RNA sequencing (snRNA-seq) of more than 300,000 claustrum cells and spatial transcriptomic analysis (Stereo-seq) over 89 sections in 6 macaque monkeys (M. fascicularis), we obtained a spatially resolved, transcriptome-based single-cell atlas of the macaque claustrum. We identified 39 cell clusters in the claustrum, including 24 glutamatergic, 10 GABAergic, and 3 non-neuronal cell clusters. We also characterized the spatial distribution patterns of these cell clusters across the entire claustrum, and found that the distribution of several glutamatergic cell clusters exhibited distinct dorsoventral or core-shell preferences. These spatial distribution patterns correlated with the topographic patterns of claustrum-cortical connectivity, based on retrograde labeling of claustrum neurons by fluorescent dye-injections in 70 cortical areas. Additionally, by comparison with similar data from neighboring putamen and the insula cortex, we identified a set of claustrum-specific cell clusters. Together, these data began to reveal the cellular and molecular organization of the claustrum.

Disclosures: **Y. Lei:** None. **Y. Liu:** None. **L. Ding:** None. **N. Yuan:** None. **M. Wang:** None. **Z. Zhu:** None. **C. Li:** None. **Z. Wu:** None. **C. Li:** None. **M. Poo:** None. **J. Yao:** None. **Z. Liu:** None. **L. Liu:** None. **W. Wei:** None. **X. Xu:** None. **Z. Shen:** None.

Poster

PSTR385. Comparative Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR385.04/B56

Topic: A.10. Development and Evolution

Support: National Key R&D Program of China" (2021YFA0805100)

Title: Spatial transcriptomics reveals the convergent evolution of mammalian and avian pallium

Authors: *S. LIU, *S. LIU;
BGI-Research, Shenzhen, China

Abstract: Amniotes have evolved a complex brain organization, particularly in the telencephalon. However, the identity and evolutionary conservation of the telencephalon at the genoarchitecture scale remains largely unknown. We constructed zebra finches, turtles, mice, and macaques using the Stereo-seq with single-cell resolution spatial transcriptome telencephalon atlases. For the first time, we presented a complete single-cell and spatial atlas of the turtles and birds, where we identified some novo brain sub-regions and predicted the mechanism of function or structures change with the gene expression pattern. Moreover, comparative analysis of spatial atlas across the species in amniotes, we identified two major types of gene regulatory models during the evolution of the dorsal ventricular ridge (DVR) in sauropsids: changes in the expression of transcription factors (TF), and changes in the binding motifs of TFs with no difference in expression. We reveal the mechanism by which avian DVR and mammalian neocortex recruit the same effector genes under different transcription factors, indicating their convergent evolution. Overall, our analysis yields insights into the evolutionary relationships of the telencephalon across amniotes.

Disclosures: **S. Liu:** None. **S. Liu:** None.

Poster

PSTR385. Comparative Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR385.05

Topic: A.10. Development and Evolution

Support:
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European Commission Marie Curie COFUND
NIH Grant R01NS114491
NSF Grant 1146575
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Human Frontiers Science Program

Title: Profiling cellular diversity in sponges informs the evolutionary origin of neurons and coordinated behavior in animals

Authors: *J. MUSSER[1], L. L. MOROZ[2], D. ARENDT[3];
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Dept. of Neurosci., Saint Augustine, FL; [3]Develop. Biol. Unit, European Mol. Biol. Lab., Heidelberg, Germany

Abstract: The evolutionary origin of neurons and coordinated behaviors in animals is a major unresolved question. Using whole-body single-cell RNA sequencing in a sponge, an animal without nervous system and musculature, we identified 18 distinct cell types. These include myoepithelial cells responsive to nitric-oxide and secretory neuroid cells that reside in close proximity to digestive cells expressing postsynaptic scaffolding and receptor proteins. Visualizing neuroid cells by correlative x-ray and electron microscopy revealed secretory vesicles and cellular projections enwrapping digestive cell microvilli and cilia. Our data show a communication system that is organized around sponge digestive chambers, using conserved modules that became incorporated into the pre- and postsynapse in the nervous systems of other animals.

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Poster

PSTR385. Comparative Cellular and Molecular Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR385.06/B57

Topic: A.10. Development and Evolution

Support: NIH Grant R01NS060123 (ZY)
NIH Grant R01NS117590
NIH Grant R21AG067570 (ZY and NY)

Title: Integrated proteomics reveals the landscape of autophagy degradation in neurons

Authors: X. ZHOU[1,2,6], ***H. KIM**[3,1,2], Y.-K. LEE[1,2], X. LI[1,2], X. HAN[7,8,11], L. WANG[12], H. TAN[7,8,9], S. ZHOU[9], Y. FU[10,8,9], J. PENG[10,8,9], N. YANG[4,5], Z. YUE[1,2];
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Abstract: Background: As post-mitotic cells of the central nervous system, neurons rely heavily on quality-control pathways to promote cellular homeostasis. Through seminal works in autophagy-deficient mice brains, autophagy has emerged as a critical proponent in a neuron’s repertoire in maintaining the cellular homeostasis and function. Autophagy is the quality-control catabolic pathway wherein cellular components are engulfed in a double membraned vesicle and degraded in a lysosome-dependent manner. As increasing lines of evidence have converged on

autophagy's significant role in maintaining neuronal homeostasis, our project sought to understand the degradative landscape of autophagy in neurons. **Methods**: To enrich for putative autophagy cargoes, we generated autophagy-deficient stem cell-derived neurons by knocking down *ATG7* and *ATG14* using CRISPR-inhibition technology. In addition, we established a neuron-specific *ATG7* and *ATG14* conditional knock out (cKO) mouse line to validate our findings in our stem cell-derived human-induced neurons. To profile putative autophagy cargoes in these models, we subjected our neuronal models to liquid-chromatography tandem mass spectrometry, and defined autophagy cargoes to be proteins with significant accumulations in autophagy-deficient neurons compared to controls. In addition to these models, we also performed affinity purification from *ATG7* cKO mice expressing GFP-LC3 to identify autophagy cargoes who interact with LC3, an autophagy receptor. We utilized our autophagy-deficient human-induced neurons and mouse brains for downstream experiments, including immunostaining and immunoblotting, to validate our computational findings. **Results**: By highlighting proteins significantly accumulated in autophagy-deficient neurons, our quantitative proteomic analyses identified multiple cellular organelles and pathways as autophagy targets. Our results suggest that endoplasmic reticulum (ER), synaptic vesicles (SV), and protein kinase A (PKA) pathway components as major targets of neuronal autophagy. Following up with *in vitro* experiments in the same models, we have validated the significant increase of ER, SV, and PKA pathway proteins in autophagy-deficient neurons compared to controls. **Conclusion**: Through these findings, we have conducted a comprehensive, multi-disciplinary analysis to profile the regulatory landscape of neuronal autophagy. By generating a list of putative autophagy cargoes, we hope to shed light on the molecular mechanisms for neurodevelopmental and neurodegenerative disorders associated with autophagy deficiency.

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Poster

PSTR385. Comparative Cellular and Molecular Mechanisms

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Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR385.07/B58

Topic: A.10. Development and Evolution

Support: JSPS KAKENHI 21KK0158
JSPS KAKENHI 21K09813

Title: A parasympathetic neurotransmitter contributes to the formation of functional neuro-myoepithelial cell junctions in submandibular salivary glands.

Authors: *T. NAKAMURA[1,2], Y. SHINDO[3], H. M. NAKAMURA[4], J. NAKAI[2], K. TAKAHASHI[2], T. IWAMOTO[3], M. WAKAMORI[2];
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Abstract: The parasympathetic nervous system via muscarinic receptors mainly regulates serous saliva secretion from the salivary glands (SGs) by controlling myoepithelial cell (MEC) contractility. In the developed SGs, MECs derived from epithelial cells acquire mesenchymal properties by epithelial-mesenchymal transition (EMT) and are located on the end buds and ducts of SGs as a thin layer above the basement membrane. The formation of the nerve-MEC junction is important for the parasympathetic response of SGs. During the embryonic stage, the mouse SG with neural responsiveness develops, but how the nerve-MEC junction is formed during the development remains unclear. In this study, we used *ex vivo* organ culture system of submandibular glands (SMGs) to analyze the role of parasympathetic neurotransmitters during nerve-MEC junction formation in mouse SG development. *Chrm1*, which encodes muscarinic ACh receptor M1 (mAChR M1), was preferentially expressed in developing SMG of E13 embryos. Inversely, *Chrm3*, which encodes mAChR M3 receptor, was predominantly expressed in developed SMGs of postnatal 7-day mice. In addition, the expression of neuronal tubulin beta 3 (*Tubb3*), a marker of parasympathetic nerve in developing SMG, was increased at E13.5. qPCR analysis in *ex vivo* organ culture system revealed that carbachol (CCh), a chemical that mimics ACh, promotes the expression of marker genes for EMT and *Acta2*, a marker for MECs, suggesting CCh induces the epithelial cell differentiate into MECs. On the other hand, CCh-mediated MEC induction was canceled by mAChR M1 inhibitor. Immunohistochemical analysis showed MECs were identified at the surface of endbuds close to Tubb3-positive neurons. Taken together our results suggest that ACh, secreted from parasympathetic neurons, promotes MEC differentiation limited in mAChR M1-expressing epithelial cells. This regulation could contribute to the formation of nerve-MEC junctions in neuro-innervating SGs.Our findings provide novel insights into neuro-regulated organ development and also a clue for the development of a therapeutic approach in age-related dry mouth and dysfunction SGs related to oral sarcopenia.

Disclosures: T. Nakamura: None. **Y. Shindo:** None. **H.M. Nakamura:** None. **J. Nakai:** None. **K. Takahashi:** None. **T. Iwamoto:** None. **M. Wakamori:** None.

Poster

PSTR385. Comparative Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR385.08/B59

Topic: A.10. Development and Evolution

Support: DFG Grant 424957847

Title: THRA isoform expression profile in healthy human tissues and cortical brain organoids: insights into thyroid hormone signaling during early cortical development

Authors: *E. GRACEFFO[1], R. OPITZ[2], H. KRUDE[3], M. SCHUELKE[1];
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Abstract: Thyroid hormone receptor α (THRA) is a nuclear hormone receptor that acts as a transcription factor for genes involved in cell growth and metabolism and therefore mediates the effects of thyroid hormone at the cellular level. There are two main splice isoforms that arise from alternative splicing, the canonical THRα1 and the less-known THRα2. While both isoforms share a high degree of sequence homology and retain key functional domains, only THRα1 can bind to thyroid hormone Triiodothyronine (T3) and hence activate gene expression. In contrast, THRα2 lacks the T3-binding site and consequently acts as an inhibitor. We hypothesize that the local ratio of the two isoforms dictates the overall local action of *THRA*. Limited data is available on the isoform expression levels in different tissues. For this reason, we set out to characterize the *THRA* isoform expression pattern in various healthy human tissues with a specific focus on cortical brain organoids. Considering the lack of antibodies specific for the respective isoforms, we used a bioinformatic pipeline to analyze four bulk RNA-sequencing datasets: **(A)** Newly generated dataset of pooled healthy adult human tissues (10 neuronal and 14 non-neuronal); **(B)** Publicly available human cortical organoids from Testa's lab (E-MTAB-8325) including biological triplicates at 3 time points; **(C)** Newly generated dataset of human cortical organoids including biological triplicates at 3 time points and two T3 conditions and **(D)** Publicly available human and gorilla cortical organoids (GSE153076) including 7 time points. FastQ files were mapped to the reference genome (GRCh30 v13 and Kamilah GGO v6 respectively) using STAR, QC was performed with FastQC and MultiQC, isoform abundances were quantified with StringTie and visualization was done in R. We found that *THRA* expression steadily increases over time in human cortical organoids, with a strong predominance of THRα2 at all stages. This trend was conserved in Gorilla cortical organoids. In adult human tissues, we observed a predominance of THRα2 in all the central nervous system tissues and thyroid, while THRα1 was predominant in adipose tissue and skeletal muscle. Additionally, we observed no difference in *THRA* expression in cortical organoids treated with high T3 levels. These results shed light on the enigmatic role of THRα2 and point at an additional layer of gene regulation. Considering THRα2 is T3-independent, results suggest that the brain requires protection from thyroid hormone during early development. This study may contribute to the development of targeted therapeutic interventions for neurodevelopmental disorders associated with thyroid hormone imbalances.

Disclosures: E. Graceffo: None. **R. Opitz:** None. **H. Krude:** None. **M. Schuelke:** None.

Poster

PSTR385. Comparative Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR385.09

Topic: A.10. Development and Evolution

Support: AMED under Grant Number JP21gm1310012

Title: Analysis of subplate marker genes that could lead to expansion of the human subplate layer

Authors: *Y. MATSUMURA[1], K. HIRAI[1], K. MORIYA-ITO[1], M. OKAMOTO[2], C. MARUYAMA[1];
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Abstract: During brain development, transient neurons provide the basis for developing neural networks. Subplate neurons (SpN), below the cerebral cortex, are born and mature early in the developing brain. Previous studies, including ours, have suggested that SpNs play a role in constructing neural networks, such as regulating radial migration and building connections between the thalamus and the neocortex. Furthermore, the subplate layer (SP layer) is much enlarged in primates compared to mice, indicating that SpNs in the primate have acquired a new and primate-specific function and play an essential role in neocortex enlargement. However, the molecular mechanisms that could lead to the expansion of the SP layer remain elusive. To verify the extent to which gene expression profiles in SpNs are conserved among humans, marmosets, and mice, we analyzed the expression patterns of SpN markers using spatial transcriptome data in the mouse, marmoset, and human cerebral cortex. As a result, we found some subplate marker genes commonly expressed between primates and mice. On the other hand, *Suppression of Tumorigenicity 18* (*ST18*), which encodes a transcription factor, is more expressed in the human SP layer than in the marmoset and mouse ones. This result indicates a correlation between ST18 expression and the expansion of the SP layer. Moreover, to clarify the function of *ST18*, we induced overexpression of *ST18* in mouse SpNs by *in utero* electroporation and analyzed its effect on the SP layer. Elucidating the molecular role of subplate marker genes, including *ST18*, may lead to understanding the enlargement of the SP layer and the development of the neocortex in humans.

Disclosures: Y. Matsumura: None. **K. Hirai:** None. **K. Moriya-Ito:** None. **M. Okamoto:** None. **C. Maruyama:** None.

Poster

PSTR385. Comparative Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR385.10/B60

Topic: A.10. Development and Evolution

Support: NSFC 31820103006

Title: Bmp7 drives evolutionary expansion of the mammalian cortex

Authors: *Z. YANG[1,2], Z. LI[1], G. LIU[1], L. YANG[1], Z. ZHANG[1], Z. XU[1], X. LI[1];
[2]Inst. of Brain Sci., [1]Fudan Univ., Shanghai, China

Abstract: The remarkable increase in human cortical pyramidal neurons (PyNs) is mainly because human cortical radial glial (RG) cells generate PyNs for ~130 days, whereas the same process takes only ~7 days in mice. The molecular mechanisms underlying this difference are largely unknown. Here, we found that BMP7 is expressed by increasing numbers of cortical RG cells during mammalian evolution (mouse, ferret, monkey, man). BMP7 expression in cortical RG cells promotes neurogenesis, inhibits gliogenesis, and thereby increases the length of neurogenic period, whereas SHH signaling promotes cortical gliogenesis. We demonstrate that BMP7 signaling and SHH signaling mutually inhibit each other through regulation of GLI3 repressor formation. We propose that BMP7 drives the evolutionary expansion of the mammalian cerebral cortex by increasing the length of the neurogenic period.

Disclosures: Z. Yang: None. **Z. Li:** None. **G. Liu:** None. **L. Yang:** None. **Z. Zhang:** None. **Z. Xu:** None. **X. Li:** None.

Poster

PSTR385. Comparative Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR385.11/B61

Topic: A.10. Development and Evolution

Support: NIH-NIGMS

Title: Semi-quantitative analysis neurotrophic factors and their receptors in regenerating mesentery of the sea cucumberH. glaberrima

Authors: *G. WICKERSHAM GARCIA[1,3], J. E. GARCIA-ARRARAS[2];
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Abstract: The field of regenerative medicine and precision medicine aims to use growth factors, transcription factors, and other regulators to target specific cellular mechanisms to regenerate tissues and organs. In humans, the ability to regenerate their nervous system is highly limited by many issues that are not yet fully understood. In contrast, other animal species have amazing regenerative capacities. The sea cucumber *H. glaberrima*, like other echinoderms, has been shown to be a potential model to understand nervous system regeneration. One of the components we are exploring is the reinnervation of the regenerated intestinal tissue and possible nerve-dependent regeneration initiated by the blastema. We hypothesize that fibroblasts and mesenchymal cells inside the blastema are sending growth factors and motility factors to the neurons in the mesentery, thus modulating their behavior. Exploration of these putative factors identified from regeneration transcriptomes has allowed the identification of several holothurian orthologues of vertebrate genes. Two differentially spliced sequences were discovered for the

growth factor neurotrophin-3 and two sequences were also found for the GDNF family related receptor 4. These were present in both radial nerve cord and intestine transcriptomes. GFRα-4 sequences contained the binding domains for the GFRα family as well as two hydrophobic regions at the end and beginning of the sequence. NT-3 sequences contained the binding domains for the NGF superfamily. Neighbor-Joining Trees demonstrated groupings of TrkC receptors, GFRα-4 and NT-3 among echinoderm sequences. Using differential gene expression sets generated from RNA-sequencing log-2-fold change analysis, the expression levels of the holothurian GFRα-4 and NT-3 genes were determined at different intestinal regeneration stages. Both were found to be underexpressed before 7 days-post evisceration (dpe) and overexpressed at 14 dpe suggesting their association with intestinal re-innervation. Further studies of these genes could lead to the determination of their roles in regenerating tissue and their possible importance in the regeneration of the organs.

Disclosures: G. Wickersham Garcia: None. **J.E. Garcia-Arraras:** None.

Poster

PSTR385. Comparative Cellular and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR385.12/B62

Topic: A.10. Development and Evolution

Support: NIH Grant R15EY029866-01

Title: The Long-Term Molecular Effects of Tributyltin on Estrogen Signaling In The Zebrafish Retina

Authors: *M. SANDERS[1], V. CONNAUGHTON[2];
[1]Biol., American Univ., Rockville, MD; [2]Biol., American Univ., Washington, DC

Abstract: The development of the retina involves a delicate process that requires careful completion to prevent disruption in proper functioning. A vital component of this careful process involves the production of aromatase, an enzyme that is required for the synthesis of estrogen. Endocrine Disrupting Compounds (EDCs) affect the inhibition or upregulation of hormones like estrogen, which can cause cell apoptosis (cell death), thinning of the retina and cornea, as well as delayed development that emphasizes the importance of estrogen during this process. Unfortunately, not much is known about the long-term implications of changing the estrogenic signaling involved in retinal development. The purpose of this work was to examine if transient developmental exposure to tributyltin (TBT), an antifouling EDC and aromatase inhibitor, can disrupt estrogenic signaling pathways in the brain and retinas of adult zebrafish (Danio rerio*)*. Zebrafish larvae aged 72 hours postfertilization (hpf) or 7 days (d) pf were exposed to either water, vehicle (0.1% ethanol), low TBT (0.2uM) or high TBT (2uM) for 24 hr. After exposure, larvae were returned to control conditions and raised to adulthood, when retinal and brain tissue was collected. qPCR analysis examined expression of estrogenic genes of interest: aromatase B,

estrogen receptor alpha (ERalpha), and ERbe*ta. Our results indicate that developmental TBT exposure at 72hpf had little effects on brain and retina gene expression.* One-way ANOVA was used for statistical analysis. At 72hpf, Aromatase expressions in retina and brain were statistically insignificant (retina; p=.437, brain; p=.939). Similarly, at 7dpf aromatase expression was also statistically insignificant (retina; p=.334, brain; p=.486) However, the results at 7dpf were slightly more significant than 72hpdf. These results indicate the possibility of deeper biological processes triggered by TBT to compensate for the effects of TBT. It is also possible that TBT may be acting on non-genomic pathways. This work will help to uncover estrogen signaling mechanisms in the retina by better understanding aromatase regulation, as aromatase inhibitors are used clinically as a breast cancer therapeutic.

Disclosures: M. Sanders: None. **V. Connaughton:** None.

Poster

PSTR386. Non-NMDA Receptors: Regulation, Pharmacology, and Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR386.01/B63

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: Colorado State University
Boettcher Foundation's Webb-Waring Biomedical Research Program
BrightFocus Foundation
NIH grant 1R03AG072102

Title: Ketamine's rapid antidepressant effects are mediated by ca^{2+}-permeable ampa receptors

Authors: *M. WILES, A. ZAYTSEVA, E. BOUCKOVA, M. H. WUSTRAU, H. MENDEZ-VASQUEZ, I. G. SCHMIDT, S. KIM;
Colorado State Univ., Fort Collins, CO

Abstract: Ketamine is shown to enhance excitatory synaptic drive in multiple brain areas, which is presumed to underlie its rapid antidepressant effects. Moreover, ketamine's therapeutic actions are likely mediated by enhancing neuronal Ca^{2+}signaling. However, ketamine is a noncompetitive NMDA receptor (NMDAR) antagonist that reduces excitatory synaptic transmission and postsynaptic Ca^{2+} signaling. Thus, it is a puzzling question how ketamine enhances glutamatergic and Ca^{2+} activity in neurons to induce rapid antidepressant effects while blocking NMDARs in the hippocampus. Here, we find that ketamine treatment in cultured mouse hippocampal neurons significantly reduces Ca^{2+} and calcineurin activity to elevate AMPA receptor (AMPAR) subunit GluA1 phosphorylation. This phosphorylation ultimately leads to the expression of Ca^{2+}-Permeable, GluA2-lacking, and GluA1-containing AMPARs (CP-AMPARs). The ketamine-induced expression of CP-AMPARs enhances glutamatergic activity and glutamate receptor plasticity in cultured hippocampal neurons. Moreover, when a sub-anesthetic dose of ketamine is given to mice, it increases synaptic GluA1 levels, but not GluA2, and GluA1

phosphorylation in the hippocampus within one hour after treatment. These changes are likely mediated by ketamine-induced reduction of calcineurin activity in the hippocampus. Using the open field and tail suspension tests, we demonstrate that a low dose of ketamine rapidly reduces anxiety-like and depression-like behaviors in both male and female mice. However, when *in vivo* treatment of a CP-AMPAR antagonist abolishes the ketamine's effects on animals' behaviors. We thus discover that ketamine at the low dose promotes the expression of CP-AMPARs via reduction of calcineurin activity, which in turn enhances synaptic strength to induce rapid antidepressant actions.

Disclosures: M. Wiles: None. **A. Zaytseva:** None. **E. Bouckova:** None. **M.H. Wustrau:** None. **H. Mendez-Vasquez:** None. **I.G. Schmidt:** None. **S. Kim:** None.

Poster

PSTR386. Non-NMDA Receptors: Regulation, Pharmacology, and Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR386.02/C1

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: Colorado State University
NIH/NCATS Colorado CTSA Grant UL1 TR002535
Boettcher Foundation's Webb-Waring Biomedical Research Program
BrightFocus Foundation
NIH grant 1R03AG072102

Title: Ketamine's rapid action on chronic stress-induced mental disorders

Authors: *E. BOUCKOVA, M. WILES, M. WUSTRAU, J. FLOWERS, P. VETTER, R. LEE, S. KIM;
Colorado State Univ., Fort Collins, CO

Abstract: Repeated stress affects brain functions, which contributes to the development of anxiety and depression, cognitive dysfunction, and social avoidance. After demonstrating rapid and robust antidepressant efficacy, the FDA approved esketamine (the S enantiomer from of ketamine) for the treatment of depression in 2019, sparking a surge in clinical and public interest around the world. In addition to reducing depression, some research indicate that ketamine may have neuroprotective effects against chronic stress-related neuropsychiatric conditions in both humans and animals. However, the effects of ketamine on these behaviors have not been fully investigated. Low-dose ketamine can enhance excitatory synaptic drive and neuronal Ca^{2+} signaling in the hippocampus, which are presumed to underlie its rapid antidepressant effects. However, ketamine is a noncompetitive antagonist for NMDA receptors (NMDARs), major Ca^{2+} channels in excitatory synapses. Therefore, it is a puzzling question how ketamine enhances glutamatergic and Ca^{2+} activity while blocking NMDARs. Our recent work has discovered a new mechanism of ketamine's antidepressant effects, in which ketamine at the low dose promotes the

expression of Ca^{2+}-Permeable AMPA Receptors (CP-AMPARs) a rare subtype of AMPARs that have larger single channel conductance. Therefore, the ketamine-induced expression of CP-AMPARs can compensate for reduced NMDAR-mediated synaptic Ca^{2+}signaling and enhance synaptic strength enabling ketamine's rapid antidepressant actions in naïve mice. This further suggests that the ketamine-induced expression of CP-AMPARs can enhance synaptic activity to be neuroprotective against chronic stress. Here, 3-month-old C57Bl6 female and male mice received chronic restraint stress (CRS) for 2 weeks, and we performed 4 behavior tests - open field test, reciprocal social interaction, contextual fear conditioning (CFC), and tail suspension test (TST), to determine whether CRS induced behavioral dysfunction. We found that CRS was sufficient to induce anxiety- and depression-like behaviors, fear memory loss, and social dysfunction in mice. Next, we injected 10 mg/kg and 5 mg/kg ketamine to male and female mice following CRS, respectively, and performed social interaction tests, CFC, and TST and found that ketamine treatment improved these behaviors in stressed mice. These demonstrate that ketamine shows strong neuroprotection against chronic stress.

Disclosures: **E. Bouckova:** None. **M. Wiles:** None. **M. Wustrau:** None. **J. Flowers:** None. **P. Vetter:** None. **R. Lee:** None. **S. Kim:** None.

Poster

PSTR386. Non-NMDA Receptors: Regulation, Pharmacology, and Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR386.03/C2

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH Grant R01NS036715

Title: Interaction of GluA2 and NSF regulates Ca^{2+} permeable AMPA receptors

Authors: *Q. ZHU[1], H. L. TAN[2], R. R. JOHNSON[1], R. L. HUGANIR[1];
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Abstract: Synaptic plasticity at excitatory synapses relies on the mobilization of AMPA receptors, which is known to be mediated by the interaction of GluA2 and NSF. Previous studies have shown that NSF facilitates the synaptic insertion of AMPA receptors by directly binding to GluA2. However, there is little comprehensive characterization regarding how the GluA2-NSF interaction impacts synaptic transmission and rodent behaviors. To address it, we generated a knock-in (KI) mouse line called GluA2ΔNSF, in which endogenous GluA2 does not bind to NSF. First, we quantified the basal levels of AMPAR subunit in hippocampus through biochemical analysis. We found that synaptic GluA1 number and its S831 phosphorylation were increased in KI mice compared to WT, but the amount of GluA2 remained unchanged. These findings were consistent with the electrophysiological data obtained from spontaneous release rather than evoked release, suggesting that the disruption of GluA2-NSF interaction may promote the synaptic insertion of calcium-permeable AMPARs (CP-AMPARs) primarily at

spontaneous release sites. Since an enriched environment (EE) can modify synaptic plasticity, we placed both KI and WT mice in EE cages as well as normal control cages. In the WT, EE led to a higher insertion of CP-AMPARs at evoked release sites. However, in the KI mice, EE did not have any significant effects on AMPAR number and synaptic transmission, suggesting GluA2-NSF interaction is required for EE-induced synaptic plasticity. Thus, this study implies that the GluA2-NSF interaction plays a role in regulating the trafficking of a specific pool of AMPARs, potentially involved in the modulation of AMPAR nanodomains.

Disclosures: Q. Zhu: None. **H.L. Tan:** A. Employment/Salary (full or part-time):; Howard Hughes Medical Institute. **R.R. Johnson:** None. **R.L. Huganir:** None.

Poster

PSTR386. Non-NMDA Receptors: Regulation, Pharmacology, and Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR386.04/C3

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: AG070255

Title: Electrophysiology of AMPA receptors in individuals resilient to Alzheimer's disease Neuropathology

Authors: *B. GUTIERREZ GREBENKOVA[1], S. WIDEN[1], W. RUSSELL[1], C. KEENE[2], A. LIMON[1];
[1]Univ. of Texas Med. Br., Galveston, TX; [2]Univ. of Washington, Seattle, WA

Abstract: Alzheimer's disease (AD) is a prevalent form of dementia characterized by the accumulation of amyloid beta and pTau proteins in the brain. While clinical observations are typically used for AD diagnosis, postmortem studies have revealed individuals without dementia symptoms but with high AD pathology, referred to as resilient individuals. Calcium permeable AMPA receptors (CP-AMPARs) have been implicated in the calcium dyshomeostasis of AD, but their presence and electrophysiological response in resilient individuals remain unknown. To address this, we analyzed proteomic and transcriptomic data from a cohort of 30 individuals, including resistant (3M,4F; non-demented/no neuropathology), AD match resistant (2M, 5F; age-matched AD individuals), resilient (2M, 6F; non-demented with AD neuropathology), and AD match resilient (2M, 5F; age-matched AD individuals). Our analysis demonstrated alterations in the expression, organization, and synaptic remodeling of AMPARs across all groups. Notably, resilient individuals exhibited robust involvement in cellular respiration pathways, indicating compensatory mechanisms and enhanced energy management. We further investigated the synaptic responses of CP-AMPARs in these groups by isolating and transplanting synaptic membranes from the parietal cortex into Xenopus laevis oocytes for analysis. Through activation and subsequent inhibition of AMPARs, we determined the contribution of CP-AMPARs to the overall response, revealing reduced CP-AMPARs responses

in resilient individuals. Collectively, our findings provide insights into the structural and functional differences of CP-AMPARs in resilient individuals, potentially reflecting underlying structural or signaling changes identified in our proteomic analysis. Further investigations are warranted to fully understand the mechanisms underlying these alterations and their implications for the progression of AD.

Disclosures: B. Gutierrez Grebenkova: None. **S. Widen:** None. **W. Russell:** None. **C. Keene:** None. **A. Limon:** None.

Poster

PSTR386. Non-NMDA Receptors: Regulation, Pharmacology, and Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR386.05

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NSF Grant IOS 1941073
NIH Grant NS120586
NIH Grant P40 OD10440

Title: The insulin-like peptide INS-27 mediates an inter-tissue feedback pathway that couples muscle activity with AMPA receptor trafficking in distal upstream neurons in C. elegans

Authors: B. J. RENNICH[1], M. HODUL[2], ***P. JUO**[2];
[1]Grad. Program In Neuroscience, Tufts Univ. Sch. of Med., Boston, MA; [2]Tufts Univ. Sch. of Med., Boston, MA

Abstract: Regulation of the number of AMPA receptors (AMPARs) at the synapse controls synaptic strength and is a major mechanism underlying synaptic plasticity. While much has been learned about the intracellular trafficking mechanisms that control AMPAR levels at synapses, less is known about extracellular factors that control AMPAR trafficking, especially those secreted from distal, non-neuronal tissues. Here, we identify an inter-tissue signal in *C. elegans* that couples muscle activity with GLR-1/AMPAR surface levels at synapses in AVA pre-motor interneurons, which reside two synaptic layers upstream of the neuromuscular junction (NMJ) in a circuit that controls locomotion. Mutants lacking NMJ acetylcholine receptor (AChR) subunits *unc-29*/AChR or *unc-38*/AChR exhibit a compensatory increase in surface GLR-1 levels in AVA neurons. This increase in GLR-1 can be rescued by expressing wild-type *unc-29*/AChR specifically in muscle, revealing a feedback pathway that couples NMJ signaling with GLR-1 trafficking in AVA neurons. Similarly, chronic loss of muscle contraction in *unc-54*/muscle myosin mutants results in increased surface GLR-1 levels in AVA, suggesting that loss of muscle activity is sufficient to trigger the feedback pathway. Acute inactivation of muscle, using conditional mutations in *unc-54*/muscle myosin or *twk-18*/potassium channels, is also sufficient to increase surface GLR-1 levels after development is complete. Interestingly, mutations in *unc-31*/Calcium Activator of Protein Secretion (CAPS), which mediates the release of neuropeptide-

containing Dense-Core Vesicles (DCVs), blocks the feedback pathway. This block can be rescued by expressing wild-type *unc-31*/CAPS specifically in muscle suggesting that a muscle-secreted factor contained in DCVs mediates the feedback pathway. We performed a focused RNAi screen of muscle-expressed neuropeptides and identified the insulin-like peptide INS-27 as a potential mediator of the feedback pathway. Consistent with this role, we found that INS-27 is secreted from muscle in an *unc-31*/CAPS-dependent manner. Furthermore, *ins-27* loss-of-function mutants block the feedback pathway and this defect can be rescued by expression of wild-type *ins-27* in muscle. Together, our data reveal a novel inter-tissue feedback pathway mediated by INS-27 that couples muscle activity with AMPA receptor trafficking in upstream neurons. We propose that muscle-secreted INS-27 mediates a compensatory signal to adjust motor circuit excitability in response to changes in muscle activity.

Disclosures: B.J. Rennich: None. **M. Hodul:** None. **P. Juo:** None.

Poster

PSTR386. Non-NMDA Receptors: Regulation, Pharmacology, and Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR386.06/C4

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH Grant F32NS110154
NIH Grant R01NS113948
NIH Grant R01NS105438
Civitan International Research Center Emerging Scholars Award

Title: Afferent convergence to a shared population of synaptic AMPA receptors

Authors: *R. PENNOCK[1], X. YAN[2], L. S. OVERSTREET-WADICHE[3], J. I. WADICHE[4]; [1]Neurobio., Univ. of Alabama at Birmingham Chapter, Birmingham, AL; [2]Univ. of Alabama Birmingham Chapter, Birmingham, AL; [3]Neurobio., Univ. Alabama Birmingham, Birmingham, AL; [4]Neurobio., Univ. of Alabama, Birmigham, Birmingham, AL

Abstract: Precise alignment of pre- and postsynaptic elements optimizes the activation of glutamate receptors at excitatory synapses and is thought to maintain synapse specificity. Nonetheless, glutamate that diffuses out of the synaptic cleft can have actions at distant receptors, a mode of transmission called spillover. To uncover the extrasynaptic actions of glutamate, we localized AMPA receptors (AMPARs) mediating spillover transmission between climbing fibers and molecular layer interneurons (MLIs) in the cerebellar cortex. Climbing fibers provide a functional synaptic connection to MLIs where neurotransmission is mediated entirely by glutamate spillover in the absence of anatomically defined synapses. Using electrophysiology and 2-photon imaging in ex vivo murine brain slices, we show that climbing fiber-MLI transmission occurs in restricted microdomains along MLI dendrites that are reminiscent of parallel fiber synapses. Using pharmacological and physiological manipulations, we found that

spillover transmission is indeed mediated by activation of synaptic Ca^{2+}-permeable AMPA receptors at parallel fiber synapses. Despite the convergence of these afferent pathways to a common population of postsynaptic receptors, release from each pathway is differentially modulated by presynaptic GABAB receptor activation. This allows activation of synaptic AMPARs even when glutamate release from parallel fibers is suppressed. Additionally, activation of either pathway in close temporal and spatial proximity to the other facilitates recruitment of perisynaptic NMDA receptors. Together, these findings demonstrate a circuit motif that relies on the activation of a common population of synaptic receptors by two separate afferent pathways, which contrasts with the notion of synapse specificity.

Disclosures: R. Pennock: None. **X. Yan:** None. **L.S. Overstreet-Wadiche:** None. **J.I. Wadiche:** None.

Poster

PSTR386. Non-NMDA Receptors: Regulation, Pharmacology, and Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR386.07/Web Only

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Title: Activity dependent induction of presynaptic homeostatic potentiation

Authors: *C. BECKERS, J. YU-STRZELCYZK, C. ZHANG, S. GAO, M. HECKMANN, S. DANNHÄUSER;
Inst. of Physiol., Julius Maximilians Univ. of Würzburg, Würzburg, Germany

Abstract: The neuromuscular junction (NMJ) of *Drosophila melanogaster* is a versatile model for the study of glutamatergic transmission. At the NMJ of *Drosophila* four subunits assemble to one heterotetrametric receptor. Each receptor contains GluRIIC, GluRIID, GluRIIE and additionally GluRIIA or GluRIIB as a fourth subunit. Receptors containing GluRIIA as fourth subunit and receptors which incorporate GluRIIB as fourth subunit, differ in their functions and their physiological parameters. The perturbation of the postsynaptic glutamate receptor subunit GluRIIA leads primarily to a decrease of the postsynaptic response, which is consequently rescued by an increase in presynaptic transmitter release (referred to as presynaptic homeostatic potentiation, PHP)[1].One of the most basic questions has remained elusive so far: Why does the knockout of GluRIIA leads to the induction of PHP and a knockout of GluRIIB does not? A recent study laid focus on the intracellular tail of GluRIIA which recruits CamKII and activates it to pCamKII which then serves as a stop signal for PHP [2].In this study we describe the effect of a GluRIIA-pore-chimera which resembles a native GluRIIA receptor with the pore building region of GluRIIB. Beginning with outside-out-patch-clamp analysis of these receptor constructs in *Xenopus laevis* oozytes, we show that the desensitization kinetics of the GluRIIA-pore-chimera assimilates to the kinetics ofGluRIIA (10.8 ± 0.3 ms vs 14.4 ± 3.2 ms, n=4). In the next step we expressed this GluRIIA-pore-chimera with an additional ALFA-tag and a wildtype GluRIIA with an ALFA-tag at the endogenous locus of GluRIIA in *Drosophila melanogaster*.

Both constructs are expressed at the NMJ, shown by applying SIM-imaging. Interestingly a strong fluorescens signal for pCamKII could be detected as well in GluRIIA-ALFA and GluRIIA-pore-chimera-ALFA. To check how the pore chimera behaves functionally, we applied two-electrode-voltage-clamp (TEVC) of the larval NMJ. The GluRIIA-pore-chimera leads to a decrease of the postsynaptic response to a singly released vesicle. The evoked response is fully restored, thereby undergoing PHP. These findings are giving rise to the idea that an activity-dependent induction mechanismfor PHP exist.References(1) Graeme W. Davis and Martin Müller. Homeostatic Control of PresynapticNeurotransmitter Release. Annual Review of Physiology 2015 77:1, 251-270(2) Perry S, Han Y, Qiu C, Chien C, Goel P, Nishimura S, Sajnani M, Schmid A, SigristSJ, Dickman D. A glutamate receptor C-tail recruits CaMKII to suppress retrogradehomeostatic signaling. Nat Commun. 2022 Dec 10;13(1):7656.

Disclosures: C. Beckers: None. **J. Yu-Strzelcyzk:** None. **C. Zhang:** None. **S. Gao:** None. **M. Heckmann:** None. **S. Dannhäuser:** None.

Poster

PSTR386. Non-NMDA Receptors: Regulation, Pharmacology, and Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR386.08/C5

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: PAPIIT, UNAM, IN228420

Title: Does prolactin induce neuroprotection against Kainic Acid excitotoxicity by modulating intracellular Ca^{2+}concentration?

Authors: *V. RODRIGUEZ CHAVEZ[1], G. MOLINA-SALINAS[1], E. FLORES-SOTO[2], B. ROMERO MARTÍNEZ[2], L. MONTAÑO RAMÍREZ[2], M. CERBÓN CERVANTES[1]; [1]Dept. de Biología, [2]Dept. de Farmacología, Univ. Nacional Autónoma De México, Ciudad de Mexico, Mexico

Abstract: It has been described that PRL induces neuroprotection against kainic acid (KA) excitotoxicity damage, both *in vivo* models and *in vitro* models, but this mechanism is not fully described. This study aimed to determine the neuroprotective role of PRL administration in primary cultures of hippocampal neurons assessing intracellular Ca^{2+} concentration and iGluRs expression. For this study, immunofluorescence, and Western Blot methods were used to assess the expression of iGluRs and their cellular localization. To determine the effect of PRL on iGluRs and intracellular Ca^{2+} concentration, primary hippocampal neurons obtained from 17.5-day-old rat embryos were used. Cultures were divided into Control (CTRL, saline), PRL 20 ng/ml, glutamate (20 μM), PRL+Glu (20 ng/ml/ 20μM), kainic acid (20 μM), and PRL+AK (20 ng/ml/ 20μM) groups. It was found that PRLR localizes to hippocampal neurons, iGluRs subunits are observed at the protein level in primary cultures of hippocampal neurons, and the intracellular Ca^{2+} concentration was modulated by excitotoxic events. Understanding the

neuroprotective effects of PRL will allow the generation of new therapeutic targets for damage caused by the excitotoxicity of iGluR agonists.

Disclosures: V. Rodriguez Chavez: None. **G. Molina-Salinas:** None. **E. Flores-Soto:** None. **B. Romero Martínez:** None. **L. Montaño Ramírez:** None. **M. Cerbón Cervantes:** None.

Poster

PSTR386. Non-NMDA Receptors: Regulation, Pharmacology, and Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR386.09/C6

Topic: B.04. Synaptic Transmission

Support: NICHD/NIH grant Z01 HD008914, awarded to MS.

Title: Auxililaty protein Neto-a modulates subcellular distribution and functional properties of KaiR1D autoreceptor

Authors: *W.-C. HSIEH[1], T. HAN[3], R. VICIDOMINI[4], P. NGUYEN[2], Z. LI[5], M. SERPE[6]; [2]NICHD, [1]Natl. Inst. of Health, HHS, Bethesda, MD; [3]NICHD/Eunice Kennedy Shriver Nat'l Inst. of Child H, Bethesda, MD; [4]NIH/NCHID, NIH/NCHID, Bethesda, MD; [5]NIMH, NIH, Natl. Inst. of Mental Hlth., Bethesda, MD; [6]NIH, NIH, Bethesda, MD

Abstract: At excitatory synapses, glumatergic autoreceptors provide a feedback mechanism that modulate glutamate release and ensure stable neuronal networks. Disruptions of this feedback have been linked to various neuronal disorders. Detection and functional investigation of these low abundant presynaptic proteins have been challenging. At *Drosophila* NMJ, an autoreceptor containing the KaiR1D subunit has been implicated in the control of glutamate release; *KaiR1D*^{null} animals have reduced basal neurotransmission. We and others previously showed that KaiR1D requires at least two auxiliary proteins, Neto-α and Sol1. Here, we focus on the roles of Neto-α in modulating KaiR1D properties and distribution. Neto-α limits KaiR1D *in vivo* activities: Basal neurotransmission is reduced by 50% in *neto-α*^{null} and neuronal overexpression of KaiR1D cannot rescue this defect. At molecular level, Neto-α modulates homotetrameric KaiR1D channel properties, including desensitization rates and polyamine-induced channel rectification. Neto proteins are highly conserved auxiliary subunits with conserved extracellular domains, including two CUB domains and a LDL motif, and divergent intracellular domains (CTD). We demonstrated that CUB1 domain is required for modulation of KaiR1D gating properties as well as *in vivo* autoreceptor activities, while the CTD modulates KaiR1D gating properties. We found reduced KaiR1D presynaptic distribution at *neto-α*^{null} NMJs. To investigate how Neto-α modulates the subcellular distribution of KaiR1D, we expressed *Drosophila* protein in primary rat hippocampal neurons and examined KaiR1D localization. KaiR1D localizes to dendrites, with or without Neto-α, but it cannot efficiently enter the axon by itself even when overexpressed. Neto-α or ΔCUB1, but not ΔCTD, promote KaiR1D axonal localization,

indicating the intracellular domain of Neto-α promote KaiR1D axonal entry. All Neto variants distributed at both neurites and formed puncta largely colocalizing with KaiR1D. Also, Neto-α and KaiR1D colocalize in the proximity of the active zones suggesting that Neto-α may stabilize KaiR1D at the site of autoreceptor function. Our data indicate that auxiliary protein Neto-α modulates multiple aspects of KaiR1D distribution and function to ensure proper autoreceptor activities.

Disclosures: W. Hsieh: None. **T. Han:** None. **R. Vicidomini:** None. **P. Nguyen:** None. **Z. Li:** None. **M. Serpe:** None.

Poster

PSTR386. Non-NMDA Receptors: Regulation, Pharmacology, and Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR386.10/C7

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH/NINDS R01NS105502
NIH/NINDS R01NS115471

Title: A pathogenic mutation in GluK2 kainate receptor alters dendritic and neuronal excitability through dysregulation of K+ channels in the hippocampus

Authors: *T. NOMURA[1], S. TANIGUCHI[2], Y.-Z. WANG[2], N.-H. YEH[2], A. WILEN[3], C. C. CASTILLON[2], K. FOOTE[2], J. XU[2], J. N. ARMSTRONG[2], J. N. SAVAS[2], G. T. SWANSON[2], A. CONTRACTOR[2];
[1]Northwestern Univ. - Chicago, Chicago, IL; [2]Northwestern Univ., Chicago, IL; [3]Northwestern Univ., Northwestern Univ., Evanston, IL

Abstract: Human genetic studies have identified several pathogenic mutations in the *GRIK2* gene, encoding for the GluK2 subunit of kainate receptors (KARs), in individuals with neurodevelopmental disorders. Several pathogenic missense mutations cause amino acid substitutions clustered in critical functional domains of the receptor protein associated with channel gating (Guzman et al. 2017; Stoltz et al. 2021). We engineered a GluK2 mutant mouse model which carries a homologous mutation described in humans; GluK2(A657T) mouse. The present study focused on *in vitro* electrophysiological analyses of heterozygous GluK2(A657T) mice to better understand the synaptic and circuit level pathology in *GRIK2* related disorders. The KAR-mediated EPSC ($EPSC_{KAR}$) at the hippocampal mossy fiber (MF) - CA3 synapse had significantly slower decay kinetics in GluK2(A657T) mice, which is consistent with previous studies in non-neuronal cells (Kohda et al. 2000; Guzman et al. 2017) and suggests that mutant GluK2 subunits are incorporated into functional KARs in these mice. While there was a clear alteration in KAR channel function, neither short-term nor long-term synaptic plasticity, in which KARs play a critical role, was affected in GluK2(A657T) mice. Unexpectedly, we found that CA3 neurons fire spontaneous action potentials (APs) at an abnormally high frequency in

GluK2(A657T) mice. Examination of this hyper-excitability phenotype of CA3 neurons revealed that the coupling of distal associational-commissural (AC) - CA3 EPSPs to AP firing was elevated suggesting an increase in dendritic excitability. This was confirmed by the analysis of back-propagating action potential (bAP) by measuring the dendritic Ca^{2+} signal which demonstrated less attenuation more distal to the soma in mutant mice. There was a reduction in the activity of Ca^{2+} activated K^+ channels (SK), which is known to regulate dendritic excitability, in mutant mice. Pharmacological inhibition of SK channels in wild type mice in part mimicked cellular phenotypes of GluK2(A657T) mice, increasing EPSP-AP coupling and the propagation of dendritic bAPs. These results indicate that a disease causing A657T missense mutation in *Grik2* does alter KAR function, but the consequences directly attributable to KAR malfunction are relatively inconsequential. Instead, the dominant circuit pathophysiology in this *Grik2* disorder is through an unexpected alteration in K^+ channels, which results in a change in dendritic excitability and neuronal firing.

Disclosures: **T. Nomura:** None. **S. Taniguchi:** None. **Y. Wang:** None. **N. Yeh:** None. **A. Wilen:** None. **C.C. Castillon:** None. **K. Foote:** None. **J. Xu:** None. **J.N. Armstrong:** None. **J.N. Savas:** None. **G.T. Swanson:** None. **A. Contractor:** None.

Poster

PSTR386. Non-NMDA Receptors: Regulation, Pharmacology, and Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR386.11/C8

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: Startup Account

Title: Activation of glutamate receptors increases activity of female kisspeptin neurons.

Authors: *R. BEARSS[1], R. PIET[2];
[1]Sch. of Biomed. Sci., [2]Biol. Sci. Dept., Kent State Univ., Kent, OH

Abstract: Kisspeptin (kiss1) neurons in the arcuate nucleus (ARN) stimulate gonadotropin releasing hormone (GnRH) neuron activity and GnRH release, which subsequently promotes luteinizing hormone (LH) release from the anterior pituitary gland. ARN^{kiss1} neurons co-release kiss1, neurokinin B (NKB), dynorphin A (dyn A), and the neurotransmitter glutamate. ARN^{kiss1} neurons are thought to coordinate their activity through reciprocal connections, thereby generating episodic bouts of activity and driving GnRH and LH pulsatile secretion. This is necessary in both sexes for fertility and gonadal function. Ionotropic glutamate receptor antagonists suppress ARN^{kiss1} neuron coordinated activity and pulsatile LH release. Although these lines of evidence indicate a role of glutamatergic neurotransmission in kisspeptin neurons, little is known about the impact of glutamate receptor activation on these cells. We sought to examine the effect of ionotropic AMPA and NMDA receptors, and group 1 metabotropic glutamate receptors (mGluRs) on ARN^{kiss1} neuron activity in female mice.

We used calcium imaging to monitor the activity of kiss1 neurons in brain slices. We crossed Kiss1-Cre mice—expressing Cre recombinase (Cre) in kisspeptin neurons—with Cre-dependent GCaMP6f mice to generate Kiss1-Cre x GCaMP6f mice that express GCaMP6f in kiss1 neurons. Coronal brain slices (200 µm thickness) obtained from female diestrus Kiss1-cre x GCaMP6f mice containing the ARN were placed in a recording chamber. Changes in individual kisspeptin neuron GCaMP6f fluorescence were measured using epifluorescence microscopy to estimate variations in intracellular calcium concentrations ($[Ca^{2+}]_i$) as a proxy for electrical activity. Kiss1 neurons were exposed for 2 minutes, via bath application, to either AMPA (10µM), NMDA (50µM), or DHPG (50µM). On average, applications of AMPA (10 µM), DHPG (50 µM), or NMDA (50 µM) to female ARN^{Kiss1} neurons transiently increased normalized fluorescence by 0.386 ± 0.016 (161 cells, 6 slices, and 4 animals), 0.076 ± 0.007 (111 cells, 7 slices, and 4 animals), and 0.150 ± 0.011 (103 cells, 6 slices, and 4 animals), respectively. The proportion of cells activated for each drug was 100 (n=161), 94 (n=111), and 100% (n=103) for AMPA, DHPG, and NMDA application, respectively. This indicates that activation of ionotropic AMPA and NMDA receptors as well as group 1 mGluRs increases $[Ca^{2+}]_i$ in female ARN^{kiss1} neurons and, thus, stimulates activity in these cells. Further investigation into the role of endogenous glutamatergic signaling and into the respective role of glutamatergic receptors in regulating kiss1 neuron activity is needed.

Disclosures: **R. Bearss:** None. **R. Piet:** None.

Poster

PSTR386. Non-NMDA Receptors: Regulation, Pharmacology, and Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR386.12/C9

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Title: Pharmacology of $P2X_3$ and $P2X_{2/3}$ receptors in cell lines and hiPSC-derived neurons: An automated patch clamp study

Authors: E. SIRTORI[1], J.-F. ROLLAND[1], E.-L. BUDUSAN[2], M. G. ROTORDAM[3], J. RATHJE[3], T. A. GOETZE[3], N. BECKER[3], ***A. OBERGRUSSBERGER**[3], I. LU[4], T. STRASSMAIER[4], A. RANDOLPH[4], V. TRUONG[5], P. WALSH[5], N. FERTIG[3];
[1]Axxam S.p.A, Milan, Italy; [2]Univ. of Queensland, Brisbane, Australia; [3]Nanion Technologies GmbH, Munich, Germany; [4]Nanion Technologies Inc., Livingston, NJ; [5]Anatomic Inc., Anatomic Inc., Minneapolis, MN

Abstract: P2X receptors are ligand-gated ion channels activated by extracellular ATP. They are permeable to small monovalent cations, some having significant divalent or anion permeability. The $P2X_2$ and $P2X_3$ subunits are predominantly expressed in primary sensory neurons and have been proposed to play a role in thermal sensation, taste and pain. They form functional hetero- or homotrimers which are activated by αβ-methylene ATP (αβmeATP). The stoichiometry of $P2X_{2/3}$ heteromers appears to be dependent on the relative abundance of the two subunits. A

mixture of $P2X_2$ and $P2X_3$ homomers as well as $P2X_{2/3}$ heteromers are likely to exist, which can be distinguished through their biophysical and pharmacological properties. The receptors open in response to an increase in extracellular ATP which occurs under pathological conditions such as tissue damage. The resulting depolarization leads to propagation of the pain signal. Due to its role in nociception and pain signaling, these receptors are considered to be important targets for pain management. Here we present data collected on automated patch clamp (APC) systems with ranging throughput from 1 up to 384 cells simultaneously. We show activation and inhibition of $P2X_{2/3}$ and $P2X_3$ receptors expressed in CHO cells with rapid and brief application of ligand. ATP was applied using the perfusion system of the Port-a-Patch or the pipette(s) of the Patchliner or SyncroPatch 384. The EC_{50} values for αβmeATP or ATP activation of $P2X_{2/3}$ receptors, and the IC_{50} of suramin, were comparable across the three devices. The kinetics of currents mediated by homomeric $P2X_3$ receptors were distinctly faster than those of heteromeric $P2X_{2/3}$ receptors. $P2X_3$-mediated currents were activated by αβmeATP and could be repetitively activated at least 6 times in the same cell with similar peak amplitudes. The $P2X_3$-selective compound A-317491 blocked $P2X_3$-mediated currents activated by αβmeATP with an IC_{50} of approximately 90 nM and was similar on all three devices. Recording at physiological temperature resulted in a faster recovery but had negligible effects on tachyphylaxis. We also recorded P2X-mediated current responses from human induced pluripotent stem cell-derived sensory neurons on higher throughput automated patch clamp devices. The fast kinetics of the ATP-activated responses is consistent with $P2X_3$ homomeric receptors, and currents could be repetitively activated with a recovery time of 10 minutes between activation with ATP. Studying $P2X_3$ and $P2X_{2/3}$ receptors in heterologous cell lines and hiPSC-derived neurons using automated patch clamp provides physiologically relevant and high throughput tools for drug discovery.

Disclosures: **E. Sirtori:** None. **J. Rolland:** None. **E. Budusan:** None. **M.G. Rotordam:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **J. Rathje:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **T.A. Goetze:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **N. Becker:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **A. Obergrussberger:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **I. Lu:** A. Employment/Salary (full or part-time):; Nanion Technologies Inc. **T. Strassmaier:** A. Employment/Salary (full or part-time):; Nanion Technologies Inc. **A. Randolph:** A. Employment/Salary (full or part-time):; Nanion Technologies Inc.. **V. Truong:** None. **P. Walsh:** None. **N. Fertig:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH.

Poster

PSTR387. Regulation of Synaptic Plasticity

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR387.01/C10

Topic: B.05. Synaptic Plasticity

Support: Institute of Biomedical Sciences, Academia Sinica
National Science and Technology Council (MOST 111-2628-B-001-017-MY3)

Title: Frequency dependence of synaptic input dynamics supporting behavioral time-scale synaptic plasticity (BTSP) in the hippocampus

Authors: *C.-T. CHEN[1,2,3], H.-Y. WANG[2], H.-P. HUANG[2,4], C.-L. HSU[2,3,4,5,6];
[1]Academia Sinica, Taipei, Taiwan; [2]Inst. of Biomed. Sciences, Academia Sinica, Taipei, Taiwan; [3]Taiwan Intl. Grad. Program-Interdisciplinary Neurosci. (TIGP-INS), Taipei, Taiwan; [4]Taiwan Intl. Grad. Program-Molecular Med. (TIGP-MMP), Taipei, Taiwan; [5]Neurosci. Program of Academia Sinica (NPAS), Taipei, Taiwan; [6]Dept. of Life Science, Col. of Life Science, Natl. Taiwan Univ., Taipei, Taiwan

Abstract: It is crucial to rapidly form episodic memories for unfolding experience with regards to spatial environments in the brain. The pyramidal neurons of the dorsal hippocampal CA1 exhibit powerful synaptic potentiation triggered by calcium plateau potentials initiated in the dendrites, which provides an elegant candidate mechanism for one-shot hippocampal learning. In this so-called behavioral time-scale synaptic plasticity (BTSP), it is commonly believed that conjunctive dendritic input processing, which reflects dynamics of the upstream circuits during spatial navigation, is critically important for the induction of plasticity. However, the exact circuit and biophysical mechanisms are largely unknown. We hypothesized that the activation kinetics of synapses to CA1 pyramidal cells constrains BTSP induction in the context of circuit operations, and focused on the particular aspect of input frequency dependence of this plasticity in acute hippocampal slices. We found that CA3 activation frequency was a strong determinant for BTSP, which might not be explained simply by the peak postsynaptic depolarizations resulting from activation patterns. Modeling approach will be taken to refine the mechanistic model proposed by Milstein et al. (2021; *eLife* **10**:e73046) through reducing the free parameter space, and identify potential biophysical mechanisms to test pharmacologically. This quantitative description may be a necessary step toward linking the natural statistics of circuit properties to a neurobiological basis of versatile memory processes under navigation in space.

Disclosures: C. Chen: None. **H. Wang:** None. **H. Huang:** None. **C. Hsu:** None.

Poster

PSTR387. Regulation of Synaptic Plasticity

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR387.02/C11

Topic: B.05. Synaptic Plasticity

Support: 4R01NS116589-02

Title: Interval learning and spontaneous replay in ex vivo cortical circuits using a dual optical training protocol

Authors: *B. LIU, D. V. BUONOMANO;
Neurobio., UCLA, Los Angeles, CA

Abstract: The ability to process the duration, interval, and temporal structure of stimuli is critical to most forms of learning, behavior, and sensory processing. While the neural mechanisms underlying temporal processing on the subsecond scale remain unknown, they have been proposed to rely on the inherent synaptic and network dynamics of all cortical circuits rather than specialized mechanisms present in specific circuits. To test this hypothesis, we differentially trained isolated cortical organotypic slice cultures on two specific temporal patterns using a chronic dual-optical stimulation approach. Excitatory neurons in the cortex were sparsely transduced with either ChR2 or ChrimsonR. Our temporal training paradigm consisted of either an Early or Late pairing. A 440 ms long train of red light pulses (25 Hz, 625nm)—representing presynaptic neurons—was paired with a 80 ms long train of blue light pulses (50 Hz, 455nm)—representing postsynaptic neurons. In the Early group, both pathways were activated with similar onset times, while in the Late group both pathways were activated with similar offset times. Slices were trained for 24 hrs in the incubator. After training, whole-cell recordings revealed differential network dynamics in response to the red stimuli. Specifically, the median event time of the evoked polysynaptic events was significantly shorter in neurons in the Early group compared to the Late group (81 and 323 ms respectively, $p < 0.001$). Similarly, there was a significant difference in the peak time of the polysynaptic responses (77 and 387 ms, $p < 0.001$). Thus, the networks appeared to have learned the temporal structure of the patterns they were exposed to during training, specifically the median peak times of the polysynaptic events were closely aligned with their respective early (10-90ms) or late (370-450ms) training intervals. Suggesting the networks were predicting or expecting the early or late arrival of the blue light. Finally, consistent with the notion of replay and consolidation, we also observed spontaneous replay of activity in the networks which mirrored the differential polysynaptic responses of early or late training. Specifically, spontaneous activity in the early group peaked early, and spontaneous activity in the late group peaked late (47 and 215 ms respectively, $p<0.001$). Our findings suggest that cortical circuits in a dish are intrinsically capable of learning temporal information, and that the learning rules and algorithms underlying spontaneous replay are functional in a dish and thus not necessarily dependent on inter-areal interactions.

Disclosures: B. Liu: None. **D.V. Buonomano:** None.

Poster

PSTR387. Regulation of Synaptic Plasticity

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR387.03/C12

Topic: B.05. Synaptic Plasticity

Support: R01 NS103993
AHA Award Number 830217

Title: Inhibitory circuitry underlying plasticity in motor cortex during skill acquisition

Authors: *T. KIM, B. M. HOOKS;
Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Learning motor skills requires structural and functional plasticity in the primary motor cortex (M1). Cortical inhibitory circuitry is important for motor function. In addition to their proposed role in cortical plasticity in sensory areas, cortical interneurons are required in motor skill acquisition because disruption of the activity of inhibitory neuronal populations interferes with motor learning. But there is a gap in our understanding of how inhibitory synaptic connections change during skill acquisition. This study aims to determine the normal developmental trajectory of motor learning and to address the inhibitory connectivity changes after motor learning. We focus on two major inhibitory interneuron types: somatostatin-expressing (SOM+) interneurons and parvalbumin-expressing (PV+) interneurons. To investigate the difference in inhibition meditated by specific interneuron types during motor learning, animals are trained to run on an accelerating rotarod at ages from postnatal day(P) 20 to P120. Learning is assessed by changes in gait pattern and time to fall. Estimated position of paws and other body parts are obtained from high speed video. To label the task-active and task-inactive cells during motor learning, CaMPARI2, a genetically encoded activity marker, is injected in M1 perinatally to distinguish neurons in an activity-dependent manner. Upon task completion, inhibitory responses (IPSCs) are optogenetically evoked in either PV+ or SOM+ neurons to measure inhibitory synaptic strength to excitatory cells that are task-active (red) or task-inactive (green). Based on time to fall, rotarod task performance improved most rapidly between P30-60. Paw position and gait patterns change with learning, though differently between age groups. PV-mediated inhibition is greater in the active cells, while SOM-mediated inhibition is not different between inactive and active cells on the first day of training. These results suggest early changes in PV-mediated inhibition may support motor skill acquisition in mice. We hypothesize that changes in SOM+ connectivity may occur at longer training periods when learning is consolidated. Further, future experiments will test whether stronger PV-mediated inhibition persists in task-active cells during longer training periods or returns to baseline.

Disclosures: **T. Kim:** None. **B.M. Hooks:** None.

Poster

PSTR387. Regulation of Synaptic Plasticity

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR387.04/C13

Topic: B.05. Synaptic Plasticity

Support: The Hartwell Foundation
NIH R01DC018621

Title: The role of the dyslexia-associated gene KIAA0319 in regulating intrinsic excitability in the zebra finch (Taeniopygia guttata) auditory cortex

Authors: *T. M. BYRON[1], C. D. DEPPMANN[2], C. D. MELIZA[2];
[1]Psychology, Univ. of Virgina, Charlottesville, VA; [2]Program in Fundamental Neurosci., Univ. of Virginia, Charlottesville, VA

Abstract: Nearly 8% of American children ages 3-17 suffer from speech- and language-learning disorders, including dyslexia. Individuals with these disorders often exhibit deficits in processing phonemes, the fundamental units of words, suggesting underlying impairments in central auditory processing. Dyslexia is strongly associated with loss-of-function mutations in *KIAA0319* (Galaburda et al., 2006), but the function of this gene remains poorly understood. Knocking down *KIAA0319* in rats produces deficits in auditory processing and dysregulation of intrinsic excitability in the auditory cortex (Centanni et al., 2014). In zebra finches, an impoverished acoustic environment during the critical period for song memorization also disrupts intrinsic excitability in the auditory cortex via the low-threshold potassium channel Kv1.1 (Chen and Meliza, 2020). We therefore hypothesized that *KIAA0319* may be involved in experience-dependent plasticity of intrinsic dynamics during early auditory development. In this exploratory study, we characterized how co-expression of *KIAA0319* and Kv1.1 depend on age and acoustic environment in fledgling and juvenile zebra finches of both sexes using in situ hybridization and immunofluorescence. We also developed adeno-associated viruses (AAVs) carrying short-hairpin ribonucleic acid (shRNA) constructs targeting *KIAA0319*, with the goal of testing whether knocking down *KIAA0319* during auditory development impacts Kv1.1 expression, intrinsic plasticity, and song memorization. Preliminary data *in vitro* demonstrate that knocking down *KIAA0319* causes overexpression of Kv1.1. These studies can reveal a mechanistic understanding of how dyslexia-associated mutations contribute to deficits in the processing of complex vocal signals used for communication.

Disclosures: **T.M. Byron:** None. **C.D. Deppmann:** None. **C.D. Meliza:** None.

Poster

PSTR387. Regulation of Synaptic Plasticity

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR387.05/C14

Topic: B.05. Synaptic Plasticity

Support: NRF-2012R1A3A1050385

Title: Increased synaptic connection in corticocortical circuit in rodent motor skill learning

Authors: *Y. KIM[1], B.-K. KAANG[2];
[2]Sch. of Biol. Sci., [1]Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: In order to adapt to changing environments, rodents actively learn new motor skills for survival. Although the central cortical region mediating motor skill learning remained elusive, it is recently shown that the primary motor cortex (M1) plays a significant role in motor learning, unlike its classical role as a simple muscle controller. The secondary motor cortex (M2) is also recently shown to be a critical region for motor learning on top of its well-known function for motor execution and planning. Although these two regions are known for their role in motor learning, the role of direct connection and synaptic correlate between these two regions still remain elusive. Here, we show that accelerating rotarod task successfully induces motor skill acquisition in mice which recruited neuronal activity in the motor cortex during their acquisition. We also show increased synaptic density between activated neurons of M2-M1 compared to synapses with unactivated random neurons using the dual-eGRASP technique. To sum up, this study suggests the potential importance of synapses during motor learning.

Disclosures: Y. Kim: None. **B. Kaang:** None.

Poster

PSTR387. Regulation of Synaptic Plasticity

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR387.06/C15

Topic: B.05. Synaptic Plasticity

Support: HHMI

Title: Brain-wide measurement of synaptic protein turnover during learning in mice

Authors: *B. MOHAR[1], G. MICHEL[1], V. HERNANDEZ[1], P. W. TILLBERG[1], K. SVOBODA[2,1], N. P. SPRUSTON[1];
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Abstract: Cellular functions are regulated by synthesizing and degrading proteins on time scales ranging from minutes to weeks. Protein turnover varies across proteins, cellular compartments, cell types, and tissues. In the brain, circuit-specific protein turnover is thought to underlie synaptic plasticity, but current methods to track protein turnover lack cellular or subcellular resolution. We are using our recently described pulse-chase method (DELTA) to measure protein turnover with high spatial and temporal resolution throughout the body. DELTA relies on the rapid covalent capture by HaloTag of fluorescent ligands that were optimized for bioavailability *in vivo* and thus overcome the unique challenges associated with labeling in the brain. DELTA allows measurement of protein turnover with subcellular resolution and at brain-wide scales. We found that the nuclear protein MeCP2 shows brain-region- and cell-type-specific turnover, and the synaptic protein PSD95 is destabilized in specific brain regions and dendritic compartments following behavioral enrichment. We are now using DELTA in knock-in mice expressing GluA2-HaloTag fusions, to localize brain regions associated with learning a virtual reality task.

Disclosures: **B. Mohar:** None. **G. Michel:** None. **V. Hernandez:** None. **P.W. Tillberg:** None. **K. Svoboda:** None. **N.P. Spruston:** None.

Poster

PSTR387. Regulation of Synaptic Plasticity

Location: WCC Halls A-C

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Program #/Poster #: PSTR387.07/C16

Topic: B.05. Synaptic Plasticity

Support: Grant-in-Aid for Scientific Research

Title: Attempt to quantify the segmentation of continuous input by a cultured neuronal network

Authors: *K. NITTA[1], S. KUDOH[2];
[1]Kwansei Gakuin Univ., Sanda Shi/Akashiadai, Japan; [2]Kwansei Gakuin Univ., Sanda, Hyougo, Japan

Abstract: Analysis of the cultured neural network, considered to be the smallest unit of the brain, will lead to an understanding of fundamental functions of the human brain, such as learning. Previous studies have shown that a cultured neural network can retain the history of the previous two stimuli when they are applied continuously at 2-second intervals. In this study, we hypothesized that the human brain learns by segmenting a sequence of stimuli into smaller units, and we tested this hypothesis by applying continuous stimuli to a cultured neural network, a model of the human brain. The stimuli applied in uniform frequency of occurrence for all stimulus patterns consisting of a set of three stimuli (triplet), and analyzed the evoked responses to each stimulus. A sequence of 974 stimuli at 2-second intervals was used as a single sequence. The bursts immediately after the stimuli were detected from the spike group at 100 ms after the stimuli, and the firing patterns of the evoked responses to the stimuli were extracted as cell assemblies. Hierarchical clustering was performed by defining each pattern as a set of firing electrodes and calculating the distance between the sets using Jaccard coefficients. The clustering threshold was set as the average of all distance values. We analyzed the response reproducibility to stimuli using our original clustering evaluation indexes, CPI (Class Purity Index), PCA (Pattern Classification Acuracy), and SiC (Similarity in Class). We analyzed the response reproducibility to stimuli using CPI (Class Purity Index), PCA (Pattern Classification Accuracy) and SiC (Similarity in Class). The results showed that the variance of all the indices in the evoked response patterns to each [triplet] set of stimuli decreased between the first and second sequences, while there was no change in the mean value of each index in the evoked response patterns to all 27 types of [triplets]. This suggests that the cultured neural network may have learned each triplet equally as a segment from the successive stimuli with equal frequency of occurrence. The number of classes obtained by hierarchical clustering using the mean of all distance values as the threshold was around 30, which was close to the number of [triplet] types. This result suggests that the clustering method used in this study is effective in classifying the electrical activity induced by the 27 triplets according to their characteristics.

Disclosures: **K. Nitta:** None. **S. Kudoh:** None.

Poster

PSTR387. Regulation of Synaptic Plasticity

Location: WCC Halls A-C

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Program #/Poster #: PSTR387.08/C17

Topic: B.05. Synaptic Plasticity

Support: 11-2311-B-001 -020 -MY3

Title: Cmtr1 regulates hippocampus-related long-term synaptic plasticity and memory consolidation through promoting NMDA receptor signaling

Authors: *S. AZEEM[1,2], N.-H. YEH[1], Y.-S. HUANG[1];
[1]Inst. of Biomed. Sciences, Academia Sinica, Taipei City, Taiwan; [2]Taiwan Intl. Grad. Program in Interdisciplinary Neuroscience, Natl. Yang Ming Chiao Tung Univ. and Academia Sinica, Taipei, Taiwan

Abstract: Eukaryotic mRNA is 5′-end capped with m7 guanosine, known as cap0 (m7GpppNpNp, N:any nucleotide). Cap methyltransferase (CMTR1) further catalyzes 2′-O-ribose methylation of the first transcribed nucleotide (N1 2′-O-Me) to produce the cap1 (m7GpppNmNp)structure in all eukaryotes except yeasts. Although the cap0 structure is essential for mRNA stability and cap-dependent translation, it is not known whether cap1 modification also plays a role in regulating posttranscriptional gene expression.Our previous study found that CMTR1 deficiency affects dendritic arborization and cortical development. Because CMTR1 is highly expressed in the hippocampus, I investigated whether CMTR1 modulates synaptic plasticity and spatial memory by using conditional knockout (cKO^{Camk2}, *Cmtr1*$^{f/f, Camk2\text{-}Cre/+}$) mice whose Cmtr1 gene is ablated in the hippocampal CA1 and certain forebrain regions after postnatal 3-4 weeks, so dendritic development is not affected in CMTR1-cKO^{Camk2} mice. We found that CMTR1-cKO mice showed impaired memory consolidation in Morris water maze assay. Although one train of high-frequency stimulation (HFS) and theta-burst stimulation (TBS)-evoked long-term potentiation (LTP) was comparable between cWT and cKO mice, protein synthesis-dependent long-lasting LTP elicited by 4 trains of HFS and TBS was diminished in the cKO group. Moreover, I found that the number but not the size of dendritic spines was reduced in CMTR1-knockdown cortical neurons, suggesting the role of CMTR1 in synaptogenesis. My transcriptome study projects the possibility of that downregulated N-methyl-D-aspartate receptor (NMDAR)-related pathways may account for behavioral and electrophysiological defects. The reduction of NMDAR subunits, NR2A and NR2B, was confirmed by qRT-PCR.Intraperitoneal injection of D-cycloserine, a NMDAR coagonist, right before spatial learning seems to rescue the behavior defect. Further study is required to understand the molecular mechanism underlying impaired memory consolidation in CMTR1-cKO mice and whetherthe catalytic activity of CMTR1 is crucial for synaptic plasticity

and memory.Keywords: CMTR1, mRNA capping, NMDARs, synaptic plasticity, hippocampus, memory

Disclosures: S. Azeem: None. **N. Yeh:** None. **Y. Huang:** None.

Poster

PSTR387. Regulation of Synaptic Plasticity

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Topic: B.05. Synaptic Plasticity

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Johns Hopkins Catalyst Award to B.D.P

Title: Biliverdin Reductase Facilitates Synaptic Plasticity via Redox Homeostasis

Authors: *S. J. TRIPATHI[1], C. VASAVDA[2], R. KOTHARI[2], R. TOKHUNTS[2], S. CHAKRABORTY[1], S. H. SNYDER[1,2,3], B. D. PAUL[1,2,3,4];
[1]Dept. of Pharmacol. and Mol. Sci., [2]The Solomon H. Snyder Dept. of Neurosci., [3]Dept. of Psychiatry and Behavioral Sci., Johns Hopkins Univ., Baltimore, MD; [4]Lieber Inst. for Brain Develop., Baltimore, MD

Abstract: Bilirubin, an essential product of heme catabolism, is a potent antioxidant and exerts protective effects at physiological concentrations. Biliverdin reductase A (BVRA) is the main biosynthetic enzyme that converts biliverdin to bilirubin. Although bilirubin is one of the most commonly measured metabolites in the blood, its exact functions in the brain are less explored. Given that the brain is lipid-rich, metabolically active, and especially susceptible to oxidative stress, we hypothesized that optimal bilirubin concentrations might complement the actions of the hydrophilic antioxidant glutathione. Here, we show that bilirubin, being lipophilic, protects the lipid-rich compartments of cells and prevents lipid peroxidation and ferroptosis. In addition, we demonstrate that mice lacking BVRA display elevated lipid peroxidation in the hippocampal CA3 region, markers of ferroptosis, mitochondrial dysfunction, enlarged ventricles, and compromised ability to neutralize free radicals and are highly susceptible to neuronal damage. Interestingly, bilirubin directly scavenges superoxide radicals (O2•−) generated during mitochondrial respiration, indicating a direct neuroprotective mechanism. In addition to oxidative stress, we show that BVRA facilitates learning and long-term potentiation in CA3-CA1 synapses through the focal adhesion kinase (FAK) and modulates phosphorylation of the N-methyl-D-aspartate (NMDA) receptor, a crucial posttranslational modification for synaptic plasticity. In summary, we demonstrate BVRA and bilirubin facilitates synaptic plasticity via redox homeostasis. Moreover, pharmacotherapies enhancing BVRA and bilirubin-mediated

effects at physiological levels may afford therapeutic benefits in the management of neurodegenerative diseases.

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Poster

PSTR387. Regulation of Synaptic Plasticity

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Program #/Poster #: PSTR387.10/C19

Topic: B.05. Synaptic Plasticity

Support: 5R00AA028298-04

Title: Binge drinking induces cross-species plasticity in the orbitofrontal cortex

Authors: *A. MEYERS[1], C. JOHNSTON[2], M. M. PINA[3];
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Abstract: According to the CDC, an estimated 17% of adults have engaged in binge drinking, where blood alcohol concentration (BAC) exceeds .08% within a short period of time. Binge drinking is associated with increased risk of Alcohol Use Disorder (AUD) and can induce alterations in brain regions important for executive function, specifically the orbitofrontal cortex (OFC). Alterations in OFC function have been previously associated with chronic, heavy alcohol consumption in humans and animal models. In the present work, we looked at the impact of binge alcohol drinking on different cell populations of the OFC to determine how it impacts components of this structure's microcircuitry. We used whole-cell patch clamp electrophysiology to measure binge-alcohol induced changes in intrinsic properties, excitability, and synaptic drive in OFC interneurons (IN) and pyramidal neurons (PN) in mice and rhesus macaques. In mice, binge drinking was modeled using a Drinking in the Dark (DID) paradigm. In short, mice were given access to both a 20% v/v alcohol solution and H2O for 2-h sessions (three days a week) followed by a four-hour session (fourth day) for three cycles. Following the final day of DID, mice were separated into 2 groups: 1) a 24-h withdrawal and 2) a 7-d withdrawal. In rhesus macaques, drinking was assessed using an alcohol self-administration model, in which 4% w/v alcohol was available under 22h/d open access conditions over a one-year period. In this paradigm, rhesus macaques self-select into stable drinking categories, which allowed us to compare monkeys categorized as low-drinkers to binge-drinkers. In mice, OFC PN were hyperpolarized at 24-hr and 7-day following DID as compared to water controls. This effect was not observed in PN of binge drinking macaques or IN. Membrane resistance was differentially altered in PN of mice and monkeys, with increased resistance in mice at both withdrawal timepoints and decreased resistance in binge drinking monkeys. Finally, rheobase

(minimum amount of current required for firing) in OFC PN was decreased in mice at 24h but not 7d after DID. Conversely, we found that the rheobase was increased in OFC PN of binge drinking macaques as compared to low drinkers. Our ongoing work in mice and macaques indicates that binge alcohol drinking does not substantially alter the intrinsic properties or excitability of OFC IN. Findings here demonstrate that binge drinking contributes to differential alterations in OFC neuronal subpopulations, which may help explain the persistence of binge drinking behavior and its risk.

Disclosures: A. Meyers: None. **C. Johnston:** None. **M.M. Pina:** None.

Poster

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Program #/Poster #: PSTR387.11/C20

Topic: B.05. Synaptic Plasticity

Support: NIH F31 MH127958
NIH R01 MH094607

Title: Synapse activity differentially regulates mitochondrial and lysosomal-related organelles bidirectional trafficking between synapse and soma of Aplysia presynaptic sensory neurons

Authors: *K. K. BADAL[1], Y. ZHAO[2], K. E. MILLER[3], S. PUTHANVEETTIL[4];
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Abstract: Cell-to-cell communication requires bidirectional trafficking of signaling and materials between the neuron's soma and axon terminal to support synapse formation and plasticity. Organelles such as mitochondria help provide energy to the cell, and lysosomes assist in recycling and degrading macromolecules and are transported bidirectionally between the soma and synapse. However, whether and how their direction, flux, and velocity of transport are modulated for synapse formation, maintenance, and plasticity remain elusive. Here, we show that in the presynaptic sensory neurons of the Aplysia gill withdrawal reflex, the formation of functional synapses leads to a bidirectional enhancement in mitochondrial flux and density and a decrease in retrograde lysosome flux and density. Enhanced mitochondrial and reduced retrograde lysosome trafficking is highly regulated by cAMP-PKA signaling in the presence of a functional synapse. Moreover, synaptic plasticity specifically increases anterograde mitochondrial transport, whereas it decreases retrograde lysosomal transport. Importantly, we find that regulation of this transport requires modulation of PKA activity. These results demonstrate the specific regulation of two different groups of organelles during various synaptic activities.

Disclosures: K.K. Badal: None. **Y. Zhao:** None. **K.E. Miller:** None. **S. Puthanveettil:** None.

Poster

PSTR387. Regulation of Synaptic Plasticity

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Program #/Poster #: PSTR387.12/C21

Topic: B.05. Synaptic Plasticity

Title: Vagus nerve stimulation frequency and its effect on plasticity in the motor cortex.

Authors: *J. J. A. ADDO[1], C. NEIFERT[1], T. DANAPHONGSE[2], S. ABE[2], V. EZHIL[2], M. P. KILGARD[2], S. A. HAYS[3];
[1]Bioengineering, [2]The Univ. of Texas at Dallas, Richardson, TX; [3]Bioengineering, The Univ. of Texas At Dallas, Dallas, TX

Abstract: Neurological injuries such as stroke, spinal cord injury, and traumatic brain injury usually result in chronic impairments in motor function. It is widely held that approaches that enhance synaptic plasticity in spared networks after neurological injury can advance recovery. Vagus nerve stimulation (VNS) paired with rehabilitation has emerged as one such approach. VNS evokes an increase in the release of plasticity-inducing neuromodulators which results in an increase in the cortical representation of areas activated during the paired task. As such, increasing the amount of plasticity could result in an increased degree of recovery. Several studies indicate that the stimulation parameters utilized during stimulation impact the degree of plasticity observed. In this study, we sought to identify the VNS frequency that results in the greatest enhancement of plasticity when delivered during a simple jaw training model in rats. Rats received 20Hz, 30Hz, and 45Hz of VNS concurrent with chewing in a jaw training task. After 5 days of VNS pairing, all rats underwent intracortical microstimulation (ICMS) to evaluate cortical movement representations. Current findings show a plateau effect in the jaw cortical area representation as a result of VNS induced plasticity at stimulation frequencies closer to 30Hz. The results of this study help further establish the optimal stimulation parameters for VNS and depict the importance of delivering VNS at the right frequency when used in conjunction with rehabilitation therapy.

Disclosures: J.J.A. Addo: None. **C. Neifert:** None. **T. Danaphongse:** None. **S. Abe:** None. **V. ezhil:** None. **M.P. Kilgard:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MicroTransponder, Inc. **S.A. Hays:** None.

Poster

PSTR388. Oscillations in Cortical Networks

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Topic: B.07. Network Interactions

Support: NIMH Grant R01MH123687
NIBIB R01EB028161
CIHR Grant MOP_102482

Title: Inter-areal Routing Dynamics in Spiking Networks Depend on Local Network Activity and Cognitive States

Authors: *K. BANAIE BOROUJENI[1], T. WOMELSDORF[2];
[1]Princeton Univ., Princeton, NJ; [2]Vanderbilt Univ., Vanderbilt Univ., Nashville, TN

Abstract: In spiking neuronal networks, single neurons act simultaneously as receiver and router of information. Neurons receive synaptic information, integrate and translate local depolarization levels into spiking output that influences postsynaptic neurons. The mechanisms by which spiking networks balance the receiving and the routing functions of single neurons is only poorly understood and requires addressing two key unresolved questions. First, what is the timescale within which neuronal spiking activity engages in routing of information to neurons in distant regions of the brain? Secondly, what are the network activity states that determine whether a neuron is primarily an information receiver or a router? We addressed these questions by first using computational modeling of spiking networks which illustrated that routing states emerge dynamically as directional interactions of spiking activity at a fast ~20 ms timescale. The temporal lead and lag of routing states between interconnected networks were dynamically gated by narrow band burst events of the local field potential (LFP). These simulation results suggest that coherent network activity states facilitate the transition from receiving to directional routing in spiking networks. In a second step, we analyzed the dynamics of routing states in electrophysiological recordings of the lateral prefrontal cortex (LPFC), anterior cingulate cortex (ACC), and striatum of nonhuman primates during a task that separated attention and decision-making processes. As predicted from simulations, routing states emerged across ACC, LPFC and striatum at a fast timescale. Spike events in the LPFC led multiunit activity (MUA) in the ACC and striatum, and in the ACC, spikes preceded stronger MUA in the striatum. These routing states dynamically switched and amplified depending on the LFP state and on cognitive demands. During oscillatory bursts and at a narrow phase range of theta/alpha frequency (8-14 Hz) activity in ACC, neural spikes switched and amplified their temporal lead relative to MUA in the LPFC and striatum. During beta band (15-25 Hz) bursts in the LPFC, spikes amplified their lead over the striatum. The cognitive state likewise switched interareal routing states. Attention increased the size of a neuronal ensemble in the ACC that led LPFC and striatum during theta/alpha LFP bursts, while decision-making increased ensembles of temporally leading neurons in the ACC and LPFC over the striatum. In summary, these results show that routing states emerge in spiking networks at fast timescales and dynamically transition based on local population activity and cognitive states.

Disclosures: K. Banaie Boroujeni: None. **T. Womelsdorf:** None.

Poster

PSTR388. Oscillations in Cortical Networks

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Topic: B.07. Network Interactions

Support: Senior Fellowship from Department of Biotechnology(DBT)-Wellcome India Alliance (To Supratim Ray - Grant IA/S/18/2/504003)
Senior Research Fellowship from Council of Scientific and Industrial Research (to Divya Gulati)

Title: Can auditory gratings excite the neuronal circuitry the way visual gratings do?

Authors: *D. GULATI, S. RAY;
Ctr. for Neurosci., Indian Inst. of Sci., Bengaluru, India

Abstract: Sensory inputs modulate local excitatory-inhibitory neuronal interactions, which, for some visual stimuli such as cartesian gratings, induce narrow-band gamma oscillations (30-70 Hz) in the visual cortex. They appear as a bump centred between 30-70 Hz with a bandwidth of 10-20Hz in the power spectra and depend highly on stimulus properties such as size and contrast (Ray & Maunsell, 2011). Interestingly, the amplitude of these oscillations has been observed to decline with healthy ageing (Murty et al., 2020) and in patients with mild cognitive impairment and Alzheimer's disease, indicating their role as a potential biomarker for detecting cognitive decline (Murty et al., 2021). However, such studies require cumbersome procedures like eye fixation and tracking, which are challenging, especially for elderly subjects. This problem can be resolved using an auditory stimulus, delivered to subjects effortlessly using loudspeakers. But it is unclear what kind of stimulus can induce such narrow-band gamma oscillations in the auditory modality.A potential stimulus can be an auditory ripple or auditory grating sounds, an acoustic analogue of visual gratings (Shamma, 2001). We hypothesized that these sounds might excite the cortex like a large-high contrast grating. Ripples span a broad frequency range, activating many neurons in the primary auditory cortex (like a large visual stimulus). Furthermore, the presence of sharp boundaries in the structure of ripples can also engage lateral inhibitory networks. To investigate if auditory ripple stimuli can generate narrow-band gamma oscillations and if they are comparable to visual gratings, we collected 64-channel EEG data from 36 subjects (mean age of 26.1±3.8 years; 18 females) as they passively listened to ripple stimuli played using loudspeakers with their eyes closed or passively fixated on the screen while presenting full-screen grating stimuli on a monitor. For the visual gratings, we observed a robust narrow-band gamma response (22-66Hz, $p<0.01$ Wilcoxon sign rank test) and alpha suppression (8-14Hz, $p<0.01$ WSR) in occipital and parieto-occipital electrodes. Contrary to our expectations, auditory gratings did not elicit narrow-band gamma. Instead, they elicited a strong broadband "high-gamma" response (70-150Hz, $p<0.05$ WSR) in the temporal electrodes near the mastoids and suppression in beta rhythm (14-26Hz, $p<0.01$ WSR) across all the electrodes. High -gamma reflects increased firing activity, indicating that the sound stimuli did excite the auditory cortex but did not engage the neural circuitry like visual gratings do.

Disclosures: D. Gulati: None. **S. Ray:** None.

Poster

PSTR388. Oscillations in Cortical Networks

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Program #/Poster #: PSTR388.03/C24

Topic: B.07. Network Interactions

Support: NSERC USRA

Title: Induction of Cortical Gamma Activation through applied Electromagnetic Fields: Influence on cognitive functioning and cerebral dynamics

Authors: *B. T. DOTTA;
Laurentian Univ., Sudbury, ON, Canada

Abstract: Cognitive functioning has been repeatedly shown to be associated with gamma band activity. In fact, learning disorders and Alzheimer's disease are both associated with significant decreases in gamma activity in the brain. Recent research has demonstrated that the application of an electromagnetic field (EMF) at 40 Hz significantly reduces Alzheimer's pathologies in mice (Arendash *et al*., 2010). Because 40 Hz rests within the gamma band, we hypothesized that the application of a low frequency EMF patterned at 40 Hz would facilitate gamma activity in the brain. To test if the application of a low intensity EMF would alter cortical activity, cerebral dynamics were assessed pre- and post- EMF exposure though the use of an electroencephalogram (EEG). The EMF application geometry was placed directly on the subjects' head, and the EMF was applied via CPU to the application geometry through customized software. The duration and intensity of the EMF were 30 minutes and 5 μT respectively. Following EMF exposure, psychometric testing from the WAIS-IV was administered to assess participant's short term and working memory scores. In total, 58 healthy participants (aged 18 - 40 years) were measured in this study. Statistical analyses indicated significant increases in gamma activity in multiple brain regions following the application of a 40 Hz EMF. These increases were seen in the: left inferior frontal gyrus, the right inferior frontal gyrus, the left superior temporal gyrus, and the right superior temporal gyrus [$p<0.05$]. Additional analysis demonstrated a significant increase in working memory scores when the EMF was applied unilaterally to the left hemisphere [$t(29)=2.001$, $p<0.05$]. In summary, our results provide support that cortical gamma activity can be entrained through application of a 40 Hz EMF. The areas affected by the EMF application are also reported in the literature as correlates of working memory and cognitive functioning. That unilateral left hemisphere EMF application was associated with increases in working memory scores supports the proposed relationship between cerebral dynamics, applied EMF, and cognitive functioning.

Disclosures: B.T. Dotta: None.

Poster

PSTR388. Oscillations in Cortical Networks

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Program #/Poster #: PSTR388.04/C25

Topic: B.07. Network Interactions

Title: Effects of oscillating weak electromagnetic fields on EEG frequency localization in the brain following bilateral temporal electromagnetic field stimulation

Authors: *A. ZANETTI, K. SAROKA, B. DOTTA;
Laurentian Univ., Sudbury, ON, Canada

Abstract: The purpose of this study was to determine if electromagnetic field exposure modelled from physiological data would increase instances of flow in participants playing a computer game, and to determine if these fields will change the relationship between challenge and skill necessary for flow. Participants (n = 39, 18-65 years, n_{female} = 20) played the arcade game *Snake* for two ten-minute periods (each with a ten-minute rest period immediately following) at the difficulty corresponding to treatment conditions (easy, medium, and hard). For one of the trials, an electromagnetic field was applied bilaterally to the temporal lobes, with the other serving as the control. Brain activity was measured using quantitative electroencephalography, flow experience was measured using the Flow Short Scale and game play scores were also recorded. Results showed deceased beta 1 activity in the left cuneus [$t = 4.650$, $p < .01$] and left precuneus [$t = 4.603$, $p < .01$], left posterior cingulate [$t = 4.521$, $p < .05$], insula [$t = 4.234$, $p < .05$], and parahippocampal gyrus [$t = 4.113$, $p < .05$] for trials when the field was active, compared to controls during rest periods irrespective of difficulty. Deceased gamma activity was found in the right inferior temporal gyrus [$t = 6.147$, $p < .05$], right middle temporal gyrus [$t = 6.118$, $p < .05$], right superior temporal gyrus [$t = 5.861$, $p < .05$] and right fusiform gyrus [$t = 5.596$, $p < .05$] for trials when the field was active, compared to controls during play periods in the easy difficulty condition. Results from the Flow Short Scale showed a statistically significant difference in mean “concentration ease” scores across electromagnetic field conditions, irrespective of difficulty [$t = 2.131$, $p < .05$]. In all, these results seem to provide evidence supporting the ability of burst pattern EMF (6 - 20 Hz) to elicit neurological correlates of flow in brain regions previously reported in the literature and facilitate concentration.

Disclosures: **A. Zanetti:** None. **K. Saroka:** None. **B. Dotta:** None.

Poster

PSTR388. Oscillations in Cortical Networks

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Program #/Poster #: PSTR388.05/C26

Topic: B.07. Network Interactions

Support: NSERC

Title: Emotionally valenced scripts and electromagnetic field effects on cerebral dynamics and working memory scores

Authors: *K. BRANIGAN, K. SAROKA, M. LARIVIERE, P. CORRADINI, B. DOTTA; Laurentian Univ., Sudbury, ON, Canada

Abstract: Current scientific literature has demonstrated that emotion has a significant influence on cognitive processing in humans. These processes include perception, attention, learning, memory, reasoning, and problem solving. Language is a pertinent facet in the processing and understanding of emotions in humans. Utilizing Whissel's *Dictionary of Affect in Language* (DAL), three stories were constructed with either positive, negative, or neutral emotional valences. Participants' cerebral dynamics were assessed using an electroencephalogram (EEG), one of the three stories were read and then participants were exposed to 30 minutes of an electromagnetic field (EMF). Following EMF exposure psychometric testing from the Wechsler Adult Intelligence Scale (WAIS-IV) was administered. A subtest of the WAIS-IV that was used was Digit Span Forward (DSF) which assesses short term memory. Majority of volunteers (N=58) were university students with an average age of 24.08 ± 4.51 (range: 19-39; gender distribution: 24 male, 34 female). Each participant was randomly assigned a condition as well as a different story. A SINE wave EMF oscillating at 40Hz was utilized because gamma activity in the brain which oscillates at 40 Hz is highly correlated with cognitive functioning in the brain. Statistical analysis revealed that with the application of a 40 Hz-based EMF the individual emotionally valenced stories had significant influences on participant's performance on cognitive assessments of the WAIS-IV. However, the control group who did not receive any EMF, were not affected on their cognitive assessment through the different stories. Volunteers who received a 40 Hz-based EMF and were read the Positive story (N=12) performed significantly higher than those who received EMF and were read the Negative story (N=14) on the DSF subtest [$t(24)=2.548$, $p=0.018$]. Additionally, those who received the Positive story and EMF performed significantly higher on the DSF than those who received the Neutral story and EMF [$t(20)=2.258$, $p=0.035$]. Following the application of EMF, those who received a positive story had significantly higher Beta activity (20-25 Hz) in the right anterior insula, when compared to those who received the negative or neutral story. The anterior insula plays a role in supporting subjective feeling states, with the ability to regulate the introduction of feelings into cognitive and motivational processes (Namkung *et al.*, 2017). In summary, these results indicate that different emotionally valenced scripts when paired with an EMF have significant effects on short term and working memory as well as areas in the brain responsible for these processes.

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Poster

PSTR388. Oscillations in Cortical Networks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR388.06/C27

Topic: B.07. Network Interactions

Support: DFG EXC 307
ERC StG 335880
ERC CoG 864491

Title: Waveform dissociates cortical rhythms

Authors: *J. GIEHL[1,3,2], M. SIEGEL[1,3,2];
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Abstract: Neural oscillations have been associated with different cognitive functions and brain disorders. Recently, the non-sinusoidal waveform of oscillations has gained interest, as the waveform of neural rhythms may be closely associated with the underlying network physiology. However, waveform analysis in the time domain has been limited to dominant neuronal oscillations and by pre-selection of waveform features. Here, we contribute a novel Fourier-series based waveform analysis that allows for a comprehensive analysis of non-sinusoidal waveforms. We applied Fourier-based waveform analysis to human cortical oscillations recorded using magnetoencephalography (MEG). This approach allowed us to robustly dissociate multiple spectrally and spatially overlapping cortical rhythms across theta, alpha and beta frequency ranges in the human brain. Fourier-based waveform analysis is a powerful new tool to distinguish neuronal rhythms, and to study their spectral fingerprints in health and disease.

Disclosures: J. Giehl: None. **M. Siegel:** None.

Poster

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Topic: B.07. Network Interactions

Support: ARC Grant DE220101019

Title: Disambiguating phase resets from additive event-related potentials by their inter-frequency phase alignment

Authors: *A. M. HARRIS;
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Abstract: Phase resets are discontinuities in ongoing neural oscillations, whereby the wave abruptly transitions to some default phase (e.g., a peak), regardless of the phase of the wave a

moment earlier. Oscillations can have their phase reset by external stimulation or internal signals, and these resets are theorised to flexibly align the oscillatory activity of different brain regions for enhanced intercommunication in response to task requirements. As such, phase resets play a key role in theoretical accounts of sensory processing, attention, multi-sensory integration, speech processing, and many other areas of cognition. They have also been hotly debated as one possible generator of the event-related potential (ERP). The role of phase resets in cognition and neural processing has been difficult to study, however, because phase resets in electrophysiological data have been impossible to disambiguate from additive wave-like signals (additive ERPs) using available analysis methods. This is a problem for our understanding of neural processing because phase resets and additive ERPs have quite different computational and theoretical implications. I will present work describing a method for detecting phase resets in electrophysiological data using an inter-frequency phase alignment metric. This method works well on simulated data, giving strong responses to phase resets but not to additive ERPs with very similar spectral properties. I will also present the results of applying this method to several real datasets (visual stimulation, eye movements, and speech processing tasks), including showing that the P1 ERP component of the EEG is caused, in part, by a reset of ongoing oscillations, whereas later ERP components are not. This method provides an exciting avenue for the empirical examination of phase resets, and to begin testing theories of the roles of phase resets in cognitively-relevant neural processing.

Disclosures: A.M. Harris: None.

Poster

PSTR388. Oscillations in Cortical Networks

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Program #/Poster #: PSTR388.08/Web Only

Topic: B.07. Network Interactions

Title: Electroencephalographic activity patterns evoked by colors and associated emotions

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Abstract: Over the last five decades, electroencephalographic (EEG) activity patterns have been correlated with emotional responses in terms of brain hemispheric lateralization: Frontal Alpha Asymmetry (FAA) (Davidson et al., 1979). That is, increased left frontal brain activity (characterized by alpha suppression), relative to right frontal brain activity, is associated with positive emotions, and the inverse occurs with the negative ones. This has been observed with several emotional stimuli (e.g., music), but color-evoked emotions appear to be left behind. This study assessed the 37 colors from the Berkeley Color Project, which have been previously scored by subjective emotions (Palmer & Schloss, 2010), but never with EEG. In that sense, 32 participants (19 females), M = 21.4 years, SD = 3.3, right-handed, and with normal color vision, observed each color in random order for 15 s, while EEG was recorded from their right and left

frontal areas (F4 and F3, respectively), and referenced to Cz. After each color, they evaluated them subjectively with the following seven bipolar continuous scales, ranging from −100 to +100: green-red, yellow-blue, brightness, saturation, valence, arousal, and pleasure. All participants read and accepted the informed consent. EEG data was corrected for eye movement artifacts, segmented into 1 s epochs, and normalized. The absolute power of the alpha band (8—13 Hz) was calculated (Alpha Power), and natural logarithm (*ln*) was then applied. Regarding the subjective data, Valence, Arousal, and Pleasure ratings per stimulus were averaged (VAP index), meaning that greater values represent joyful and pleasurable colors, whereas sad and unpleasant colors are indicated by lower ones. The results showed that *ln*(Alpha Power) at F3 was negatively correlated with Brightness ($r_s = -.35$, $p = .03$), Arousal ($r = -.38$, $p = .02$), and the VAP index ($r = -.32$, $p = .046$). Finally, no significant correlations were found at F4. It should be noted that Brightness was positively correlated with Valence ($r_s = .68$, $p = <.001$). With these results, we found that bright, joyful, and pleasurable colors were associated with an increase of left frontal brain activity (alpha suppression), as proposed by the FAA model. On the other hand, we could argue that, in general, colors are more prone to evoke positive emotions, rather than strong negative ones, which are linked to right frontal cortex activity. These findings will help to better understand the role of FAA as a model to assess electrophysiological responses to emotions. Particularly, this study fills a gap in the study of FAA with colors as emotional stimuli in the fields of cognitive psychology and neuroscience.

Disclosures: P. Valdés-Alemán: None. **B. Tellez-Alanis:** None.

Poster

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Program #/Poster #: PSTR388.09/Web Only

Topic: B.07. Network Interactions

Support: NIH Grant RF1MH117155

Title: Synchrony of ripple oscillations in the mouse visual cortex

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Abstract: Conscious perception of visual stimuli involves the precise coordination of many cortical centers. In the visual cortex, information generally flows along an anatomical and functional hierarchy, but how different visual elements are seamlessly integrated into a perceptual whole remains largely a mystery. High frequency oscillatory bursts (i.e., ‘ripples’) have recently emerged as a potential substrate for the binding of neuronal activity across distributed cortical centers. Originally described in the rodent hippocampus, ripples riding on sharp waves (SWRs) have been shown to couple with ripples in the posterior parietal cortex and

index the reconstruction of spatiotemporal neuronal firing patterns essential for the consolidation of memories during non-rapid eye movement sleep. It is not clear, however, whether ripples exist in the rodent visual cortex and whether their properties are consistent with a role in binding of visual information. Here, we analyzed local field potential and spiking data in the visual cortex of mice collected as part of the Allen Brain Observatory Visual Coding study. We found that ripples occur and co-occur with zero phase lag in primary visual cortex and all higher visual areas with a stereotyped frequency of ~160 Hz and duration of ~70ms (similar to ripples found in the hippocampus). Surprisingly, spontaneously-evoked ripples in the deeper layers of visual cortex co-occurred more frequently with ripples in other cortical parcels than with ripples in higher layers within the same parcel. Ripple co-occurrence was most pronounced between the deepest layers of posteromedial area (VISpm) and anteromedial area (VISam), which both sit atop the visual cortex hierarchy. Ripple co-occurrence also increased co-firing between involved cortical sites. These results provide preliminary evidence that ripples occur in the murine visual cortex and may help integrate neuronal activity between separated cortical centers. Future work should: (1) explore which mechanisms generate visual cortex ripples and facilitate their co-occurrence; (2) further examine the focality of ripple events; (3) characterize how ripples may facilitate neuronal interactions; and (4) quantify how ripple occurrence in different cortical sites lock to visual stimuli.

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Poster

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Title: Co-occurring ripple oscillations facilitate neuronal interactions between cortical locations in humans.

Authors: *I. VERZHBINSKY[1], D. RUBIN[2], S. KAJFEZ[5], Y. BU[6], J. N. KELEMEN[3], A. KAPITONAVA[4], Z. WILLIAMS[8], L. HOCHBERG[9], S. S. CASH[10], E. HALGREN[7];

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Abstract: Synchronous bursts of high frequency oscillations (‘ripples’) are hypothesized to contribute to binding by facilitating integration of neuronal firing across cortical locations. We tested this hypothesis using local field-potentials and single-unit firing from four 96-channel microelectrode arrays in supragranular cortex of 3 patients. Neurons in co-rippling locations showed increased short-latency co-firing, prediction of each-other’s firing, and co-participation in neural assemblies. Effects were similar for putative pyramidal and interneurons, during NREM sleep and waking, in temporal and Rolandic cortices, and at distances up to 16mm. Increased co-prediction during co-ripples was maintained when firing-rate changes were equated and were strongly modulated by ripple phase. Co-ripple enhanced prediction is reciprocal, synergistic with local upstates, and further enhanced when multiple sites co-ripple. Together, these results support the hypothesis that trans-cortical co-ripples increase the integration of neuronal firing of neurons in different cortical locations, and that enhanced integration is not secondary to increased firing but does depend in part on phase-modulation.

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Poster

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Topic: B.07. Network Interactions

Support: EU MSCA-ITN "iConn"
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USIAS 2020 Fellowship - D. Battaglia

Title: Visual working memory content is encoded by weak but spatiotemporally coordinated oscillatory power fluctuations

Authors: *V. LIMA CORDEIRO[1], S. J. HOFFMAN[2], N. DOTSON[3], C. M. GRAY[4], A. BROVELLI[1], D. BATTAGLIA[1];
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Abstract: Working memory requires large-scale coordination among widespread brain regions. Long-range coherence between the oscillatory activity of cortical regions, arising in multiple frequency bands, may be a mechanism supporting information routing and integration across distributed networks.
Here, we perform a time-resolved spectral analysis of local field potentials (LFPs) recorded simultaneously from dozens of cortical areas in non-human primates performing a visual delayed match-to-sample task. We define a novel type of neural activity events, which we call “crackles”. A crackle is a transient upward modulation of the average oscillatory power (spatially and spectrally localized). A minority of “crackles” are also “bursts” as they correspond to strong transient increases of power. However, most crackles are not bursts, because the power remains overall weak even at the fluctuation peak (and thus unlikely to generate modulations of postsynaptic excitability).
We find that a multiplicity of regions undergo task-related modulations of crackling intensity and probability across many frequency bands (alpha to gamma), with a subset of frontoparietal regions exhibiting a ramping crackling probability through the delay period of the task. Furthermore, these modulations of crackling activity carry mutual information about the identity of the visual stimulus held in working memory.
Remarkably, the nodes whose crackling modulations convey the most mutual information are not always the ones with the strongest oscillatory power and the more prominent oscillatory activity. On the contrary, the nodes with the highest stimulus-related mutual information have weak average oscillatory power, but tend to crackle in a temporally coordinated manner with other regions. This creates windows of opportunity where nonlinear integration can take place through the formation of “co-crackling assemblies”. In other words, the regions whose LFP power encodes the most working-memory content are not the one with the strongest, but with the most entangled oscillatory crackles.
We propose alternative scenarios for interpreting our results and favor the hypothesis that stimulus is encoded within low-dimensional subspaces of distributed, but coordinated system's activity. In this view, entangled power fluctuations may be the signature of the system operating within a specifically-adapted processing mode, but they would not necessarily mediate, by themselves, inter-regional communication (even if many weak crackles could still collectively boost integration via their coordination)

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Poster

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Support: European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 956669

Title: Encoding and decoding visuomotor task and behavioral parameters using spectro-laminar beta rhythm patterns in macaque motor cortex

Authors: *L. LÓPEZ-GALDO[1], S. NOUGARET[1], D. BATTAGLIA[2,3], B. E. KILAVIK[1]; [1]Inst. de Neurosciences de la Timone (INT), UMR 7289, CNRS, Aix-Marseille Univ., Marseille, France; [2]Inst. for Systems Neurosci. (INS), UMR 1106, Inserm, Aix-Marseille Univ., Marseille, France; [3]Univ. of Strasbourg Inst. for Advanced Studies (USIAS), Strasbourg, France

Abstract: Beta rhythms are ubiquitous in the motor cortex, and many functional roles were proposed. We recently described a double-dissociation between different beta bands in macaques local field potential (LFP), with low beta (<20Hz) in primary motor cortex (M1) correlating with movement preparation and spontaneous postural dynamics, and high beta (>20Hz) in dorsal premotor cortex (PMd) correlating with temporal task prediction and dynamical visuospatial attention (Nougaret et al. 2023, bioRxiv, doi:10.1101/2023.03.28.534535). Even within the low and high beta bands, individual beta events are highly heterogeneous in their temporal, spectral and spatial dynamics. We here explore single-trial spectro-spatial beta rhythm patterns in motor cortical laminar recordings in two male macaques during visuomotor behavior, to determine their relationship to different aspects of the behavioral task and performance. In order to incorporate the laminar dimension to this analysis, we applied Non-negative Tensor Factorization (NMF) to time-frequency single trial LFP data, to decompose it into spectro-spatial patterns. We found that these patterns underwent fluctuations in amplitude, and we termed moments with relatively high amplitude of a pattern "*crackles*". Crackles resemble "bursts", although their overall amplitude could be weak compared to total LFP power in the beta range. The rates of occurrences of crackles for individual patterns were characteristically modulated by the task, and encoded task-related information. Furthermore, they could be used as features for decoding. Specifically, there was significant Mutual Information (MI) between crackles and task conditions, which allowed us to build a random forest decoder able to extract the identity of the condition cue from the "instantaneous" crackling rate of the patterns within single trials. In conclusion, the power across the total beta range can be decomposed into multiple spectro-laminar patterns that correlate with the behavioral task. These spectro-laminar patterns might reflect specific neuronal subpopulations or network states.

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Poster

PSTR388. Oscillations in Cortical Networks

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Program #/Poster #: PSTR388.13/C32

Topic: E.09. Motor Neurons and Muscle

Support: MnDRIVE Brain Conditions
NSF CAREER Award

Title: Real-time EEG-TMS motor mapping to evaluate phase-specific trends in mu and beta rhythms

Authors: *Z. HAIGH[1], M. WISCHNEWSKI[1], S. SHIRINPOUR[3], I. ALEKSEICHUK[2], A. OPITZ[2];
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Abstract: Transcranial magnetic stimulation (TMS) is an increasingly popular neuromodulation technique for therapeutic and diagnostic use in research and clinical settings. However, TMS is susceptible to variability, especially in populations with neurological abnormalities. Therefore, individualized TMS treatment is warranted. The recent development of real-time electroencephalography-TMS (EEG-TMS) created novel pathways toward such patient-specific applications. One limitation to EEG-controlled approaches is the low spatial resolution of the method. As such, it is important to characterize the spatial dependencies of EEG-TMS effects. In this study, we use TMS motor mapping to explore the spatial effects of EEG-TMS in the two prominent sensorimotor EEG rhythms, mu (8-13 Hz) and beta (14-30 Hz). Twenty participants were recruited for participation. Magnetic resonance imaging (MRI) was collected to identify anatomical and functional (fMRI) relationships within the data. Then we performed EEG-TMS over a pseudorandom grid surrounding the primary motor cortex. Four brain states were targeted, specifically, peak, falling, trough, and rising phase of the sensorimotor mu and beta rhythms. Electromyography (EMG) was recorded from the first dorsal interosseus (FDI) and abductor digiti minimi (ADM) muscles to identify changes in cortical excitability. The cortical location and orientation corresponding to the TMS-induced motor evoked potentials (MEP) were recorded using neuronavigation. The spatial data was projected to a 2-D plane the MEP data is fitted to the plane. To quantify the spatial effects of EEG-TMS, we evaluated the motor map in three ways. Traditional motor mapping uses center of gravity, area of activation, and volume of activation. Cortically localized motor mapping uses electric field stimulation to regress standard motor maps to the cortical surface for anatomical comparisons. Task- and resting state-fMRI provide correlations between TMS motor maps and functional connectivity. Preliminary results suggest elevated cortical excitability at the trough of the beta oscillation (1.08 ± 0.05 mV) compared to the peak (0.96 ± 0.06 mV) in and around the center of the spatial map. Ongoing analyses will further explore the spatial extent of this effect, compare frequencies at different spatial locations, and investigate covarying effects of individual differences in MRI and fMRI. Ultimately, this study will characterize spatial heterogeneity of brain states during real-time neuromodulation, which allows us to provide recommendations for accurate translation of EEG-TMS to clinical targets in and outside the motor cortex.

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Poster

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Topic: B.07. Network Interactions

Support: USIAS-Fellows 20-D Battaglia
ANR - HippoComp

Title: Canonical cortical circuits maximize high-order synergies for multi-frequency communication

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Abstract: The functional connectivity between cortical regions can be divided into bottom-up and top-down pathways, each utilizing a specific frequency band. These frequencies emerge at the regional scale through oscillations on different cortical layers. Explanations for the origin and utilization of this frequency communication range from a structural basis, such as specific cellular types (PV, SOM) for each frequency band, to its function as multiplexed communication with high frequency for bottom-up and low frequency for top-down. By employing rate models of coupled regions embedded in a realistic multi-layer cortical organization, we demonstrate that different layers exhibit specific and segregated frequencies. Deep layers display low frequencies, while superficial layers exhibit high frequencies. This finding is surprising because, in our model, each layer includes identical excitatory and inhibitory populations without laminar-specific resonances. We demonstrate that this frequency segregation is a by-product of self-organized collective dynamics rather than hardwired anatomy or interneuronal diversity.
Next, we evaluated the communication between two hierarchically distinct cortical regions using spectral Granger causality. The model confirms empirical results, showing that communication is possible for bottom-up in high-frequencies and top-down in low frequency. However, the model's explanatory power extends beyond this, demonstrating that top-down communication can flexibly change from low to high frequency. This modulation is finely adjusted by contextual inputs (e.g. salience/attention) or the weight of interregional connections.
We find then that the emergence of dynamic regimes with frequency segregation is non-trivial and tightly related to canonic circuit wiring as not arising in randomized multilaminar circuits. We thus speculate that this special connectome has been selected because of the peculiar functional advantages it confers. Through information theoretical analyses (in terms of S- and O-information), we find that the canonical circuit has exceptional information processing properties, since it gives rise to collective dynamics simultaneously maximizing "complexity" (coexistence of integration and segregation) and inter-regional "synergy" (beyond communication). In this view, the emergence of frequency-segregation would not be an aim by itself but rather the unsought-for but necessary consequence -a spandrel- of maximizing inter-layer and inter-regional entanglement.

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Poster

PSTR388. Oscillations in Cortical Networks

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Topic: B.07. Network Interactions

Support: NIGMS R21GM143521
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Title: Brain network hypersensitivity underlies pain crises in sickle cell disease

Authors: *P. JOO[1], U. LEE[1], R. HARRIS[1,2], Y. WANG[3,4];
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Abstract: Sickle cell disease (SCD) is a genetic disorder characterized by abnormally deformed blood cells causing vessel blockages and vascular occlusion crises (VOCs). During VOCs severe pain occurs when sickle-shaped red blood cells block blood flow in small vessels. The occurrence of VOCs is repetitive and unpredictable in many patients, making it difficult to timely implement preventive strategies. Here we suggest explosive synchronization (ES), a widely observed physical phenomenon characterized by abrupt phase transitions and high sensitivity near the critical point, as a novel approach to address this challenge. We previously demonstrated that a network mechanism of ES and hypersensitivity is involved in chronic pain in patients with fibromyalgia. We hypothesized that, changes in the default mode network (DMN) connectivity as observed in patients with SCD, possibly due to the recurrent VOCs, can potentially disrupt the hub structure, leading to ES and hypersensitivity. In this study, we analyzed EEG data collected from two 24 individuals with SCD pain and 18 pain-free healthy controls. EEG recordings were acquired at rest and during painful pressure cuff application to the left calf. We aimed to investigate the relationship between frequency disassortativity (a condition for ES) of alpha waves, patient reported outcomes (PROs), and VOCs. During the eye-closed resting state, the SCD group exhibited a lower frequency of alpha waves (9.01±0.09Hz) compared to the control group (9.83±0.13Hz). During the painful stimulation, we found that the frequency assortativity of alpha waves in the SCD group displayed a significant negative correlation with three important PROs: BPI Pain Interference score (R=-0.496, p=0.010), PROMISE29 Physical Function (R=-0.539, p=0.005), and HADS Depression (R=-0.509, p=0.008). This suggests that patients with greater pain, depression and poor physical function displayed greater ES features (more disassortative frequencies). Furthermore, patients who had a higher frequency of VOCs in the preceding 12 months presented with decreased frequency assortativity (R=-0.595, p=0.001). Importantly, the occurrence of the VOCs relative to the time of EEG recordings (within 30 days) was significantly correlated with the intensity of the ES condition: the closer of the occurrence of VOCs before or after the EEG recording, the stronger frequency disassortativity. In conclusion, we provided first-of-hand evidence that supports ES as

a novel objective marker for assessing pain and VOCs in SCD. Further studies will help us to understand unique brain network features in SCD with recurrent VOCs and pre-empt the occurrence of VOCs.

Disclosures: P. Joo: None. **U. Lee:** None. **R. Harris:** None. **Y. Wang:** None.

Poster

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Topic: B.07. Network Interactions

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Title: Probing Neural Alterations in the Wall of the Abnormal Brain Cavity: An Electrophysiological Investigation in Rats

Authors: *U. KILIC[1], B. ASAMOAH[3], Z. DENG[1], M. MCLAUGHLIN[1], B. NUTTIN[2]; [1]Dept. of Neurosciences, [2]Dept. of Neurosurg., Catholic Univ. of Leuven, Leuven, Belgium; [3]Ctr. for Neurosci. and Dept. of Neurobiology, Physiol. & Behavior, Univesity of California Davis, Davis, CA

Abstract: Cortical stimulation holds promise as an effective approach for addressing post-stroke motor impairment. Previous attempts at stimulating the perilesional cortex using epidural electrodes for post-stroke impairments have encountered challenges, potentially due to limited spatiotemporal precision. Besides, the formation of an abnormal cavity (aBC) following a stroke presents an opportunity for a new strategy involving electrical stimulation to enhance motor recovery. Utilizing more compliant and flexible arrays, the immediate surface of the aBC can serve as a neuromodulation interface for treating post-stroke impairment. However, a deeper understanding of the neuronal dynamics within the aBC wall is necessary for precise targeting, thereby opening avenues for spatiotemporal stimulation of the aBC wall to alleviate post-stroke motor impairment. This project aims to investigate the altered neurophysiological dynamics within the aBC wall. In this study, local field potentials (LFP) in the forelimb motor cortex (MC) of eleven male Sprague-Dawley rats were unilaterally recorded using a 32-channel recording probe. Subsequently, stereotactic aspiration of the MC was performed at that location to mimic an aBC. LFP recordings were repeated using the same probe, both within and around the aBC wall, approximately one hour (acute) and one week (chronic) after inducing the lesion in an anesthetized setup. Pulsed electric stimulation was applied to the contralateral forelimb muscles in all conditions, and evoked potentials were recorded through the same implanted probe. The LFP measurements revealed a sudden decrease in power within the delta and alpha bands in the acute cavity plane compared to healthy conditions, which depended on the depth of electrical contact. Conversely, in the chronic cavity, a more heterogeneous response was observed, with some regions exhibiting recovery through local field potential power in specific frequency bands,

while others experienced further deterioration. Meanwhile, in the acute cavity, the amplitude of evoked potentials was significantly reduced compared to the response observed in a healthy MC. In chronic conditions, partial recovery of the evoked potential amplitude was observed. Our study reveals changes in both spontaneous and evoked neural activity following aBC formation in the rodent motor cortex. Mapping these changes within the aBC wall can help identify optimal stimulation sites to improve motor deficits.

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Poster

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Title: Alpha oscillations mediate directed interbrain synchronization in socially interacting ferrets.

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Abstract: Social interaction plays a key role in shaping human cognition and mental health. Canonical paradigms in social neuroscience focus on recording the brain activity of individual subjects performing a specific perceptual or behavioral task. Recently, the social brain has been increasingly studied in more naturalistic and interactive settings, where brain activity from multiple interacting people is simultaneously recorded, a technique known as “hyperscanning.” This has led to the discovery of interbrain synchronization in humans in various types of social interaction, from musical performances to classrooms. Greater interbrain synchronization has been correlated with greater social engagement in humans. Two commonly observed brain oscillations mediating interbrain synchronization in humans are frontal theta oscillations (4-8 Hz) and parietal alpha oscillations (8-12 Hz). To further investigate the neural mechanisms of interbrain synchronization, hyperscanning experiments emerged in the study of animal social interactions. While different forms of synchronization have been observed across the frontal cortex of interacting animals in various species, region- and frequency-specific findings analogous to those of humans are yet to be demonstrated. Here, we introduce domestic ferrets (*Mustela putorius furo*) as a novel animal model for studying interbrain synchronization. Ferrets

are gregarious animals that exhibit strong social-cognitive skills, visuomotor communication, and vocalization. They also exhibit human-homolog theta and alpha oscillations in the frontoparietal network while performing cognitive tasks. Thus, we hypothesized that frontal theta oscillations and parietal alpha oscillations of the ferret brain would mediate interbrain synchronization during social interaction. Electrophysiology in the premotor cortex (PMC) or the posterior parietal cortex (PPC) was simultaneously recorded in pairs of animals during free social interaction. Preliminary analysis of one pair of animals demonstrated that interbrain synchronization during active social interaction is primarily mediated by directed coupling between the alpha oscillations in the PPC of the two animals. We did not observe significant synchronization in PMC between the two animals in any frequency bands, including the theta band. Our findings demonstrate the importance of PPC alpha oscillations, over PMC theta oscillations, in mediating interbrain synchronization during social interaction between ferrets. This work also demonstrates the value of using ferrets as an animal model for studying interbrain synchronization comparable to that of humans.

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Poster

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Title: Causal role of frontoparietal theta oscillations in the ferret during the 5-choice serial reaction time task

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Abstract: Introduction: The dorsal frontoparietal network (dFPN), including dorsal frontal cortex (dFC) and posterior parietal cortex (PPC), generates top-down control signals through corticocortical oscillations during cognition. Theta oscillations appear to coordinate activity in the dFPN during cognitive tasks, including sustained attention. Yet, whether theta oscillations within the dFPN play a causal role in sustained attention remains unknown. **Methods:** To

investigate sustained attention, we trained animals in the 5-choice serial reaction time task (5-CSRTT), a preclinical analog of a human sustained attention task. We recorded multi-site electrophysiology and implemented frequency-specific optogenetic perturbations in the freely moving ferret (*Mustela putorius furo*) during the 5-CSRTT. We refined the 5-CSRTT into two behavioral contexts; "hard" and "easy". Task difficulty was determined by length of delay period an animal must wait to respond to a visual stimulus for reward. The peak of frontal theta activity (~5 Hz) for each animal was extracted from endogenous activity. Optogenetic stimulation was delivered via light pulses to the dFC during the 5-CSRTT, while recording local field potential and single-unit activity from dFC and PPC. **Results and Discussion:** We were successful in creating two behavioral contexts of the 5-CSRTT by modulating delay length. The "hard" version of the task significantly degraded task performance, reflected in reduced accuracy ($p = 0.008$) and increased occurrence of premature touches ($p < 0.001$). Animals responded to the visual stimulus faster in the "hard" condition compared to "easy" ($p < 0.001$), indicating that animals were more engaged in the more difficult version. We modulated single unit activity in the dFC with temporal and frequency precision during the delay period of the 5-CSRTT across conditions. We successfully drove network connectivity with theta stimulation in the "easy" task condition, measured by phase locking value. We observed improved task accuracy ($p = 0.0728$) driven by a reduction in omissions ($p = 0.068$) in the "easy" task condition, but did not observe altered functional connectivity or behavior in the "hard" condition. **Conclusion:** We demonstrated a context-dependent effect of rhythmic optogenetic stimulation on dFPN connectivity and identified a potential causal role of theta-frequency coupling within the dFPN during 5-CSRTT performance. This work was supported by the National Institute of Mental Health (R01MH124387 and R01MH122477). Disclosure*:* FF receives payments as a consultant from Electromedical Products International, Insel Spital, and the University of Michigan.

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Poster

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Topic: B.07. Network Interactions

Support: European Union's Horizon 2020 research and innovation program under Grant Agreement No. 732032 (BrainCom)

Title: Multiscale spatio-temporal organization of neocortical dynamics in freely-moving rats revealed by brain-wide surface LFP recordings using active graphene-based ECoG.

Authors: R. GARCIA-CORTADELLA[1,4], G. SCHWESIG[2], A. SHAHIDI[3], A. UMURZAKOVA[5], N. SCHAEFER[4], A. GUIMERA-BRUNET[4], J. CISNEROS[4], F. SERRA

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Abstract: Accumulating evidence from wide-field imaging suggests the temporal and spatial non-stationarity of brain dynamics. However, the limited spatiotemporal resolution and large-area coverage of available electrophysiological or imaging techniques prevent the complete characterization of these non-stationary dynamics. Particularly, imaging techniques suffer from poor temporal resolution and applicability to freely-moving animals, while electrophysiological recordings are limited to frequencies above ~0.5Hz and to a limited area/dense coverage. Thus, novel methodologies are required to understand the large-scale and wide-band non-stationary dynamics of field potentials. Here, we present the use of an active, multiplexed and flexible micro electrocorticography probe based on graphene transistors, which is operated by a dedicated implantable head stage in freely moving rats. Graphene active sensors provide a stable gain in a wide frequency band, including DC, while enhancing the scalability in terms of channel count. Using 256 and 512 channel probes we recorded broadband local field potential (LFP) across a large fraction of the neocortical surface in freely behaving rats. 3D-tracking and spatio-spectral analysis of recordings over long sessions enabled automatic brain states segmentation. We identified topographic spectral characteristics of distinct states as well as the infra-slow correlates of brain-state transitions. We developed a novel approach for the detection of the spatiotemporally non-stationary LFP sources allowing the decomposition of cortical activity into spatiotemporal waves from infra-slow to gamma time scales clustering in similar topographic regions. We identified topographically localized and frequency-diverse sleep spindle and gamma oscillations that were modulated by infra-slow and slow waves. On a cycle time scale, sleep spindles and gamma oscillations presented complex phase dynamics corresponding to standing, travelling as well as spiral waves. Characterization of non-stationary cortical waves in a wide band challenges traditional views of brain oscillations, providing an alternative framework for cross-frequency coupling analysis across spatiotemporal scales, characterization of the oscillatory dynamics, interpretation of the polarity of the surface LFP signals and their spatiotemporal properties.

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Poster

PSTR388. Oscillations in Cortical Networks

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Topic: B.07. Network Interactions

Support: European Union Horizon 2020, No. 732032 (BrainCom)

Title: An Interface for mulit-scale investigations in freely moving rats

Authors: *G. SCHWESIG[1], E. BLANCO HERNANDEZ[1], A. GENEWSKY[1], R. GARCIA-CORTADELLA[1,2], F. STOCEK[1], R. AHMED[1], R. D'ANGELO[1], A. SIROTA[1];
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Abstract: While highly integrated, large sensor count probe systems have revolutionized neuroscience in recent years, the systems for distributed deployment of such tools on a global brain scale in freely moving animals are still poorly developed. Furthermore the linkage of electrophysiological signals across spatial scales often necessitates the combination of different probe types with highly divergent form factors in the same animal, making implantation challenges currently a main bottleneck for systematic cross-scale investigations. In addition, development cycles of new probe designs and integrated multi-scale sensor systems would benefit greatly from iterative tests of different device types within one animal as well as testing of one device type in quick succession in comparable conditions across different animals. Scientific use and technological development of high sensor count, multi-scale probe systems would therefore benefit greatly from cranial interfaces that combine chronic global brain access with techniques for modular, large-scale and iterative implantation of probes and mounting of supportive equipment for recordings in freely moving animals. We therefore developed such an implantation interface for modular, semi-chronic implantation of high-density surface and depth probes in rats, designed around an easy to implant and standardizable base layer supporting full brain access and head-fixation, development of surgical approaches for large bihemispheric craniotomies, exchangeable and probe penetrable skull prosthesis, and modular chronic mounting approaches for depth and surface probes or combinations thereof.

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Poster

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Title: Alterations of cortical and subcortical high-gamma oscillations in anticipation of reward-associated cues in visual and olfactory operant tasks

Authors: E. B.-L. MANESS[1], J. CHOI[1], M. MACIVER[1], B. KOCSIS[2], J. T. MCKENNA[1], R. E. STRECKER[1], *J. M. MCNALLY[3];
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Abstract: Consciousness is comprised of a multitude of brain states that reflect varying degrees of exteroceptive awareness, which depends on specialized functional neural networks that flexibly respond to the demands of the external world. The default mode network (**DMN**) is one such large-scale brain network in humans and non-human animals that is active during introspective behavior, such as resting wakefulness. Suppression of DMN activity is essential to allow task-associated network function for goal-directed behaviors and cognitively-demanding tasks. The basal forebrain (**BF**), a subcortical region which promotes alertness and vigilance, has been hypothesized to facilitate the switch between cortical DMN activity and "task-on" network activity. DMN-like gamma activity (30+ Hz) is enhanced in both the prelimbic cortex (**PrL**), a central node in the DMN, and the BF during quiet wakefulness in the home cage, while such activity is suppressed during exploration of a novel environment, contexts that are presumed to promote or suppress DMN activity, respectively. However, such findings do not address the ability of the BF to provide instant-by-instant regulation of transitions between the DMN and network activity associated with externally guided behaviors. Thus, here we employed multi-site local field potential (**LFP**) recording techniques to measure changes in oscillatory activity in DMN-associated brain regions versus non-DMN regions during performance of two self-initiated operant tasks testing vigilant attention, the rodent psychomotor vigilance task (**rPVT**); and executive control, the odor discrimination Go/No-Go (**GNG**) task. In both tasks, we observed a reduction of broadband high gamma activity (80 - 200 Hz) in anticipation of information-bearing visual and olfactory signals in regions associated with the DMN (PrL, BF, olfactory bulb) and salience network (anterior insula). Interestingly, this suppression was more profound during correctly answered trials, such as when mice released the lever within one second of signal presentation in the rPVT and either retrieved the reward in "go" trials (hit) or refrained from responding in "no-go" trials of the GNG task (correct rejection). Together, these findings support the hypothesis that the BF is an important subcortical regulator of DMN-associated cortical regions, suppressing DMN activity during task-oriented behaviors, such as when awaiting the transient occurrence of a reward-predicting stimulus.

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Poster

PSTR388. Oscillations in Cortical Networks

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Title: Assessing the role of interhemispheric GABAergic neurons in coordinating cortical activity

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Abstract: Inhibitory neurons play an essential role in cortical computation and brain synchronization; however, they have been studied mainly in regard to their local synaptic interactions. We focused on the study of a sub-type of neocortical long-range inhibitory neurons recently described: interhemispheric long-range GABAergic neurons (Int-LRGNs) that interconnect bilateral sensory cortical areas. A substantial population of these neurons co-express parvalbumin (PV) and are fast-spiking neurons, but how they impact interhemispheric coordination of neuronal activity and shape large-scale oscillatory patterns remain unknown. To address this question, we took advantage of cell type-specific optogenetic manipulations, i.e., injection of the Adeno-Associated Virus (AAV) expressing Channelrhodopsin in transgenic mice (PV-Cre). In acute brain slices, optogenetic stimulation of Int-LRGNs evokes inhibitory postsynaptic responses in cortical neurons. In freely moving injected mice that were chronically implanted with bilateral optrodes (arrays of electrodes and optical fibers), Int-LRGNs synchronize large-scale oscillatory activity at gamma, impacting interhemispheric coupling. The results of this research contribute to understanding how Int-LRGNs shape cortical activity and impact interhemispheric coordination. Further research may shed light on their contribution to processes such as sensory perception, as well as their participation in pathological conditions in which interhemispheric synchronization is altered such as schizophrenia and autism.

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Poster

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Title: Gaba transport blockaded in the external globus pallidus disinhibits the thalamic reticular nucleus and desynchronized cortical beta oscillation.

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Abstract: The external globus pallidus (GP) is a GABAergic core that coordinatesfiring and synchronization in the basal ganglia–thalamic–cortical networkthrough afferents and spiking rate. In this sense, the GP sends axons to therostral sector of the reticular thalamic nucleus (RTn); however, thefunctional contribution of this pathway is unknown. The existence of theGP-RTn pathway is important since the RTn controls the flow of informationbetween the thalamus and cortex, suggesting that it contributes to corticaldynamics. In Parkinson's disease, high GABA levels alter electrical activityin the GP and contribute to motor symptoms. Under normal conditions,GABA levels are regulated by GABA transporters (GATs). GAT type 1(GAT-1) is highly expressed in the GP, and pharmacological blockade ofGAT-1 induces tonic inhibition. In this work, we investigated the effect ofincreased GABA levels (by pharmacologically blockading GAT-1 into GPwith 200 nm of tiagabine) on firing pattern in the RTn by obtaining single-unit extracellular recordings from anesthetized rats and on the motor cortex(MCx) by corticography (n=16). Our results show that high GABA levelsincrease the spontaneous activity rate of RTn neurons by 138.02 ± 19.37%and desynchronize oscillations in the beta frequency band in the MCx(diminishing the mean power spectra by 59.89 ± 11.39%). Our findingsprovide evidence that the GP exerts tonic control over RTn activity throughthe GP–RTn pathway and functionally contributes to cortical oscillationdynamics.

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Poster

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Topic: B.07. Network Interactions

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R01 MH124695

Title: Sex differences in LFP bursts and phase discontinuities in the Shank3B-/- mouse

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Abstract: Autism spectrum disorder (ASD) is a developmental disorder characterized by corticostriatal circuit dysfunction. Hyperconnectivity between frontal cortical regions such as the cingulate cortex and the dorsomedial striatum (DMS) has been found in several ASD experimental cohorts. Corticostrial hyperconnectivity is hypothesized to drive behavioral disturbances in ASD such as repetitive behaviors, language development, motor learning and social development. One model to study corticostriatal hyperconnectivity is the Shank3B loss-of-function model. Shank3B is a highly penetrant cause of ASD and the mouse model has increased corticostriatal structural connectivity during development. However, it is unknown whether there are functional changes in corticostriatal connectivity in the Shank3B mouse. Furthermore, it is unknown whether there is increased functional connectivity in the corticostriatal circuit of both male and female mice. We used dual in vivo setup to record neural activity from the anterior cingulate cortex (ACC) and dorsal striatum (DMS) in juvenile male and female Shank3B+/+ and Shank3B-/- mice. We analyzed different properties of the LFPs such as LFP bursts and phase discontinuities. Bursts represent periods of increased LFP activity and show increased power in the theta and gamma bands. Phase discontinuities may represent the disruption of fluid coordination of neural information processing. Preliminary data suggests that the LFP bursts are less frequent but longer in the Shank3B-/- ACC and DMS with a stronger effect in females. When we analyzed phase discontinuities, we found that females had increased phase discontinuities in the beta band (12-30 Hz). In the DMS we found a potential interaction effect where Shank3B-/- males have increased phase discontinuities in the gamma band (30-80 Hz) and Shank3B-/- females have decreased phase discontinuities in the gamma band (30-80 Hz). These data suggest that there are changes in processing of neural information that differs across sexes. There are very few studies analyzing the differences between sexes in both normal and abnormal circuit thus these studies provide new insights into the functional circuit differences between males and females. Future work will include analyzing spike data from the same recordings to see if there are any changes at the single neuron level and determining synchronization between the ACC and DMS.

Disclosures: L. Nelson: None. **R. Peixoto:** None.

Poster

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Glut1 Deficiency Foundation

Title: Isolation of the murine Glut1 deficient thalamocortical circuit: wavelet characterization and reverse glucose dependence of low and gamma frequency oscillations

Authors: *E. SOLIS[1], L. B. GOOD[1], R. GRANJA-VAZQUEZ[1], S. PATNAIK[1], A. G. HERNANDEZ-REYNOSO[1], Q. MA[2], G. ANGULO[2], A. DOBARIYA[2], S. F. COGAN[1], J. J. PANCRAZIO[1], J. M. PASCUAL[2], V. JAKKAMSETTI[2];
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Abstract: Glucose represents the principal brain energy source. Thus, not unexpectedly, genetic glucose transporter 1 (Glut1) deficiency (G1D) is synonymous with encephalopathy. G1D seizures, which constitute a prominent disease manifestation, often prove refractory to medications but may respond to therapeutic diets. These seizures are associated with aberrant thalamocortical oscillations as inferred from human electroencephalography and functional imaging. Mouse electrophysiological recordings indicate that inhibitory neuron failure in the thalamus and cortex underlies these abnormalities. This provides the motivation to develop a neural circuit testbed to characterize the mechanisms of thalamocortical synchronization and the effects of known or novel interventions. We used *ex-vivo* mouse thalamocortical slices on microelectrode arrays and characterized spontaneous low (0.1-4 Hz) frequency oscillations and gamma (30-60 Hz) oscillations under near physiological (2.5mM) and high (20mM) bath glucose concentrations. Using the cortical recordings from layer IV, we quantified oscillation epochs via an automated wavelet-based algorithm. Acknowledging that both persons and mice with G1D exhibit neurophysiological changes upon neural fuel administration, we asked if our slice model captured relevant changes in low and high frequency oscillations. Under near physiological bath glucose, G1D slices exhibited low frequency oscillations at a rate of 0.03 ± 0.02 per second (mean ± SEM, n=6 slices from 3 mice) with an average duration of 1.57 ± 0.03 seconds. Gamma oscillations, which were sparser, occurred at a rate of 8 ± 0.7 mHz, with an average duration of 1.63 ± 0.26 seconds. High bath glucose resulted in a significant ($p < 0.05$, paired t-test) decrease in low frequency oscillations by 13.4 ± 7.2 % (or a rate of 24 ± 20 mHz), whereas the gamma oscillatory events rose by 53.2 ± 16.9 % (or a rate of 12 ± 0.1 mHz). There was no significant change in epoch duration for these broad bin low (1.55 ± 0.23 seconds) or high (1.49 ± 0.1 seconds) frequency oscillations under high bath glucose. However, specifically for 2 to 4.8 Hz oscillations a significant decrease in epoch duration under high glucose was exhibited (2.5 mM glucose: 1.34 ± 0.03 seconds; 20 mM glucose:1.19 ± 0.02 seconds, $p < 0.01$, paired t-test). Increased bath glucose reduced the low frequency oscillations while augmenting the gamma oscillations, likely reflecting strengthened inhibitory neuron activity. This approach provides an *ex vivo* method for the evaluation of mechanisms, fuels, and pharmacological agents in a crucial G1D epileptogenic circuit.

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Poster

PSTR388. Oscillations in Cortical Networks

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Topic: C.01. Brain Wellness and Aging

Title: Phase lock of gamma wave by aurally presenting tones amplitude-modulated at 40 Hz

Authors: *Y. NAGATANI[1], K. TAKAZAWA[1], K. MAEDA[2], A. KAMBARA[3], Y. SOETA[3], K. OGAWA[2];
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Abstract: [Motivations] The gamma-band brain activities or entrainments corresponding to sound stimuli are expected to be effective for preventing cognitive diseases. In many studies, a pulse train, the intermittent sound, is used as the stimuli. In this study, we have focused on the type or the modulation functions. [Methods] Eight stimuli were used: sinusoidal waves at 40 Hz (low frequency sound) and 1 kHz (normal tone as control); sinusoidal waves at 1 kHz which are amplitude-modulated (modulation ratio was 100 %) by 20, 40, and 80 Hz sinusoidal wave, 40 Hz sawtooth wave, and 40 Hz inverse-sawtooth wave; a pulse train containing single 1 kHz sinusoidal pulses with a repetition period of 25 ms. The duration of all stimuli was 500 ms including 0.3 ms tapers. All stimuli were presented to the participants at the A-weighted equivalent noise level of 60 dB via headphones. Each stimulus was randomly presented 100 times. For the measurement of EEG signals, 26 participants with no medical history on hearing were employed (22.7 ± 2.1 years). The experiment was performed in accordance with the protocol approved by an ethical review committee. The result at T6 channel is reported as representative in this abstract. The time-frequency analysis of the EEG signals was performed by using continuous wavelet transform with complex Morlet wavelet. Then, the phase locking index (PLI) of each stimulus was calculated. Finally, the PLI values within the presenting area were temporally averaged with phase adjustment to derive a representative scalar value of the degree of phase locking for each stimulus. [Results and Discussions] First, the temporal mean of the PLI at 35 Hz, which is out of the modulation frequency of the stimuli, is checked. The PLIs of all stimuli was around 0.3 without showing any statistical significance. It implies that the phase was moderately locked to the onset timing of the stimuli regardless of the signal component and/or the shape of the envelope since the duration of the stimuli was short enough to keep the synchronization. The continuing duration of the locking should be checked in the future work. In

contrast, the result of 40 Hz showed different tendency. All stimuli with 40 Hz modulation or 40 Hz pulse as well as the 40 Hz resulted in stronger locking with statistical significance to the non-modulated sound while the 20 Hz and 80 Hz modulation result in no difference. In addition, the inverse-sawtooth modulation did not statistically show difference to the pulse train. The result leads us to the expectation that the target sound to be modulated can be arbitrarily selected for the purpose of brain wave synchronization, which may be useful for the future clinical use for dementia prevention.

Disclosures: **Y. Nagatani:** A. Employment/Salary (full or part-time):; Pixie Dust Technologies, Inc. **K. Takazawa:** A. Employment/Salary (full or part-time):; Pixie Dust Technologies, Inc. **K. Maeda:** A. Employment/Salary (full or part-time):; Shionogi & Co., Ltd. **A. Kambara:** 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.; Pixie Dust Technologies, Inc.. **Y. Soeta:** None. **K. Ogawa:** A. Employment/Salary (full or part-time):; Shionogi & Co., Ltd..

Poster

PSTR389. Brain Networks: *In Vitro* Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR389.01/C45

Topic: B.07. Network Interactions

Support: euSNN MSCA-ITN-ETN H2020-860563
HBP-SGA3 No.945539
PRE2018-086203

Title: Spatiotemporal characterization of human cortical network dynamics and modulation in vitro

Authors: ***J. COVELO**[1], J. SANCHEZ-SANCHEZ[1], A. CAMASSA[1], L. DALLA PORTA[1], R. M. ROBLES[1], N. CANCINO-FUENTES[1], A. MANASANCH[1], S. TAPIA-GONZÁLEZ[2,3], P. GOROSTIZA[4,5], M. CARREÑO MARTINEZ[6], E. CONDE[6], J. RUMIÀ ARBOIX[6], P. ROLDÁN[6], J. DEFELIPE[2,3], M. V. SANCHEZ-VIVES[1,4];
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Abstract: Human neurons exhibit distinctive structural and functional qualities, making studies using human tissue particularly valuable for gaining mechanistic insights into the functioning and pathologies of the human brain. The use of *in vitro* human brain slices preserves the complex

cytoarchitecture and functional local microcircuits, therefore comprising a close representation of local networks *in vivo*. Here, we aimed to provide a two-dimensional characterization of human cortical dynamics during spontaneous cortical activity and in response to pharmacological and electrical modulation. We resorted to cortical tissue from patients with pharmacoresistant epilepsy and brain tumors undergoing resective surgery, cut into 400 μm thick slices. Local field potentials were simultaneously recorded from multiple cortical columns and layers using 16 and 32 multielectrode arrays. Following electrophysiological recording, cortical layers were histologically reconstructed, enabling a two-dimensional spatiotemporal profiling of spontaneous cortical events. We also studied the progressive effects of blocking $GABA_A$ receptors on cortical activity, which was transformed into epileptiform patterns. Finally, we investigated the effect of cholinergic activation by means of light, using the photoswitchable muscarinic agonist (Phthalimide-Azo-Iper (PAI)). Human slices expressed spontaneous slow oscillations, characterized by alternating periods of activity (Up states) and silence (Down states), at a frequency <1Hz. This activity presented layer-specific dynamics, with Up states initiating preferentially in deep cortical layers. Gradual blockade of $GABA_A$ receptors increased the firing rate and initially shortened Up states, eventually leading to epileptiform discharges with high network synchronization. The horizontal propagation of spontaneous slow oscillations was ca. 50 mm/s, an order of magnitude faster than the one described in other mammals. Slice illumination with white light induced the photoconversion of PAI from inactive (*cis*-) to the active isoform, *trans*-PAI. The subsequent activation of M2 muscarinic receptors of acetylcholine resulted in an increase in slow oscillation frequency. Exploring network dynamics in local circuits broadens our understanding of human cortex network mechanisms and mesoscale organization. Even when structurally the recorded slices showed no signs of dysplasia, the potential impact of patients' epileptic discharges on the tissue will be discussed. Comprehending the fundamental properties of the human cortex is a crucial first step towards the development of effective treatment strategies.

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Poster

PSTR389. Brain Networks: *In Vitro* Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR389.02/C46

Topic: B.07. Network Interactions

Title: Optimization of a multiplexed, cell-based assay of neuronal function

Authors: P. ELLINGSON, B. STREETER, D. SULLIVAN, A. PASSARO, S. CHVATAL, *D. MILLARD;
Axion Biosystems, Atlanta, GA

Abstract: The flexibility and accessibility of induced pluripotent stem cell (iPSC) technology has allowed complex human biology to be reproduced in vitro at high throughput scales. Indeed, rapid advances in stem cell technology have led to widespread adoption for the development of in vitro models of neuron electrophysiology to be used in screening applications in drug discovery and safety. As in vitro neuronal models become more mature, the functional electrophysiological activity may begin to reproduce low-frequency brain rhythms, in addition to the spiking, bursting, and synchronization already observed. Also, the nature of the functional activity, whether desynchronized or synchronized, may impact the utility of a model for a given application. The objective of this work is to develop and validate a functional neuronal assay that quantifies the relationship between cell health, the spiking activity of individual neurons, and the network activity embedded in the local field potential (LFP). Further, we provide a rationale for optimizing an in vitro model, including the cells and the media system. To accomplish these aims, we utilized multiwell microelectrode array culture plates, with a planar array of microelectrodes embedded into the substrate of each well. Impedance measurements were used to quantify the attachment of cells to the substrate and microelectrodes, as a measure of cell viability. Broadband (1 - 5000 Hz) electrophysiological data was acquired and then separately processed for action potential detection (200 - 5000 Hz) and low frequency oscillations (1 - 50 Hz). The power spectral density was computed from the low frequency signal sampled after network burst events, and then absolute power was computed in the delta (1-4 Hz), theta (4-8 Hz), alpha (8-14 Hz), beta (14-30 Hz), and gamma (30-50 Hz) bands. The spectral power within the LFP was correlated with the emergence of bursting activity within the rodent cortical neurons by 14 days in culture, and intra-burst oscillations in the alpha band were detected at 21 days in culture. Known reference compounds were then added to the cultures and the change in LFP features were quantified and compared with more traditional functional endpoints based on spiking activity. For example, Amoxapine reduced the burst duration and increased the network burst frequency, while also eliminating the intra-burst oscillations and thus decreasing the LFP power in the alpha band. These results support the continued development and use of in vitro neural assays for high throughput drug discovery and safety assessment.

Disclosures: **P. Ellingson:** A. Employment/Salary (full or part-time):; Axion Biosystems. **B. Streeter:** A. Employment/Salary (full or part-time):; Axion Biosystems. **D. Sullivan:** A. Employment/Salary (full or part-time):; Axion Biosystems. **A. Passaro:** A. Employment/Salary (full or part-time):; Axion Biosystems. **S. Chvatal:** A. Employment/Salary (full or part-time):; Axion Biosystems. **D. Millard:** A. Employment/Salary (full or part-time):; Axion Biosystems.

Poster

PSTR389. Brain Networks: *In Vitro* Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR389.03/C47

Topic: B.07. Network Interactions

Support: NIMH 101634
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NICHD P50 HD 103536
NSF 1749772
Cystinosis Research Foundation
Schmitt Foundation

Title: The Effect Of Network Size On The Structure Of Population Activity In Human-iPSC Derived Neurons

Authors: *Y. UZUN[1,3], R. SANTOS[4,5], C. MARCHETTO[6], K. PADMANABHAN[2,3,7,8,9]; [1]Physics and Astronomy, [2]Univ. of Rochester, Rochester, NY; [3]Univ. of Rochester Sch. of Med., Del Monte Inst. for Neurosci., Rochester, NY; [4]Binstitute of Psychiatry and Neurosci. of Paris, Paris, France; [5]Cnrs, Inst. des Sci. Biologiques, Paris, France; [6]Salk Inst., La Jolla, CA; [7]Dept. of Neurosci., Univ. of Rochester Sch. of Med. an Dent., Rochester, NY; [8]Ctr. for Visual Sci., Univ. of Rochester Sch. of Med. and Dent., Rochester, NY; [9]Intellectual Develop. and Disability Res. Ctr., Univ. of Rochester Sch. of Med., Rochester, NY

Abstract: Cell culture methods for studying population activity in human neural networks offer unprecedented opportunities to understand how the structure of circuits can give rise to complex activity patterns. Although it is generally thought that the size of the network, defined as the number of neurons that comprise the circuit, is critical for determining the dynamics of the population activity, the relationship between this structural parameter and the resultant activity patterns is unclear. Using multi-electrode arrays, we characterized the spontaneous activity in human iPSC-derived neural networks of different size as these circuits matured. We found rather counterintuitively, that networks with fewer neurons generated more complex patterns as compared to networks with a larger density of cells. Furthermore, a model of the global structure built by examining the dynamics and interactions of pairs of neurons was better at predicting the structure of activity in large networks of neurons as compared to cultures with fewer cells, suggesting that larger networks are highly interconnected and therefore more simply organized. Beyond the implications these results have for determining how to experimentally study human brain circuits in vitro, this work illustrates how a property of neuronal cultures such as the size of the network, can self-organize circuits whose dynamics differ not just in degree, but in kind.

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Poster

PSTR389. Brain Networks: *In Vitro* Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR389.04/C48

Topic: B.07. Network Interactions

Support: The NeuroDeRisk consortium has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No

821528.
Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation program, and EFPIA.

Title: Comparative Assessment of Network Level Ca^{2+} Oscillations in 2- and 3-Dimensional hiPSC Derived and Primary Neuronal Cultures with the NeuroDeRisk Compound Set

Authors: *J. IMREDY[1], G. ROUSSIGNOL[2], H. CLOUSE[1], G. SALVAGIOTTO[3], L. MAZELIN-WINUM[2];
[1]Nonclinical Drug Safety, Merck & Co., Inc., Rahway, NJ; [2]Preclinical Safety, Sanofi R&D, Montpellier, France; [3]Fujifilm Cell. Dynamics, Inc., Madison, WI

Abstract: Human induced Pluripotent Stem Cell (hiPSC) derived neural cells offer great potential for modeling neurological diseases and toxicities and have found application in drug discovery and toxicology. As part of the European Innovative Medicines Initiative (IMI2) NeuroDeRisk (Neurotoxicity De-Risking in Preclinical Drug Discovery), we here explore the Ca^{2+} oscillation responses of 2- and 3-dimensional (2D, 3D/neurospheroid) hiPSC derived neuronal networks composed of mixtures of astrocytes and neurons of mixed glutamatergic and GABAergic activity. The test compound set (33 in total) encompasses established seizurogenic tool compounds as well as drugs that have a higher association with seizurogenic adverse events (positive) than other drugs (negative) based on the Food and Drug Administration Adverse Event Reporting System (FAERS) database. Both types of hIPSC networks were scored against Ca^{2+} responses of a mouse neonatal primary cortical neuron 2D network model serving as an established comparator assay. Parameters of frequency and amplitude of spontaneous global network Ca^{2+} oscillations and the drug-dependent directional changes to these were assessed, and predictivity of seizurogenicity scored using contingency table analysis. In addition, responses were compared between both 2D models as well as between 2D and 3D models. Concordance of parameter responses was best between the hiPSC neurospheroid and the mouse primary cortical neuron model (77% for frequency and 65% for amplitude). Decreases in spontaneous Ca^{2+} oscillation frequency and amplitude were found to be the most basic shared determinants of risk of seizurogenicity between the mouse and the neurospheroid model based on testing of the seizurogenic compound set. Increases in spontaneous Ca^{2+} oscillation frequency were primarily observed with the 2D hIPSC model, though the specificity of this effect to seizurogenic clinical compounds was low (33%), while decreases to spike amplitude were more predictive of seizurogenicity. Overall predictivities of the models were similar, with sensitivity of the assays typically exceeding specificity due to high false positive rates. Higher concordance of the hiPSC 3D model over the 2D model when compared to mouse cortical 2D responses may be the result of both a longer maturation time of the neurospheroid (84-87 days for 3D vs. 22-24 days for 2D network maturation) as well as the 3-dimensional nature of network connections established. The simplicity and reproducibility of spontaneous Ca^{2+} oscillation readouts support further investigation of hiPSC derived neuronal sources and their 2D and 3D networks for neuropharmacological safety screening.

Disclosures: **J. Imredy:** A. Employment/Salary (full or part-time):; Merck & Co., Inc. **G. Roussignol:** A. Employment/Salary (full or part-time):; Sanofi R & D. **H. Clouse:** A. Employment/Salary (full or part-time):; Merck & Co., Inc. **G. Salvagiotto:** A. Employment/Salary (full or part-time):; Fujifilm Cellular Dynamics, Inc. **L. Mazelin-Winum:** A. Employment/Salary (full or part-time):; Sanofi R & D.

Poster

PSTR389. Brain Networks: *In Vitro* Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR389.05/C49

Topic: B.07. Network Interactions

Support: NIH/NINDS Grant R01NS110590

Title: Ca^{2+}-induced Zn^{2+}spikes are generated via Zn^{2+}liberations from metallothionein III in neurons

Authors: *L. SALVAGIO[1], C. ZHANG[1], Y. QIN[2];
[2]Univ. of Denver, [1]Univ. of Denver, Denver, CO

Abstract: Ca^{2+}-induced Zn^{2+} spikes are generated via Zn^{2+} liberations from metallothionein III in neuronsCrosstalk between Ca^{2+} and Zn^{2+} ions has been demonstrated in numerous cellular processes, and previous research from our lab showed synchronous Ca^{2+} spikes that occur during critical stages of neurodevelopment can induce large increases in cytosolic Zn^{2+}, termed “Zinc spikes” in primary hippocampal neurons (Chen et al., Journal of Neurochemistry 2021). These Zn^{2+} spikes are always preceded by Ca^{2+} spikes and were found to be dependent on calcium-induced intracellular acidification. Zn^{2+} has the ability to interact with proteins as a structural element or enzymatic co-factor and has recently been thought to act as a signaling molecule. Changes in Zn^{2+} concentration may have the ability to alter the activity of proteins related to neuronal development and activity. The observation of zinc spikes introduces a new form of crosstalk between Ca^{2+} and Zn^{2+} ions and raises the question of uncovering potential roles for Zn^{2+} during neurodevelopment stages where spontaneous and synchronous Ca^{2+} spikes occur. It was hypothesized that intracellular acidification can liberate Zn^{2+} from Zn^{2+} binding proteins, like metallothiones (MT), or ligands, such as antioxidant glutathione or cysteine residues. This work aims to discover the mechanism behind how neuronal Ca^{2+} spikes produce Zn^{2+} spikes. Using fluorescence microscopy imaging, we found that Zn^{2+} spikes were evoked using various methods to induce Ca^{2+} influx, such as depolarization, glutamate activation, and optical activation in primary rat hippocampal neurons. MT3 is a brain specific isoform of the metallothiones Zn^{2+} binding proteins and can sequester up to seven Zn^{2+} ions. Reduction in MT3 expression by shRNAi reduced Zn^{2+} spikes in primary hippocampal neurons. This evidence demonstrates that MT3 may release Zn^{2+} ions upon Ca^{2+} influx and cellular acidification and therefore be responsible for large increases in cytosolic Zn^{2+} in neurons, which may be important signaling events during brain development.

Disclosures: L. Salvagio: None. **C. Zhang:** None. **Y. Qin:** None.

Poster

PSTR389. Brain Networks: *In Vitro* Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR389.06/C50

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: A human brain organoid-on-chip model meeting biological and industrial requirements of neurological preclinical studies

Authors: *J. RONTARD[1], H. CASTIGLIONE[1], C. BAQUERRE[1], L. MADRANGE[2], J. RENAULT[1], Y. CALDERINI[1], D. DEBIS[1], F. LARRAMENDY[1], F. YATES[2], P.-A. VIGNERON[2], T. HONEGGER[1];
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Abstract: Whilst still widely used, animal experimentation shows some limits, especially in the field of neuroscience. In the last few years, cerebral organoids have appeared as relevant alternative solutions to model human cerebral cellular organization and development in 3D. Nevertheless, the implementation of organoids for large-scale drug screening in terms of efficacy and toxicity requires reproducibility and scalability. To address this challenge, we combine cerebral organoid culture with a microfluidic system, called Brain Organoid-on-Chip (Castiglione *et al*., 2022), which enables us to improve organoid culture conditions thanks to the precise modeling of cellular microenvironment. Here we present the establishment of the conditions for cerebral organoid culture in the NETRI Duplex Well microfluidic device, composed of two compartments separated by a porous membrane: 1/ a 3D organoid-culture chamber, and 2/ a perfusion channel enabling medium diffusion. Two different types of human cerebral organoid generation protocols have been assayed: unguided (adapted from Lancaster *et al.*, 2013) and cortical (adapted from Xiang *et al.*, 2017) organoids. Preliminary assays were performed to optimize on-chip cerebral organoids culture conditions, including the medium renewal procedure and an anti-adherence approach. We compared the Brain Organoid-on-Chip system to conventional culture, in terms of morphology, viability, cytoarchitecture, and transcript level of expression. The established procedure for culture medium renewal takes advantage of the perfusion channel to reduce direct medium changes within the organoid culture chamber. Following this procedure, unguided and cortical cerebral organoids were cultured for different long-term time points. They exhibited the characteristic morphologies expected from Lancaster *et al*, 2013 and Xiang *et al*, 2017. Immunofluorescence and RT-qPCR characterizations confirmed the presence of neural progenitors, mature neurons, and astrocytes. The chip design enables a reproducible culture protocol for cerebral organoids. This Brain Organoid-on-Chip platform paves the way for modeling the blood-brain barrier, and ultimately for studying the diffusion of compounds, for toxicological assessments, such as developmental neurotoxicity testing, and for efficacy screening of novel therapeutics.

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Poster

PSTR389. Brain Networks: *In Vitro* Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR389.07/C51

Topic: B.07. Network Interactions

Support: AHA Allen
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Bert and Ethel Aginsky Research Scholar award

Title: Diversity of network burst patterns in astrocyte enriched brain organoids

Authors: *S. E. FERNANDES[1], Y.-M. LIN[2], K. S. PRADEEPAN[3], L. ZHANG[4], M. WANG[1], J. MARTINEZ-TRUJILLO[3], F. H. GAGE[1];
[1]LOG-G, Salk Inst., La Jolla, CA; [2]Florida Intl. Univ., Miami, FL; [3]Univ. of Western Ontario, London, ON, Canada; [4]LOG-G, The Salk Inst., La Jolla, CA

Abstract: Brain organoids demonstrate some aspects of spatial-temporal brain development, including complex cytoarchitecture, resulting in a broad diversity of cell types. However, the ratio of neurons to glial cells is often disproportionately higher in brain organoids than in typical brains. Our lab has developed protocols for the generation of astrocyte-enriched organoids (AEO) containing amounts of astrocytes exceeding those of available organoid protocols. The astrocytes exhibit complex morphology by approximately day 150 in culture. Here, we first performed an immunohistochemical analysis to demonstrate that AEOs are composed of a variety of cell types including astrocytes. Second, we used multielectrode array (MEA) technology to explore neuronal and network activity patterns in the AEO. By nine months, astrocyte-enriched organoids showed spontaneous activity consisting of network bursts as well as sparsely distributed spikes. We used a burst detection algorithm that allowed detection of bursting patterns with high temporal resolution. In some AEO we found bursting patterns that evolve to repetitive high frequency reverberations resembling those previously described on monolayer excitatory networks (Abstract #12085; Pradeepan et al., 2023). However, different from patterns isolated in monolayer networks, AEO showed a much greater variety of network bursts and reverberating phenotypes, likely arising from the diversity of cell types. Third, we are exploring mechanisms that may be contributing to the diverse repertoire of network bursting and reverberating burst patterns through pharmacological treatment corroborated with immunohistochemistry. Pharmacological treatments include bicuculline (GABAAR antagonist), EGTA-AM (slow-kinetic calcium chelator), Strontium chloride (calcium-like divalent cation), artificial cerebrospinal fluid, and Sachez-Vivez (SV) solution (mimicking ionic composition of brain interstitial fluid). Our preliminary findings suggest SV solution promotes hyperexcitability in the network, resulting in increased frequency of patterned bursts. Taken together, AEOs provide evidence of mature cellular networks, diverse cell types, and complex,

electrophysiologically active neuronal networks, all of which are essential elements when modeling neurodegenerative to neuropsychiatric disorder pathophysiology.

Disclosures: **S.E. Fernandes:** None. **Y. Lin:** None. **K.S. Pradeepan:** None. **L. Zhang:** None. **M. Wang:** None. **J. Martinez-Trujillo:** None. **F.H. Gage:** None.

Poster

PSTR389. Brain Networks: *In Vitro* Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR389.08/C52

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

Title: A blinded compound study on a human cortical tri-culture model using isogenic iPSC-derived cortical excitatory neurons, cortical inhibitory interneurons and astrocytes.

Authors: *S. HUMPHREYS[1], S. BROADBENT[1], S. SPRINGE[1], S. YANG[2], A. GHOSHAL[2], N. DEDIC[3], A. BARNES[1];
[1]Axol Biosci., Cambridge, United Kingdom; [2]Sunovion Pharmaceuticals Inc., Marlborough, MA; [3]Sunovion Pharmaceuticals, Sunovion Pharmaceuticals, Marlborough, MA

Abstract: As the global population ages the prevalence of neurodegenerative diseases (NDD) continues to rise to around 2% in people over 80. Despite this for many of the most common types, such as Alzheimer's, there is no cure and current treatments only management of the declining symptoms. One of the key brain areas affected by NDD is the cortex and as a result, a major area of interest for drug discovery. However, the cortex is also a complex structure and a significant site of potential neurotoxicity leading to 25% of drug attrition in full development and causing major side-effects in over 400 registered medications. The lack of physiologically relevant cortical models is a major challenge for developing better treatments for NDDs and identifying potential neurotoxic liabilities during drug development. In drug discovery, categorising the phenotypic effect of small molecules on a target tissue is a common approach. Axol have used iPSC-derived neuronal and brain immune cells in *in vitro* co-culture to recreate a cortex-like tissue for research and drug discovery approaches. In partnership with Sunovion, Axol performed a blinded study with eight reference compounds on Axol's cortical tri-culture model using an Axion MEA system. The tri-culture was maintained to Day 26 post thaw. On Day 26, 10 minutes baseline data was recorded before adding the first concentration of each compound. 10-minute recordings were taken immediately to capture any acute effects and after 60 minutes to capture longer-term effects. Following this a second concentration of each compound was added to the same wells in an accumulative manner and the process repeated. In total three concentrations of each compound were tested. Axol analysed the data and categorised each blinded compound from their observed actions into one of six groups/sub-groups. The main groups were Group 1 - "Little Effect", Group 2 - "Activators" and Group 3 - "De-Activators" which were further sub-divided into Group 2A - "Hyperpolarising", Group 2B - "Excitatory", Group 2C - "Pro-convulsant", Group 3A - "Blockers" and Group 3B - "Inhibitors". On

unblinding the study, all eight compounds were found to be correctly identified although one compound, *A* was placed into the right group as a “De-Activator” but into the wrong sub-group as it’s mode of action would be expected to be an “Inhibitor” rather than a “Blocker”. Overall, this supports the ability of Axol’s human iPSC-derived cortical tri-culture model in predicting the action of new drugs and identifying potential neurotoxic liabilities.

Disclosures: **S. Humphreys:** A. Employment/Salary (full or part-time):; Axol Bioscience Ltd. **S. Broadbent:** A. Employment/Salary (full or part-time):; Axol Bioscience Ltd. **S. Springe:** A. Employment/Salary (full or part-time):; Axol Bioscience Ltd. **S. Yang:** A. Employment/Salary (full or part-time):; Sunovion Pharmaceuticals Inc. **A. Ghoshal:** A. Employment/Salary (full or part-time):; Sunovion Pharmaceuticals Inc. **N. Dedic:** A. Employment/Salary (full or part-time):; Sunovion Pharmaceuticals Inc. **A. Barnes:** A. Employment/Salary (full or part-time):; Axol Bioscience Ltd.

Poster

PSTR389. Brain Networks: *In Vitro* Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR389.09/C53

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Functional Interactions of iPSC-Derived Neuronal and Astrocytic Networks: Insights from MEA and Patch Clamp Recordings

Authors: A. MANOLE, A. RHEE, C. GAO, A. PANKONIN, S. CHIN, J. URRESTI, ***N. LIN**; Ixcells Biotechnologies, SAN DIEGO, CA

Abstract: Understanding the complexity of brain function depends on our ability to comprehend the complicated interactions that take place between various cell types in neural networks. In this study, we used multi-electrode array (MEA) and patch clamp techniques to examine the functional characteristics and interconnections of dopaminergic, sensory, motor, glutamatergic and GABAergic neurons, and astrocytes that are capable of releasing cytokines. Our findings give insight into the underlying mechanisms and shed light on the functional effects of astrocyte-neuron interactions. By employing MEA recordings, we evaluated the synchronization patterns and network activity of these neuronal groups using MEA recordings. Every neuronal subtype exhibited unique firing patterns and network dynamics that were indicative of their functional specialization. The electrical characteristics and synaptic connections within these neural circuits were also examined using patch clamp recordings. Notably, we discovered synapse-specific traits and various synaptic transmission patterns in several neuronal populations. Our research also demonstrated the critical part astrocytes play in controlling neuronal activity. We found that astrocytes secreted cytokines that affected the activity and plasticity of the nearby neurons in addition to their well-known supporting roles. The functional impacts of astrocyte-neuron crosstalk were shown by the cytokine-mediated effects on neuronal excitability and neurite outgrowth. Overall, our research offers thorough understandings of the characteristics and

interconnections of dopaminergic, sensory, motor, glutamatergic and GABAergic neural networks produced from iPSCs. Furthermore, we stress the crucial part astrocytes play in determining neuronal activity and the possible influence astrocytic cytokines may have on neuronal function. The intricate dynamics of brain networks are better understood thanks to these discoveries, which also have implications for neurodegenerative and neurodevelopmental diseases.

Disclosures: **A. Manole:** None. **A. Rhee:** None. **C. Gao:** None. **A. Pankonin:** None. **S. Chin:** None. **J. Urresti:** None. **N. Lin:** None.

Poster

PSTR389. Brain Networks: *In Vitro* Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR389.10/C54

Topic: B.07. Network Interactions

Support: Simons Foundation Autism Research Initiative (SFARI) Research Grant #514918
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Provincial Endowed Academic Chair in Autism and Behavioural Science

Title: Calcium dependent reverberating super bursts in human Rett syndrome excitatory neuronal networks

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Abstract: Rett syndrome (RTT) is a rare neurodevelopmental disorder caused by mutations of *MECP2*, which encodes a global transcriptional regulator. RTT patients show abnormal developmental trajectories that include epileptic seizures. Previous studies have shown human stem cell derived excitatory *MECP2* mutant single neurons have smaller cell bodies with shorter dendrites and decreased branching, decreased synaptic potentials, and hyperexcitability. Using multielectrode array (MEA) technology, researchers have begun investigating patterns of in vitro spontaneous and synchronous activity that reflect neuronal and synaptic development. Abnormal patterns of spontaneous activity reflect altered normal development. Using MEAs and standard

burst detection algorithms, MEAs demonstrate progressive synchronization between electrodes over development - indicative of neurons connecting within a network. Previously, *MECP2* mutant networks have showed decreased burst frequency interpreted as a decrease in network activity and functional connectivity compared to isogenic controls. Here, we reveal that *MECP2* deletions lead to an increase in frequency of reverberating super-bursts (RSBs), consisting of an initial large amplitude network burst followed by high frequency mini-bursts resulting in an increase in burst frequency and number of bursts - indicating hyper-activity. Pharmacological treatment of Ca^{2+} chelator EGTA-AM selectively eliminated RSBs, implicating elevated pre-synaptic calcium dynamics and rescuing the network burst phenotype. RSB were not affected by bicuculline (GABAAR antagonist). Taken together, reverberating super bursts (RSBs) present as a temporally complex dynamic that warrants careful consideration when defining bursts. Our results show that RSBs emerge early in developing neuronal networks of RTT, likely a consequence of enhanced excitability in single neurons and networks. *MECP2* mutant networks exist in a genetically pre-disposed hyperexcitable state, such that physiologically relevant stimuli are more likely to persist within the network as a reverberation. These abnormal dynamics may lead to deviations from the typical developmental trajectory that facilitate the emergence of hyper-synchronic states that contribute to seizure onset.

Disclosures: K. Pradeepan: None. **F. McCready:** None. **W. Wei:** None. **J. Ellis:** None. **J. Martinez-Trujillo:** None.

Poster

PSTR389. Brain Networks: *In Vitro* Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR389.11/C55

Topic: B.07. Network Interactions

Support: UK Research & Innovation
MSD (UK) Limited

Title: An in vitro investigation of cortical network hyperexcitability in mouse models of ALS

Authors: *M. DOULOUDI[1], A. URBANEK[1], A. J. GRIERSON[1], J. WATKINS[2], J. DUCE[2], M. R. LIVESEY[1], R. J. MEAD[1];
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Abstract: Amyotrophic lateral sclerosis (ALS) is an incurable neurodegenerative disease that leads to the progressive death of cortical and spinal motor neurons that comprise the locomotor circuit. Growing evidence indicates that cortical hyperexcitability, an early detectable electrophysiological disturbance in ALS and a driver of neuronal toxicity, is a strong candidate for driving locomotor circuit dysfunction and neuronal injury/loss. Cortical hyperexcitability, therefore, represents an attractive therapeutic target. However, the underlying drivers and

mechanisms of cortical hyperexcitability are not well understood. Multi-electrode arrays combined with *in vitro* dissociated cortical neuronal cultures provide a powerful platform to decipher mechanisms which drive excitability in transgenic mouse models of ALS and thus, pave the way for the discovery of novel therapeutics and *in vivo* testing. Using this technology, we performed a longitudinal analysis in cortical neuronal networks of different ALS mouse models with the aim of detecting changes in network excitability. We have analysed our excitability data using a multi-parameter approach, which took into consideration spike frequencies, burst duration, inter-burst intervals, individual bursts per array and number of network bursts per array, amongst other parameters. Specifically, we found early and late network excitability alterations in G93A-SOD1 derived cortical neurons consistent with a hyperexcitable phenotype, that were evidenced by decreased burst intervals (days *in vitro* 14: $p = 0.009$, days *in vitro* 21: $p = 0.012$), but not spiking frequency (days *in vitro* 14: $p = 0.396$, days *in vitro* 21: $p = 0.456$) compared to control neurons. Pharmacological interrogation of the G93A-SOD1 network disturbances by glutamatergic and GABAergic receptors blockers, uncovered that cortical hyperexcitability was accompanied by inhibitory circuitry deficits, but not changes in the excitatory input, suggesting that inhibitory transmission-related physiology is a promising therapeutic target for further investigation in rescuing the early hyperexcitable phenotype that is present in the disease. We also extended our analysis to two different C9ORF72-BAC mouse models which present molecular phenotypes only. Our results report early excitability changes in G93A-SOD1, but not in C9ORF72-BAC, cortical neurons, confirming that cortical hyperexcitability is only observed in models which go on to display a motor phenotype associated with motor neuron loss.

Disclosures: **M. Douloudi:** Other; MSD (UK) Limited. **A. Urbanek:** None. **A.J. Grierson:** None. **J. Watkins:** None. **J. Duce:** None. **M.R. Livesey:** None. **R.J. Mead:** None.

Poster

PSTR389. Brain Networks: *In Vitro* Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR389.12/C56

Topic: B.07. Network Interactions

Support: NIH Grant RO1-HL-141560

Title: Vagus nerve stimulation is differently integrated at the nucleus of the solitary tract (NTS) neurons during heart failure in rats.

Authors: *L. G. FERNANDES, E. BEAUMONT;
Biomed. Sci., East Tennessee State Univ., Johnson City, TN

Abstract: Vagus nerve stimulation (VNS) therapy is currently use to treat heart failure with reduced ejection fraction (HFrEF). VNS is typically used at a frequency of 5 Hz with an intensity that is sufficient to induce a slight bradycardia during stimulation. We previously shown that all

myelinated and none of the unmyelinated afferents are activated during VNS. The current study investigates the integration of vagal afferents on NTS neurons in control and HF rats (ejection fraction &lt 60%) following different VNS protocols. Sprague-Dawley rats with pressure overload underwent a thoracic aortic constriction twelve weeks prior to terminal electrophysiology experiments and were compared to healthy controls. For terminal patch clamp experiments, the brainstem was removed under isoflurane anesthesia and a horizontal brainstem slice containing the solitary tract (ST) and medial NTS was obtained. Brain slice was maintained in physiological CSF and whole-cell voltage-clamp recordings was performed. Electrical shocks to the ST produced fixed latency evoked excitatory postsynaptic currents (eEPSCs) with a jitter &lt 200usec that identified NTS neurons with monosynaptic afferent input. EPSCs with jitter &gt 200usec were considered polysynaptically innervated. ST stimulation protocols were used to mimic in vivo VNS using the following protocols: 1) continuous 5 Hz stimulation for 14 secs, 2) continuous 20 Hz stimulation for 14 secs, 3) 300Hz burst stimulation (4 pulses) for 14 secs, interburst interval (IBI) of 1 sec, 4) 300Hz burst stimulation (7 pulses) for 14 secs, IBI of 1 sec. Results showed that spontaneous EPSCs from second-order neurons in HF rats had smaller amplitude ($p < 0.05$) and frequency (p &lt 0.05) compared to sEPSCs observed control rats. VNS at 300 Hz with 4 pulses elicited an increase in evoked EPSC amplitude compared to other protocols in control rats (p &lt 0.05), but this effect was not observed in HF rats. All VNS protocols elicited asynchronous release of glutamate in TRPV1+ ST terminals, and these events were significantly more frequent (p &lt 0.05) and larger in amplitude (p &lt 0.05) in second-order neurons in controls compared to HF animals. In conclusion, second-order NTS neurons in HF rats showed significantly depressed synaptic features compared to controls. Since VNS therapy is beneficial in improving cardiac function, its effect may rely on restoration of synaptic properties for central integration of vagal afferents at the NTS level.

Disclosures: L.G. Fernandes: None. **E. Beaumont:** None.

Poster

PSTR389. Brain Networks: *In Vitro* Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR389.13/C57

Topic: B.07. Network Interactions

Support: Air Force Office of Scientific Research under award number FA9550-22-1-0000

Title: Learning in closed-loop neural cultures embodied in virtual worlds

Authors: *W. CLAWSON, M. LEVIN;
Allen Discovery Ctr., Tufts Univ., Medford, MA

Abstract: All brains are collectives of cells embodied in a larger collective (the body) that together form an organism, whether non-vertebrate, vertebrate, mouse, or human. These bodies

are in an environment which is detected through a number of sensors, and that sensation is passed to the brain through electrical signaling. Much of learning and memory occurs in, or is directed by, the brain itself, where through neural firing and electrochemical interactions, the brain utilizes its plasticity to adapt to the environment. However, the full extent of brain plasticity - what elements of brain composition change, when, and how - is not fully understood. Additionally, the technical difficulty of in-vivo experimentation leaves much to assumption or indirect measurement. Here we propose to study the environment-brain relationship through closed-loop electrical stimulation of neural tissue culture, which is capable of the sensing, processing, learning, and action loops present in all organisms as well as being experimentally more accessible than in-vivo brains. We grow both primary cultures from wildtype embryonic mice as well as human induced neural stem cells (hiNSCs) as monolayers on a high density multielectrode array (HD-MEA) which records local field potentials (LFPs) and spiking activity in high temporal and spatial fidelity. These cultures are embodied in a virtual world after 21 days in vitro (DIV) via a closed-loop system in which neural electrical activity guides an agent's behavior in a simple computer game and the agent's interactions inside the game are encoded as stimulations given to the culture. Crucially, both the agent-environment stimulation ruleset and the location of the stimulations remain fixed throughout the training. Therefore, any change in behavior comes from the neural cultures' plasticity efforts, rather than a change of software. With this, we aim to probe a form of tissue level intelligence as only a subset of plasticity changes is available to the neural culture. We report and classify a number of traditional electrophysiological measures as cultures develop over weeks, how these measures change in response to embodiment, and propose a novel platform for future study of fundamental principles of learning and memories in neural systems.

Disclosures: **W. Clawson:** None. **M. Levin:** None.

Poster

PSTR389. Brain Networks: *In Vitro* Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR389.14/C58

Topic: B.07. Network Interactions

Title: Comparative study of neural cell culture conditions and their impact on synchronized bursting

Authors: *J. L. TANCREDI, A. M. IBRAHIMI, M. A. DERR, S. SHIN;
Cell Biol. R&D, Thermo Fisher Scientific, Frederick, MD

Abstract: In vitro culture of neural cells requires an ideal energy source to maintain their viability and functionality. Glucose and pyruvate are commonly utilized as energy sources for sustaining neurons in vitro. However, culturing neurons in a low glucose-containing medium has been associated with significant degeneration. Thus, providing optimal culture conditions to support neural cells in vitro is crucial.

This study compared the effects of different culture conditions on neuronal health and functionality. B27 System was specifically designed to culture neurons while suppressing the growth of glial cells. In contrast, B27 Plus system aimed to support matured neurons and was found to be capable of supporting both neurons and astrocytes. It has been previously reported that glial cells can enhance synchronized bursting, a phenomenon observed in neuronal networks.
To evaluate the impact of culture conditions on synchronized bursting, electrophysiological measurements were conducted. Calcium imaging analysis revealed that B27 Plus system resulted in the development of synchronized bursting when compared to B27 system. The emergence of synchronized bursting signifies enhanced network activity and coordination among neurons. Furthermore, astrocytes, a type of glial cell, were found to produce L-serine and glycine. A subsequent "add back" study was performed, where glycine and L-serine were supplemented in the culture medium. This supplementation led to an increase in both the frequency and amplitude of spontaneous synaptic currents, indicating a positive impact on neuronal communication. These findings emphasize the importance of selecting appropriate culture conditions for neural cell studies in vitro. Understanding the influence of different conditions on neuronal health and functionality can contribute to the development of optimized culture protocols, ultimately enhancing our understanding of neural networks and their dynamics.

Disclosures: **J.L. Tancredi:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **A.M. Ibrahimi:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **M.A. Derr:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **S. Shin:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific.

Poster

PSTR389. Brain Networks: *In Vitro* Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR389.15/C59

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

Support: Fitzgerald Translational Neuroscience Fund

Title: Linking physically isolated in vitro neural specimens on separate three-dimensional microelectrode arrays via virtual white matter

Authors: ***M. KHANTAN**[1,2], J. T. LIM[1], A. NAPOLI[1], I. OBEID[2], M. D. SERRUYA[1]; [1]Raphael Ctr. for Neurorestoration, Thomas Jefferson Univ., Philadelphia, PA; [2]Dept. of Electrical and Computer Engin., Temple Univ., Philadelphia, PA

Abstract: Closed-loop stimulation of the nervous system, in which activity recorded from one area triggers focal stimulation in a separate target area, has emerged as a potential therapeutic modality to enhance recovery after neurological disease and injury. In addition to in vivo investigations in non-human animals, e.g., Neurochip3 developed by Fetz and colleagues [1],

this technique has also been applied to individual neural specimens in vitro, such as in the European Project ‘BrainBow’ ‘Bidirectional Bridging’ system developed by Chiappalone et al [2] and closed-loop optogenetic stimulation by Jackson and colleagues [3]. To our knowledge, neural activity-driven microstimulation across two physically separate in vitro specimens has not been reported. If shown to be feasible, a software-driven virtual white matter platform could enable neuroscientists to scale up an increasing number of specimens to link to each other to create more sophisticated circuits and feedback loops, and to test the effects of temporarily virtually disconnecting or reconnecting specimens to each other.We created an in vitro model composed of two ex-vivo brain tissue samples cultured in two separate dishes, and a computer-based system to record and stimulate them. The activity of a neural specimen was recorded from one dish, action potentials (APs) and field potentials (FPs) were identified and used to focally modulate artificial electrical stimulation in the second dish. The neural activity evoked in the second dish is also recorded and used in turn to modulate electrical stimulation delivered back to the first dish. This focal stimulation therefore functions as a bi-directional virtual white matter connection.The system collects APs and FPs from neural specimens with preserved cytoarchitecture (organotypic slices and organoids) using three-dimensional multielectrode arrays (MEAs). The system selectively and focally stimulates desired regions of the brain tissue, and stimulation can be modulated in real-time based on neural activity recordings. Following stimulation, the system can detect changes in neural activity in the surrounding tissue, demonstrating the possibility of virtually recreating white matter.These findings demonstrate the viability of using electrical circuitry to virtually recreate the connections between various neural specimens. In addition to providing a novel methodology to study emergent properties from dynamically linked neural specimens in two or more separate dishes. Furthermore, the system could function as a testbed to develop in vitro brain-computer interfaces that rely on activity-triggered microstimulation and bidirectional feedback.

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Poster

PSTR389. Brain Networks: *In Vitro* Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR389.16/C60

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

Support: CAMS Innovation Fund for Medical Sciences 2021-I2M-1-020

Title: Multi-scale Organization of Neural Networks in a Three-Dimensional Bioprinted Mini-Brain Model

Authors: *H. YANG[1,3], J. ZHANG[2,3], Z. ZHONG[1,3], Y. LI[2], N. YANG[4], L. GU[1], Y. WANG[1], Y. LIU[4], Y. MAO[2], W. MA[1], H. YANG[2];
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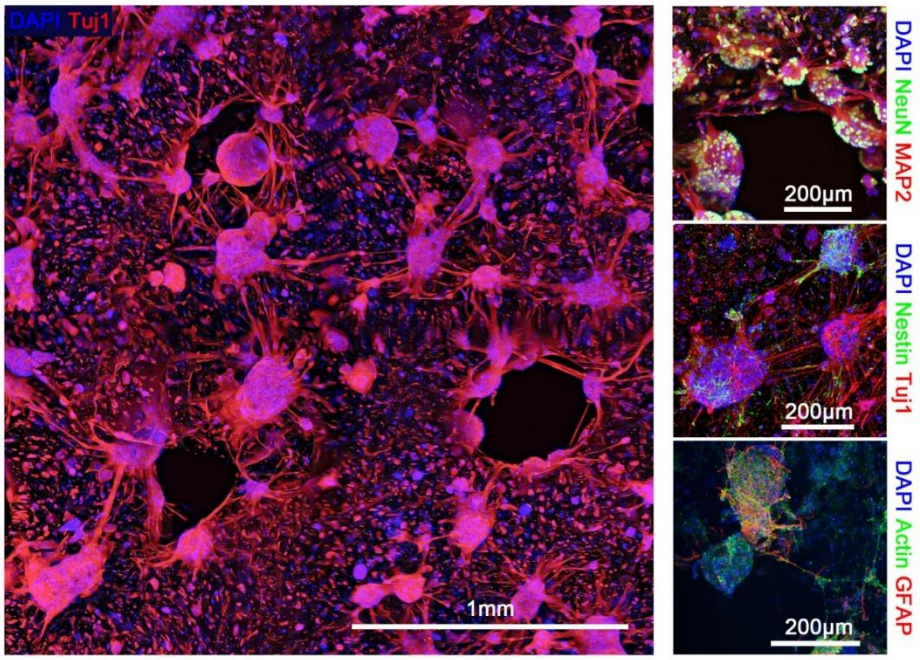
Abstract: Efficiently establishing *in vitro* neural models that accurately mimic the structural and functional connectivity of neural networks is of paramount importance in neuroscience research. Three-dimensional (3D) bioprinting, an emerging biofabrication technology, offers great potential for constructing sophisticated *in vitro* models. Here, we employed extrusion-based 3D bioprinting to fabricate 7×7×1.5 mm GelMA-based constructs containing E18 rat cortical neurons, which was subsequently referred to as 3DP. ATP quantification and CaM/PI staining indicated favorable neuronal viability within the 3DP over 14 days *in vitro*. Immunofluorescence staining targeting specific markers, such as NeuN, MAP2, and Tuj1, revealed progressive aggregation of neurons into spherical clusters of 50-200 μm in diameter (Figure). These clusters were accompanied by distinct axonal interconnections within and between clusters, resulting in the formation of a brain-like network. Fluo-4 mediated 3DP calcium signal recording enabled the visualization of spontaneous discharges within neuronal clusters at a micrometer scale, as well as synchronized calcium signals at a millimeter scale. Further investigation of pharmacological perturbation by antagonists of glutamate receptor, GABA receptor, and ion channel on the 3DP neurons exhibited significant alterations in firing rates and global correlation. RNA-seq analyses on both 3DP and conventional two-dimensional (2D) cultured neurons were performed. Gene Set Enrichment Analysis (GSEA) showed that the 3DP gene expression profiles were more similar to the adult rat cortex compared to the E18 cortex and 2D-cultured neurons, suggesting that 3DP neurons manifest an adult-like expression pattern despite being originated from fetal rats. These findings demonstrate the formation of multi-scale neural circuits within the 3D bioprinted mini-brain structure.



Disclosures: **H. Yang:** None. **J. Zhang:** None. **Z. Zhong:** None. **Y. Li:** None. **N. Yang:** None. **L. Gu:** None. **Y. Wang:** None. **Y. Liu:** None. **Y. Mao:** None. **W. Ma:** None. **H. Yang:** None.

Poster

PSTR389. Brain Networks: *In Vitro* Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR389.17/C61

Topic: B.07. Network Interactions

Support: NEUREKA project, GA 863245

Title: Label free functional characterization of ipsc derived neurons at subcellular resolution

Authors: *S. OLDANI[1], E. GUELLA[1], S. RONCHI[2], M. J. OBIEN[3];
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Abstract: In recent years, brain models derived from pluripotent stem cells have become a fundamental tool for studying common neurological disorders, such as epilepsy, Alzheimer's disease, and Parkinson's disease. The ability to measure the electrical activity of human iPSC derived neurons in real time and label free can provide much needed insights into the complexity of the neuronal networks. Nowadays, combining single cell resolution with high throughput physiological assays, which can potentially deepen our understanding of subtype specific neuronal activity, is especially valuable and yet difficult to achieve.In this study, the MaxTwo System (MaxWell Biosystems, Switzerland), a multi well high density (HD) MEA platform was used. MaxTwo HD MEA System allows *in vitro* extracellular recordings of action potentials at different scales, ranging from network through single neuron to subcellular features. Moreover, we showed the advantages of having an HD-MEA system featuring 26,400 electrodes per well, which are key to increase the statistical power of the data collected from iPSC derived neurons over multiple days, weeks and to capture the smallest neuronal signals.Finally, we present the Axon Tracking Assay, a tool for automated recording and analysis of individual axonal arbors of many neurons in parallel. The Axon Tracking Assay enables to measure action potential conduction velocity, axonal length, and number of axonal branches. With this unique method, we characterized the function and axonal structure of different iPSC derived neuronal cell lines.Our HD MEA platforms and the extracted metrics, such as firing rate, spike amplitude, and network burst profile among several others, provide an extremely powerful and user friendly approach for *in vitro* drug screening and disease modelling.

Disclosures: S. Oldani: None. **E. Guella:** None. **S. Ronchi:** None. **M.J. Obien:** None.

Poster

PSTR390. Circuit-Based Antiepileptic Therapies

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR390.01/C62

Topic: B.08. Epilepsy

Support: NIH R01NS118091

Title: Responsive closed-loop neurostimulation to prevent focal epilepsy progression and associated long-term memory impairment.

Authors: *J. J. FERRERO[1], A. HASSAN[2], Z. YU[2], Z. ZHAO[3], L. MA[2], S. SHAO[2], J. ENGEL[1], D. KHODAGHOLY[3], J. GELINAS[2];
[2]Neurol., [3]Electrical Engin., [1]Columbia Univ., New York, NY

Abstract: Long-term memory impairment significantly impacts patients with temporal lobe epilepsy (TLE), but no targeted treatment exists. The hippocampus and associated mesial temporal lobe structures are essential for declarative memory, and uncontrolled epileptic activity in these brain areas is a strong risk factor for progression of epilepsy and memory dysfunction. Temporal coupling of neural oscillations is critical for physiologic brain functions, and is dysregulated in the presence of interictal epileptiform discharges (IEDs).
We used a rodent kindling model to investigate the electrophysiologic activity patterns in hippocampus and medial prefrontal cortex (mPFC) as animals experienced worsening of seizures and cognitive decline. Progression of epileptic activity in the hippocampus was associated with creation of an independent focus of interictal epileptic activity in the mPFC. Emergence of this independent cortical IED focus was correlated with a defined shift in hippocampal-cortical interactions that promoted intra-cortical hypersynchronous neural spiking. We found that spatiotemporally targeted closed-loop mPFC stimulation triggered on hippocampal IED occurrence eliminated this hypersynchronous response, effectively preventing recruitment of the mPFC into the epileptic network and ameliorating long-term spatial memory deficits.
These results suggest a causal role for IED-mediated hippocampal-cortical communication in epilepsy progression and memory impairment, and propose interictal closed-loop electrical stimulation as a potential therapy for epilepsy and its comorbidities.

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Poster

PSTR390. Circuit-Based Antiepileptic Therapies

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR390.02/C63

Topic: B.08. Epilepsy

Support: RGC/ECS 21103818
RGC/GRF 11104320
RGC/GRF 11104521

JCYJ20190808182203591
internal funds from City University of Hong Kong 9610354

Title: Activation of parvalbumin interneurons in the anterior piriform cortex alleviates seizures in temporal lobe epilepsy

Authors: *H. ZHANG, A. CHIU, H. JIANG, H. CHOI, C. G. LAU;
City Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: Epilepsy is a pathological state that involves altered excitation-inhibition in the brain and exhibits recurrent seizures. The anterior piriform cortex (APC) is a limbic area that is closely associated with temporal lobe epilepsy (TLE). In the APC, parvalbumin-expressing (PV^+) interneurons, which form abundant perisomatic synapses on principal neurons, can provide strong local inhibition. However, the causal relationship between APC-PV neurons and TLE is unclear. Using the pilocarpine model, we first found reduced local inhibition and PV synaptic density in the APC. To examine the causal role of APC in TLE, we applied chemogenetics and multi-site local field potential (LFP) recording in both the pilocarpine (i.p.) and hippocampal kainic acid (KA) injection models. In the pilocarpine model, we showed that APC-PV neuronal activation induced an anti-ictogenic effect and afforded seizure protection but did not influence an ongoing status epilepticus. In the chronic KA model, activation of APC-PV neurons significantly weakened focal seizure (FS) expression in the hippocampus. Additionally, brain dynamics analysis indicated that APC-PV neuronal activation took effect by modulating the long-range functional connectivity of the seizure network. Specifically, we found decreased local excitability and increased phase synchronization in ictal states when APC-PV was activated. Phase synchronization was measured by inter-regional phase locking value (PLV) of LFP. Moreover, there was a strong, negative correlation between FS number and PLV in ictal states. These results identify the APC-PV neuron as a target for treating TLE and highlight long-range functional connectivity as an endophenotype to report seizure severity. Importantly, our research provides novel insights into how a cortical microcircuit can exert antiepileptic benefits.

Disclosures: H. Zhang: None. **A. Chiu:** None. **H. Jiang:** None. **H. Choi:** None. **C.G. Lau:** None.

Poster

PSTR390. Circuit-Based Antiepileptic Therapies

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR390.03/D1

Topic: B.08. Epilepsy

Support: CIRM TRAN1-11611
CIRM CLIN2-13355

Title: NRTX-1001: Human inhibitory interneuron cell therapy suppresses seizures and reduces histopathology in a mouse model of chronic focal epilepsy with high repeatability across multiple studies and manufacturing lots

Authors: *P. HAMPEL[1], M. B. PAREKH[2], H. K. KIM[2], E. T. SEVILLA[2], F. PORKKA[2], A. VOGEL[2], S. LEE[2], J. H. JUNG[2], M. W. L. WATSON[2], S. HAVLICEK[2], S. KRIKS[2], M. BERSHTEYN[2], Y. MAURY[2], L. FUENTEALBA[2], A. BULFONE[2], G. BANIK[2], C. A. PRIEST[2], C. R. NICHOLAS[2];
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Abstract: One-third of people with epilepsy have drug-resistant seizures. Surgical resection or ablation of a seizure focus can be effective options for chronic focal epilepsy; however, these procedures are tissue-destructive and are not indicated for all. As a restorative therapeutic alternative, the administration of cells that deliver GABA to the seizure focus could suppress chronic seizures without tissue destruction. NRTX-1001 comprises GABAergic, post-mitotic interneurons of a specific MGE pallial-type lineage derived from human pluripotent stem cells. Previous work examining NRTX-1001 transplantation into a mouse model of chronic focal epilepsy demonstrated seizure suppression and reduced hippocampal histopathology. The studies described here demonstrate that the disease-modifying effects of NRTX-1001 transplantation are consistent across multiple studies with independent manufacturing lots of NRTX-1001. Clinically-compliant processes were used to manufacture and cryopreserve NRTX-1001. Multiple preclinical studies were performed in the intrahippocampal kainate mouse model of drug-resistant focal epilepsy using independent lots of NRTX-1001. Single intrahippocampal administration of NRTX-1001 was performed in the chronic phase of the disease model, which is characterized by spontaneous recurrent seizures and hippocampal sclerosis. Focal seizures were detected by continuous (24/7) EEG recording from bipolar hippocampal electrodes at several timepoints up to 7 months post transplantation. Intrahippocampal administration of NRTX-1001 into mice with chronic mesiotemporal seizures resulted in pronounced reduction of focal seizures across multiple independent cell lots and studies. NRTX-1001 transplantation consistently resulted in stable seizure-freedom in approximately two-thirds of epileptic mice. At 7.5 months post transplantation of NRTX-1001, cell engraftment and hippocampal histopathology were assessed. NRTX-1001 interneurons persisted for the duration of the studies and distributed throughout the hippocampus. NRTX-1001 administration led to significantly reduced hippocampal dentate granule cell dispersion. No ectopic tissues, tumors or teratomas were observed following transplantation of NRTX-1001. The results of these preclinical studies support the ongoing phase I/II clinical trial (NCT05135091) to evaluate NRTX-1001 in people with drug-resistant temporal lobe epilepsy. Funding: CIRM (TRAN1-11611; CLIN2-13355)

Disclosures: **P. Hampel:** A. Employment/Salary (full or part-time):; Neurona Therapeutics. **M.B. Parekh:** A. Employment/Salary (full or part-time):; Neurona Therapeutics. **H.K. Kim:** A. Employment/Salary (full or part-time):; Neurona Therapeutics. **E.T. Sevilla:** A. Employment/Salary (full or part-time):; Neurona Therapeutics. **F. Porkka:** A. Employment/Salary (full or part-time):; Neurona Therapeutics. **A. Vogel:** A. Employment/Salary (full or part-time):; Neurona Therapeutics. **S. Lee:** A. Employment/Salary (full or part-time):; Neurona Therapeutics. **J.H. Jung:** A. Employment/Salary (full or part-time):; Neurona Therapeutics. **M.W.L. Watson:** A. Employment/Salary (full or part-time):; Neurona Therapeutics. **S. Havlicek:** A. Employment/Salary (full or part-time):; Neurona Therapeutics. **S.**

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Poster

PSTR390. Circuit-Based Antiepileptic Therapies

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR390.04/D2

Topic: B.08. Epilepsy

Support: NIMH RF1MH114126-01
NIMH UG3-MH120095-01
NIMH UG3-MH120095-02
NIMH UG3-MH120095-03

Title: Cell class-specific gene therapy for Dravet syndrome

Authors: J. K. MICH[1], J. RYU[2], B. B. GORE[3], A. D. WEI[2], R. A. MARTINEZ[3], R. GUO[3], E. M. LUBER[3], R. L. CHRISTIAN[3], M. BARD[2], ***Y. BISHAW**[3], J. RAMIREZ[2,4], J. T. TING[3], E. S. LEIN[3], B. P. LEVI[3], F. KALUME[2,4];
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Abstract: Dravet syndrome (DS) is a devastating developmental and epileptic encephalopathy marked by treatment-resistant seizures, developmental and motor deficits, and a high rate of premature death. In over 80% of patients with DS, the disease is caused by heterozygous loss-of-function mutations in SCN1A, the gene encoding Nav1.1 channels. SCN1A mutations impair GABAergic interneuron excitability, and mouse models recapitulate key DS phenotypes when SCN1A mutations are targeted to GABAergic interneurons. Taken together these observations suggest interneurons constitute a promising target for precision gene therapy in DS. Here we developed and validated cell class-specific enhancer AAV gene replacement therapy driving expression of SCN1A in GABAergic interneurons. Using these tools, we overcame the AAV carrying capacity limitations and achieved full-length SCN1A expression in forebrain GABAergic neurons with high efficiency and specificity. Patch-clamp recordings and western blot in cell lines showed that these constructs produce a functional Nav1.1 protein. After packaging these vectors into AAV and administration in mice, immunohistochemical analyses showed dose-dependent biodistribution of the therapeutic cargo in targeted cells and brain regions. Remarkably, these vectors displayed strong dose-dependent protection against mortality

and thermally induced seizures to DS mouse models carrying nonsense alleles of Scn1a. We observed protection in two independent model mouse lines and at two independent research sites, with no overt toxicity observed in littermate control mice. Immunohistochemistry on these testing cohorts allowed us to directly correlate vector biodistribution with functional rescue. Furthermore, we also tested additional vectors for pan-neuronal expression of Nav1.1, and these vectors provided weaker protection from DS symptoms, and showed safety concerns including preweaning lethality. These findings suggest that cell class specificity might not only boost effectiveness of gene therapy in DS but could also enhance the safety of therapy. In summary, these findings demonstrate proof-of-concept that cell class-specific AAV-mediated SCN1A gene replacement therapy could provide an effective precision therapy in DS.

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Poster

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Topic: B.08. Epilepsy

Support: NINDS NS112500
Dravet Syndrome Foundation

Title: Histological analysis of hippocampal interneuron populations in the Scn1b knockout mouse model of Dravet syndrome

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Abstract: Dravet syndrome (DS) is a severe and devastating developmental epileptic encephalopathy characterized by early age of onset, refractory seizures, and cognitive comorbidities. Homozygous loss-of-function mutations in the *SCN1B* gene have been linked to DS, and *Scn1b* loss specific to interneurons in mice has been shown to cause early mortality. Given the involvement of the protein products of *SCN1B*— β1 and β1B—in neuronal migration and pathfinding, it is possible that loss of *SCN1B* may contribute to the DS phenotype by interfering with the migration and/or integration of GABAergic interneurons. GABAergic parvalbumin (PV), somatostatin (SST), and calretinin (CR) interneurons play significant roles in hippocampal processing and are involved in the pathology of other forms of DS and epilepsy. To address the hypothesis that hippocampal interneuron displacement contributes to DS pathology, we compared the number and anatomical locations of PV, SST, and CR hippocampal interneurons between *Scn1b* knockout DS model mice and their wild-type littermates. We bred

independent mouse lines to label specific interneuron subtypes, crossing *Scn1b*$^{+/-}$ mice with interneuron-specific Cre lines and the Ai-14 TdTomato (TdTom) reporter line. Between ages P15-20, we perfused and fixed the brains of *Scn1b*$^{-/-}$;Cre^{+};TdTom^{+} (knockout) and *Scn1b*$^{+/+}$;Cre^{+};TdTom^{+} (wild-type) mice and performed immunohistochemistry to—in combination with Cre-mediated TdTom labeling—increase the accuracy of PV, SST, and CR interneuron identification. Manual cell counts were conducted using ImageJ software, and the numbers and locations of cells throughout hippocampal subregions were analyzed. Preliminary data (N=2 mice for each interneuron type) showed no obvious difference between the number or placement of PV, SST, or CR hippocampal interneurons in *Scn1b* knockout mice compared to their wild-type littermates. These data indicate that, even in the absence of *Scn1b*, GABAergic PV, SST, and CR interneurons are still able to migrate and incorporate into the expected subregions of the hippocampus. Thus, it is not likely that alterations in hippocampal physiology found in *Scn1b* knockouts are caused by a deficit in GABAergic interneuron migration. Additional detailed cell counts will be performed to verify this conclusion.

Disclosures: **J. Edwards:** A. Employment/Salary (full or part-time):; University of Texas at Austin. **J.H. Chancey:** A. Employment/Salary (full or part-time):; University of Texas at Austin. **M.A. Howard:** A. Employment/Salary (full or part-time):; University of Texas at Austin.

Poster

PSTR390. Circuit-Based Antiepileptic Therapies

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Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR390.06/D4

Topic: B.08. Epilepsy

Support: NS112500
Dravet Syndrome Foundation

Title: Behavior analysis in a Purkinje cell-specific SCN1B deleted mouse model of Dravet syndrome

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[1]Neurosci., Ctr. For Learning & Memory, Univ. of Texas, Austin, Austin, TX; [2]Neurosci., Univ. of Texas at Austin, Austin, TX; [3]UNIVERSITY OF TEXAS AT AUSTIN, Austin, TX

Abstract: *SCN1B* encodes the β1 protein, an auxiliary subunit to voltage-gated ion channels, cell adhesion molecule, and transcriptional regulator, as well as the secreted β1B protein. Mutations in *SCN1B* are associated with developmental epileptic encephalopathies including Dravet syndrome (DS). DS is a severe infantile epilepsy with frequent and difficult to treat seizures and a range of comorbidities including movement disorders, cognitive, and social delays.The cerebellum is involved in coordination of voluntary movements and motor learning, as well as cognition and social behavior. *SCN1B* expression is high in cerebellar Purkinje and granule cells, but the role of this protein in the cerebellum, and the cerebellum's role in DS, are understudied.

We hypothesized that loss of *SCN1B* causes cerebellar dysfunction, leading to the ataxia and social behavioral changes frequently reported in people with DS even after seizures are medically controlled.Global *Scn1b* knockout mice die by age P22, so are inadequate for studying DS in the adult. Thus, we crossed a conditional *Scn1b* knockout mouse with a Purkinje cell-specific Cre line creating an *Scn1b* mouse model that lives into adulthood (>P150). To quantify motor and social deficits, we performed a three-phase behavioral paradigm including open field and 3-chamber tests on Purkinje cell-specific *Scn1b* KO and WT littermate mice, repeated at 8, 10, and 12 weeks of age. Phase 1 was open field testing of locomotor activity. Distance traveled, velocity, and time spent in middle of the arena were scored using Ethovision XT software. In phase 2 the test mouse was placed in a 3-chamber arena with the choice of spending time with either a novel mouse or a novel object. In phase 3, the 3-chamber choice was spending time with a familiar mouse or a novel mouse. Sociability is the propensity of the test mouse to spend more time in the chamber containing the novel mouse vs novel object; social preference is the propensity to spend more time in the chamber containing the novel mouse vs the familiar mouse. Our preliminary findings show that conditional KO mice exhibit clear ataxia and balance issues by 8 weeks of age compared to wild type. These mice moved more slowly than their WT peers and would fall over when rearing up. Importantly, these mice do not show signs of failure to thrive, seizures, or early mortality which severely afflict the constitutive *Scn1b* KO mice. These findings indicate that cerebellar function is directly altered by loss of *Scn1b* from Purkinje cells, which may be an underlying cause of some of the comorbidities of DS.

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Poster

PSTR390. Circuit-Based Antiepileptic Therapies

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Program #/Poster #: PSTR390.07/D5

Topic: B.08. Epilepsy

Support: NINDS NS112500

Title: Sex-specific aggressive behavior in the pilocarpine mouse model of temporal lobe epilepsy

Authors: A. PANT, M. A. HOWARD, *J. H. CHANCEY;
Univ. of Texas At Austin, Austin, TX

Abstract: Temporal lobe epilepsy (TLE) is one of the most common forms of epilepsy, affecting over 50 million people worldwide. In addition to seizures, TLE has a significant impact on quality of life, affecting cognitive function and emotional well-being. While individuals with TLE are not inherently more prone to aggression than the general population, up to 40% experience postictal aggression in the hours or even days following seizures. Understanding the neurophysiological pathways responsible for this behavior is crucial for providing

comprehensive care to individuals with TLE. Although studies have reported aggression in rodent models of TLE, there are a limited number of systematic investigations examining aggressive behavior in relation to the disease state. Additionally, there is a significant lack of TLE studies in females despite existing sex differences in epilepsy and its comorbidities, even in extensively studied animal models of chemically induced TLE. Our objective is to understand sex differences in postictal aggression and establish a correlation between the severity of epilepsy and aggressive behavior in a mouse model of TLE. The pilocarpine mouse model of TLE was employed, wherein a 2-hour episode of status epilepticus (SE) was induced, then pharmacologically stopped. The mice were then continuously monitored using 24-hour video recordings to evaluate the frequency, duration, and intensity of seizures. Aggressive behavior was assessed using the resident intruder test at two time points: 5-10 days after SE, representing the latent period characterized by ongoing epileptogenesis without spontaneous seizures, and 30-35 days post-SE, the epileptic period. In this test, an unfamiliar (intruder) mouse is introduced into the home cage of the test (resident) mouse, creating a social interaction that can trigger territorial aggression. Latency to attack was recorded as a measure of aggressive behavior. We found that males in the pilocarpine group averaged a lower latency, i.e., more aggressive behavior, than controls at both time points. This pattern was not observed in the female mice. These results suggest that there is an increased likelihood of postictal aggressive behavior that is sex dependent, and that the pilocarpine model provides a tool to study the underlying neurophysiological mechanisms of TLE associated aggression.

Disclosures: **A. Pant:** None. **M.A. Howard:** A. Employment/Salary (full or part-time):; University of Texas at Austin. **J.H. Chancey:** A. Employment/Salary (full or part-time):; University of Texas at Austin.

Poster

PSTR390. Circuit-Based Antiepileptic Therapies

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR390.08/D6

Topic: B.08. Epilepsy

Support: CBI - Brain Institute - Data Bank

Title: Thalamic connectivity with epileptogenic neural circuitry in patients implanted with responsive neurostimulators

Authors: *P. REEDERS, N. W. SCHULTHEISS, M. LALLAS, S. WANG, P. JAYAKAR, M. DUCHOWNY, M. FAJARDO;
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Abstract: Responsive Neurostimulation (RNS) is an alternative treatment for patients with drug-resistant epilepsy who are not candidates for surgical resection. RNS detects abnormal electrical activity and delivers targeted electrical stimulation to interrupt seizure propagation. Surgical

therapies using RNS typically target the centromedian nucleus of the thalamus (CM) due to its widespread connectivity. The mechanism by which RNS aborts seizures at CM is not completely understood.
Understanding whether the anatomical network from CM includes epileptogenic zones provides important insight in how RNS reduces seizure burden. One hypothesis is that direct connectivity of CM with the region of highest amplitude of the scalp activity distribution leads to more favorable outcomes with RNS. We investigated 1) structural connectivity of CM in patients with generalized epilepsy and a RNS, 2) whether this network includes epileptogenic brain regions with predominant peaks in the spatial current density distribution (CDR hotspots) of the EEG, and 3) whether there is a relationship with the connectivity measures and seizure burden reduction post RNS.
We performed probabilistic tractography on pre-surgical diffusion weighted imaging using CM of the thalamus as our seed and whole brain cortical regions as our targets. We used streamlines from each voxel in the seed and recorded when they ended in one of the target regions. We created a seed-target distribution to investigate the connectivity profile. We then identified the target regions with direct connectivity to CM and compared them to the brain areas with CDR hotspots. Lastly, we explored whether connectivity between brain regions with early seizure activity to CM, is related to lowering of seizure burden post RNS implantation.Preliminary findings (N=2) in patient 1 revealed CDR hotspots in bilateral superior frontal gyri and left medial orbitofrontal cortex. Tractography revealed corresponding strong connectivity between CM and the superior frontal gyri, as well as to the medial orbitofrontal area. This patient had a reduction in seizure duration and intensity. Patient 2 had CDR hotspots in the rostral area of the middle frontal gyrus, and strong connectivity between CM and this region. This patient had a reduction in seizure intensity and improvement in reported behaviors. Further analyses and a larger sample are necessary to verify initial findings.
These results support a relationship between anatomical connectivity of CM with brain regions involved in early seizure activity, and seizure burden reduction post RNS. This relationship may aid in candidate selection, surgical targeting and outcome prediction.

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Poster

PSTR390. Circuit-Based Antiepileptic Therapies

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Program #/Poster #: PSTR390.09/D7

Topic: B.08. Epilepsy

Support: NC 123240.1

Title: High frequency stimulation modifies the firing activity of thalamic reticular nucleus

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Abstract: Deep brain stimulation, specifically high-frequency stimulation (HFS), is an alternative and promising treatment for intractable epilepsies; however, the optimal targets are still unknown. The thalamic reticular nucleus (TRN) occupies a key position in the modulation of the cortico-thalamic and thalamo-cortical pathways. High-frequency stimulation (HFS) in TRN retards the generalization of PTZ-induced seizures, however, the mechanisms of action are still unknown. To explore the mechanism underlying HFS-TRN we recorded the multiunit activity of TRN in response to HFS-TRN and PTZ. Male Wistar rats, anesthetized with urethane (1.2 g/kg) and atropine sulfate (0.05 mg/kg), were used. A borosilicate microelectrode (4-8 MOhms) for extracellular recording, and a bipolar electrode for biphasic HFS (100 Hz, 0.1 ms, 200 mA), were introduced, both in the left TRN. At the end of the experiment, the rats were sacrificed and the brain was removed for histological analysis. The total frequency, the firing type, and the firing rate were analyzed. PTZ caused a significant decrease in firing rate, 15 neurons changed their firing from tonic to burst, while an increase in firing rate was observed in another five neurons. HFS decreased firing frequency compared to baseline and the second dose of PTZ, with no change in firing rate observed in three neurons. The results suggest that the TRN participates in the maintenance of the characteristic spike-wave discharges of absence seizures, while the HFS in the TRN can induce the inhibition of the synchronization of oscillatory activity. In conclusion, the HFS in the TRN may block the burst firing in TRN, and may be enough to protect against spread of convulsive seizures.

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Poster

PSTR390. Circuit-Based Antiepileptic Therapies

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Topic: B.08. Epilepsy

Support: NSTC 111-2314-B-006-100
NCKUH-11202033

Title: Cathodal transcranial direct current stimulation decreases the epileptic excitability: From neuronal firings to local field potential oscillations

Authors: *Y.-J. WU[1], C.-C. CHIANG[2], M.-E. CHIEN[1], Y.-J. HUANG[1], S.-F. LIANG[3], K.-S. HSU[4], D. DURAND[2];
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Abstract: Seizure is a hyperexcitability brain disorder. Cathodal transcranial direct current stimulation (ctDCS) has been applied to treat refractory seizures. However, the mechanism of how ctDCS modulates seizure excitability remains not fully understood. We used local field potential (LFP) and extracellular single-unit recordings with one session *in vivo* ctDCS in the kainic-acid induced hippocampal seizure animals to investigate the neurophysiological changes following ctDCS compared with sham stimulation. The neuronal unit firings recorded at CA1 coupled with the LFP epileptic discharges. In tDCS-treated rats, the amplitudes of LFP epileptic spikes were reduced and the LFP oscillations were shifted toward delta-to-theta ranged low-frequency oscillations compared with sham. Neuronal unit spike numbers at CA1 were reduced in tDCS-treated rats compared with sham, among which the putative interneuron spikes decreased to a more significant degree than those of the putative pyramidal cells. High unit spike-LFP coherences were observed in the putative interneurons while the coherences decreased during ctDCS treatment. The short-term plasticity measured by paired-pulse stimulation showed enhanced paired-pulse depression and reduced paired-pulse facilitation in ctDCS-treated rats. These ctDCS-induced neurophysiological changes occurred during tDCS and lasted for 90 minutes following stimulation. c-Fos activations increased in GAD^+ inhibitory neurons whereas reduced in $CaMKII^+$ excitatory neurons. Collectively, ctDCS decreases seizure excitability by reducing the neuronal unit firings and altering LFP toward low-frequency oscillations. These underpinnings exert the inhibitory effects of ctDCS with the short-term plasticity changes in an epileptic brain.

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Poster

PSTR390. Circuit-Based Antiepileptic Therapies

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Topic: B.08. Epilepsy

Support: The Health and Medical Research Fund (HMRF), Food and Health Bureau, Hong Kong Special Administrative Region Government (Ref. No.: 08193956)

Title: Investigation of Therapeutical Potential of Optimized Low Frequency Deep Brain Stimulation at Novel Anatomical Target Area in the Treatment of Epilepsy

Authors: *V. Y. Y. CHU[1], **S. CHAN**[2], G. KUMAR[2], C. H. E. MA[2,3];
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Abstract: Epilepsy is a debilitating neurological disorder and is caused by abnormal hyperexcitability of neurons which disrupts normal neurocircuitry and brain functions. It affects

over 60 million people globally with more than 40% of the patient population developing antiepileptic drug resistance (ARD). Deep brain stimulation (DBS) is typically prescribed to patients with ARD using Anterior thalamic nucleus (ATN) as the target brain stimulation site. However, stimulation in this area results in side effects such as memory impairment, and depression. We have previously reported a novel anatomical DBS target in the midbrains that has demonstrated therapeutic efficacy when DBS is administered at 20Hz frequency, 100µA pulse amplitude, 80µs pulse width for 1 hour daily for 7 days. To further examine the neurocircuitry involved, an AAV fluorescent tracer is injected at the novel DBS target site, and we observed presence of fluorescent signal in the motor cortex, substantia nigra par reticular (SNr) and ATN; suggesting that the therapeutic effect is largely due to neuronal innervation from the new DBS site. We then optimize DBS parameters by varying DBS current and frequency using acute mouse model of pentylenetetrazol (PTZ)-induced epilepsy. The optimized DBS parameters increased myoclonic latency by ~200% and reduced general tonic clonic (GTC) duration by 80%. Furthermore, our optimized DBS treatment has suppressed hyper excitation in the delta band by ~67% and that of theta band by ~70%. Reduction of GTC duration holds clinical relevance as prolonged sessions often leads to irreversible brain damages and ultimately resulting in death. To dissect the molecular mechanisms involved in the pathogenesis of PTZ or kainic acid (KA) induced epilepsy and the effects of DBS, we investigate the role of excitatory (vGLUT) and inhibitory (GAD67) neurons in the new DBS site and their contribution to the pathogenesis of epilepsy. Immunohistochemical staining of vGLUT and GAD67 demonstrate a 50% reduction of vGLUT-positive neurons and 30% reduction of GAD67-positive neurons after DBS in the new DBS site. Our results suggest the involvement of glutaminergic and GABAergic neurons in disease progression of epilepsy. In summary, our study demonstrates the therapeutic potential of low frequency DBS at our proposed novel DBS site via its neuronal connection to the motor cortex, SNr and ATN. Further molecular and electrophysiological studies are required to investigate the involvement of vGLUT and GAD67 neurons in our proposed target region and their roles in orchestrating the pathogenesis of epilepsy.

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Poster

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Topic: B.08. Epilepsy

Support: R01NS114120

Title: Low-frequency deep brain electrical stimulation of callosal white matter tracts provides an antiepileptic effect in the cortex by activating inhibitory $GABA_B$ and slow afterhyperpolarization mechanisms

Authors: *N. PAKALAPATI, D. DURAND;
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Abstract: Epilepsy is a neurological disorder characterized by abnormal brain electrical activity and poses significant treatment challenges when it becomes drug resistant. Deep brain stimulation (DBS), particularly low-frequency stimulation (LFS) targeting white matter tracts, has emerged as a potential therapy for drug-resistant epilepsy. However, the precise mechanisms underlying LFS-induced suppression of epileptic activity in the cortex remain unknown. This study investigates how LFS, when applied to the corpus callosum, provides an antiepileptic effect in the cortex in coronal rodent brain slices. We first identified that 5 Hz LFS maximally suppressed epileptic activity in the cortex (>80%) relative to other stimulation frequencies in a 4-aminopyridine (4-AP) seizure model. Upon assessing changes to tissue excitability resulting from LFS using a paired-pulse test, we observed a reduction in tissue excitability across a wide range of interspike intervals (ISI) (20-1000 ms), as indicated by the decrease in the P2/P1 ratio of evoked potentials relative to no stimulation (n = 8). Furthermore, we observed that the maximum reduction in tissue excitability occurs at an ISI of 200 ms, suggesting the involvement of specific receptors or mechanisms contributing to the anti-epileptic effect. Notably, tissue excitability remains depressed at 1000 ms, indicating the activation of persistent long-term inhibitory mechanisms. To explore the underlying mechanisms contributing to the reduction in tissue excitability, we employed various specific and non-specific pharmacological blockers in combination with LFS to study changes in the resulting antiepileptic effect. Independent administration of $GABA_B$ receptor blockers and slow afterhyperpolarization (sAHP) blockers (n = 8 each) reduced the LFS-induced antiepileptic effect by over 85%. When specific blockers for these mechanisms and receptors are used in conjunction, LFS fails to provide an antiepileptic effect—indicating that LFS primarily depends on these two mechanisms to provide an antiepileptic effect. Our findings suggest that $GABA_B$-mediated inhibition maximally reduces tissue excitability at an ISI of 200 ms, while sAHP provides a weaker, persistent antiepileptic effect observed at an ISI of 1000 ms. Overall, our study reveals that 5 Hz LFS targeting white matter tracts activates $GABA_B$ receptors and sAHP mechanisms, leading to a potent reduction in cortical epileptic activity. These insights contribute to the understanding of LFS-induced suppression of epileptic activity and provide a basis for the development of novel therapeutic approaches for drug-resistant epilepsy.

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Poster

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Topic: B.08. Epilepsy

Support: R01NS097762-06

Title: Optogenetic activation of the dorsal striatum suppresses generalized absence epilepsy and limbic seizures

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Abstract: Generalized seizures are the most common type of epilepsy in humans. The striatum is the main basal ganglia input structure, and it has important connections with cortical and limbic brain sites, suggesting that modulation of striatal activity may be a viable approach for control of generalized seizures. Here, we tested if optogenetic activation of the striatum can attenuate generalized absence seizures induced by gama-butyrolactone (GBL) and generalized limbic seizures induced by the amygdala kindling. 25 Sprague-Dawley (SD) rats were submitted to stereotaxic surgery for bilateral cortical electrodes implantation over the frontal, parietal, and cerebellum (reference and ground). 16 SD rats were implanted with bipolar electrode within the basolateral amygdala nucleus (BLA). All animals received a virus injection in the striatum. rAAV-hSyn-ChR2 (H134R)-mCherry was used for activation of striatal neurons and AAV-CAG-GFP was used for control rats (opsin-negative). Viruses were allowed to express for 3 weeks. For limbic seizures, animals were submitted to daily electrical stimulation of the BLA. Once animals were fully kindled, they were tested under different light frequencies (blue light 473 nm, 5 and 100 Hz). For absence seizures, rats received intraperitoneal injection of GBL (100 mg/kg) and were tested under 5 and 100 Hz conditions. In the GBL model we also performed additional test sessions to compare open loop (continuous) and closed loop (responsive neuromodulation) light delivery. In previously kindled rats, both 5 and 100 Hz suppressed the afterdischarge ($p<0.05$) and the behavioral manifestation of limbic seizures ($p<0.05$). In the GBL model of absence epilepsy, the continuous light delivered (open loop, both 5 and 100 Hz) reduced the number of spike-and-wave discharges (SWD), mean SWD duration, and the percentage of time seized during session ($p<0.5$). In the closed loop protocol, 100 Hz attenuated absence epilepsy manifestation, reducing SWD mean duration ($p<0.05$), but 5 Hz was ineffective. Control animals showed no alteration in any of the parameters assessed in the GBL and kindling models. In separate sessions we monitored electrographic activity changes in the cortex and BLA during and after light delivery without evoking seizures; these data suggest an increase in high frequency, low amplitude activity during optogenetic stimulation. These results support our hypothesis that activation of the striatum can attenuated generalized seizures. Additionally, our closed loop data suggest that, under specific testing conditions, on-demand activation of the striatum is sufficient to shorten SWDs displayed in the GBL model of absence epilepsy.

Disclosures: W.L. Lopes: None. **S. Hyder:** None. **P.A. Forcelli:** None.

Poster

PSTR390. Circuit-Based Antiepileptic Therapies

Location: WCC Halls A-C

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Program #/Poster #: PSTR390.14/D12

Topic: B.08. Epilepsy

Support: NIH Grant R01NS124592
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Title: Subthreshold oscillating waves induced by optogenetic stimulation can suppress epileptiform activity in the hippocampal slice

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Abstract: Subthreshold oscillating waves have been observed in different regions across the brain and are thought to influence the timing of neural spikes. Our previous study shows that optogenetic stimulation can induce a subthreshold oscillating wave propagating across the whole hippocampal slice, even without synaptic transmission. Moreover, the induced subthreshold waves can generate interference patterns and suggest that the neural tissue could process signals with a very low amplitude below the threshold of firing action potentials. In the current study, we further characterize the induced subthreshold waves and investigate the interaction between the induced subthreshold waves and other endogenous neural activity. We found that the induced subthreshold waves decay in amplitude along the propagating direction. The propagating speed of the subthreshold waves also decreased with reduced amplitude but became constant at very low amplitudes. Furthermore, the speed and amplitude of the subthreshold waves can be modulated by changing the osmolarity in the environment. To study the interaction between subthreshold and suprathreshold neural activity, we generated subthreshold waves in the theta frequency traveling from the septal hippocampus towards an epileptic focus in the temporal region. We found that the subthreshold waves could suppress interictal spiking activity induced by 4-aminopyridine (4-AP). The suppression of epileptiform activity is dependent on the amplitude of subthreshold waves. These results suggest that the low amplitude subthreshold waves traveling by ephaptic coupling non-synaptically could suppress much larger amplitude epileptiform activity. The mechanism of this effect is not known, but it suggests that theta waves are involved in promoting a low excitability level in the brain. The subthreshold waves and epileptiform activity could possibly explain the seizure suppression by theta waves.

Disclosures: C. Chiang: None. **D.M. Durand:** None.

Poster

PSTR390. Circuit-Based Antiepileptic Therapies

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Program #/Poster #: PSTR390.15/D13

Topic: B.08. Epilepsy

Support: PAPIIT IN215923

Title: Short-term calorie restriction reduces the spectral power of seizures induced by microinjection of kainic acid in rat hippocampus

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Abstract: Calorie restriction (CR) defines as a reduced calorie intake from food without causing nutritional deficiencies. Even though long-term CR has been described to have an anticonvulsant effect in generalized seizure models, the mechanisms that produce this effect are not clear in short-term. The objective of the present work was to evaluate the effect of short- term CR (SCR) on seizures induced by microinjection of kainic acid (KA) in the hippocampus of anesthetized rats. Male Wistar rats (250-300g) were divided into two groups: the first received food and water ad-libitum; the second, a short-term CR schedule that consisted of restricting calories to 40% in each animal for 30 days. A recording electrodes and microinjection by cannula system directed to the right hippocampus was implanted. Subsequently, each group was subdivided into two: one received the vehicle microinjection (0.9% NaCl) and the other the KA microinjection (1µg/µl). The electroencephalogram (EEG) was recorded at the microinjection site in the animals. The percentage of protection against status epilepticus (SE) was determined and the EEG signal was analyzed at different times (30,60 and 120 min). In the group subject to SCR, an increase in protection was found when presenting SE. In the spectral analysis of the EEG signal, the spectral power was lower in SCR rats compared to ad-libitum [$F(30,60) = 3.182$, $P<0.05$] at 120 min. These results suggest that SRC reduces the voltage of seizures induced by KA microinjection in the rat hippocampus.

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Poster

PSTR390. Circuit-Based Antiepileptic Therapies

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Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR390.16/D14

Topic: B.08. Epilepsy

Title: Downregulation of Wnt/B-catenin pathway in kindled rats subjected to caloric restriction

Authors: *E. URIBE[1], A. PORTILLA[1], D. HUERTA[1], W. MORENO[1], M. RUBIO-OSORNIO[2], C. RUBIO[1];
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Abstract: Several of the antiepileptic effects of caloric restriction (CR) have been described, however, no relationship has yet been established within the *Wnt* molecular pathway, whose

status has been observed to be particularly altered in epilepsy in recent years. Continued research into these types of adjuvant therapies is essential as they could unveil new therapeutic targets for better disease control. We analyzed electroencephalographic changes and Racine´s stages behavior using the amygdalin Kindling experimental model (t-test), as well as the concentrations of key proteins within the *Wnt*/β-catenin signaling pathway (β-catenin, cyclin D, *GSK3β, Wnt*) using Western Blot and immunofluorescence technique in hippocampus and motor cortex of male Wistar rats (n=24) randomly divided into a control group (n=6), a Sham group (n=6) and two groups subjected to the Kindling model, one with CR (n=6) and the other *Ad libitum* (n=6). Data were analyzed using the software ImageJ for obtaining arbitrary optical units for protein´s density in grayscale and then analyzed in Excel using ANOVA and Tukey post hoc. After the Kindling model, we observed both a decrease in the duration of epileptic activity and the behavioral threshold in rats kindled with CR compared to those kindled *ad libitum* ($p<0.05$). Statistically significant differences were obtained in the densitometric analysis of proteins with an elevated concentration of all proteins in the Kindling *ad libitum* group compared to the rest ($p<0.01$) in both the motor cortex and hippocampus. Likewise,
a marked decrease of proteins close to control levels was observed in the Kindling group with CR ($p<0.01$), particularly in the hippocampus. Our results evidence a clear stabilizing effect of the *Wnt*/β-catenin pathway in rats subjected to CR after being exposed to seizures with the Kindling model, promoting a downregulation of the pathway in general, thus exerting a counterbalance to the overregulation obtained in the *ad libitum* Kindling group. It is important to consider the regulation of this intracellular signaling pathway as another molecular target within the antiepileptic effects of caloric restriction, as well as to continue investigating its potential relationship with other intracellular pathways such as mTOR, the ubiquitin-proteasome system or mitochondrial biogenesis.

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Poster

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T32NS007453
American Epilepsy Society Postdoctoral Fellowship 506835

Title: Female sex influences microRNA-silencing and anti-seizure efficacy of an antagomir in mice

Authors: *D. TIWARI[1,4], V. RAJATHI[2], J. RYMER[2], L. N. BEASLEY[1], A. MCGANN[2,5], A. BUNK[2], E. V. PARKINS[2,5], M. F. RICE[2], K. SMITH[3], D. M. RITTER[2], A. WHITE[2], C. M. DOERNING[3], C. GROSS[2,4];
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Abstract: About one third of individuals living with epilepsy have treatment-resistant seizures. Alternative therapeutic strategies are thus urgently needed. One potential novel treatment target is microRNA-induced silencing, which is differentially regulated in epilepsy. Inhibitors (antagomirs) of specific microRNAs have shown therapeutic promise in preclinical epilepsy studies; however, these studies were mainly conducted in male rodent models, and research into microRNA regulation in females and by female hormones in epilepsy is scarce. This is problematic because female sex and the menstrual cycle can affect the disease course of epilepsy and may, therefore, also alter the efficacy of potential microRNA-targeted treatments. Here, we used the proconvulsant microRNA miR-324-5p and its target, the potassium channel Kv4.2, as an example to test how microRNA-induced silencing and the efficacy of antagomirs in epilepsy are altered in female mice. We showed that Kv4.2 protein is reduced after seizures in female mice similar to male mice; however, in contrast to male mice, microRNA-induced silencing of Kv4.2 is unchanged, and miR-324-5p activity, as measured by the association with the RNA-induced silencing complex, is reduced in females after seizure. Moreover, a miR-324-5p antagomir does not consistently reduce seizure frequency or increase Kv4.2 in female mice. As a possible underlying mechanism, we found that miR-324-5p activity and silencing of Kv4.2 in the brain were differentially correlated with plasma levels of 17β-estradiol and progesterone. Our results suggest that hormonal fluctuations in sexually mature female mice influence microRNA-induced silencing and could alter the efficacy of potential future microRNA-based treatments for epilepsy in females.

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Poster

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Program #/Poster #: PSTR390.18/D16

Topic: B.08. Epilepsy

Support: RF-2021-12372526

Title: Identification of non invasive biomarkers to improve diagnosis and prognosis of subjects with suspected autoimmuno encephalitis with seizure.

Authors: *L. LIBRIZZI[1], D. VILA VERDE[2], F. DELEO[1], G. D'AMBROSIO[1], C. REGONDI[1], M. DE CURTIS[1];
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Abstract: Seizures frequently occur in Autoimmune Encephalitis (AE), but the factors underlying their development have not been fully identified. AutoAbs, indeed, could in principal account for the synaptic dysfunction, but only incompletely explain the generation of seizures that does not invariably occur in all the AE patients sharing the same autoAb, and can occur even in AE-seronegative cases (Bien,2017; Dalmau and Graus,2018). Growing evidence suggests that inflammatory mediators contribute to seizure development in epilepsy (Vezzani,2019; Librizzi,2021). In this study we investigated the specific role of peripheral blood mononuclear cells (PBMCs) in combination with serum albumin in initiating brain inflammation and in inducing seizure-like events (SLEs). We propose to identify novel mechanisms of seizure generation and new immunological markers specific to AE subtypes. We used the in vitro guinea pig brain preparation (de Curtis,2016) to investigate the effect of blood brain barrier impairment consequence, such as PBMC and serum albumin extravasation, on brain inflammation and excitability. We collected plasma samples from healthy volunteers and from AE patients who respond to these inclusion criteria: a) age 18 years; b) clinical onset no more than 9 months before inclusion in the study and at least one seizure (Graus,2021); c) APE2 score 4 (Dubey, 2019). HPBMCs were isolated and cultured in a standard medium. At t0 and at t6, supernatants were analyzed by ELLA® system, for IL10, IL17A, IL1β and IL6. Healthy volunteers (20) and AE patient-derived (7) hPBMCs ($10x10^6$) and their supernatant were perfused intra-arterially in the in vitro brain preparation. LPS (100ng/ml) and human recombinant albumin (4mg/ml) were previously perfused into the arterial stream to respectively favor and mimic a mild BBB impairment (Librizzi,2021). The specific ability of the experimental treatment to induce SLEs and brain inflammation was investigated. Morphological analysis and reconstruction of GFAP- and Iba1-positive glial cells were performed. Only AE patient-derived PBMCs treatment was able to evoke seizure activity and glial cells activation in the isolated brain preparation. ELLA immunoassay revealed an increased level of inflammatory mediators such as IL10, IL1β and IL6 in AE patient- derived PBMC supernatants compared with healthy ones.Preliminary results suggest an impact on brain inflammation and excitability by intrinsically activated, AE patient-derived PBMCs. Our experiments might shed light on identifying key mediators involved in seizure generation in order to identify novel treatments in epilepsies due to inflammatory and dysimmune causes.

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Poster

PSTR391. Microglia-Neuron Interactions Health and Disease

Location: WCC Halls A-C

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Program #/Poster #: PSTR391.01/D17

Topic: B.09. Glial Mechanisms

Support: R35NS132326
R01NS088627

Title: Cortical microglial dynamics promote pain hypersensitivity

Authors: *M.-H. YI[1], D. B. BOSCO[1], P. HANUMAIHGARI[4,1], S. PARUSEL[1], L. WANG[1], J. ZHENG[1], L. EAUCHAI[1,5], L.-J. WU[1,2,3];
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Abstract: Chronic pain following peripheral nerve injury is accompanied by increased neuronal activity in the somatosensory cortex. However, whether and how cortical microglia contribute to these changes are less understood. To this end, we applied the optogenetic strategy to specifically target cortical microglia and their function in pain behavioral sensitization. We found that in vivo optogenetic activation of cortical microglia via red-activated channelrodopsin (ReaChR) triggered pain hypersensitivity and spontaneous pain in mice. Remarkably, S1-targeted microglial optogenetic stimulation increased microglial landscape change and ATP release events. In addition, optogenetic stimulation of cortical microglia up-regulated neuronal c-Fos expression and neuronal Ca^{2+} hyperactivity in the S1. We further found that optogenetic stimulation increased number of microglia derived extracellular vehicles (EVs) with proteomic alterations. The data obtained from the profiling of differentially expressed proteins (DEPs) provides mechanistic evidence how cortical microglia involved in development of chronic pain. Together, our results suggest that cortical microglia are critical in regulating neuronal activity, which is implicated in pain hypersensitivity.

Disclosures: M. Yi: None. **D.B. Bosco:** None. **P. Hanumaihgari:** None. **S. Parusel:** None. **L. Wang:** None. **J. Zheng:** None. **L. Eauchai:** None. **L. Wu:** None.

Poster

PSTR391. Microglia-Neuron Interactions Health and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR391.02/D18

Topic: B.09. Glial Mechanisms

Support: NINDS, R01NS088627 (L.-J.W.)

Title: Chemogenetic approaches reveal dual functions of microglia in epilepsy

Authors: *A. DHEER[1], D. B. BOSCO[1], J. ZHENG[1], L. WANG[1], S. ZHAO[1], K. HARUWAKA[1], M.-H. YI[1], A. BARATH[1], D.-S. TIAN[2], L.-J. WU[1];

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Abstract: Microglia are key players in maintaining brain homeostasis and exhibit phenotypic alterations in response to epileptic stimuli. However, it is still relatively unknown if these alterations are pro- or anti-epileptic. To unravel this dilemma, we employed chemogenetic manipulation of microglia via of the artificial Gi-Dreadd receptor within a kainic acid (KA) induced murine seizure model. Our results indicate that Gi-Dreadd activation can reduce seizure severity. Additionally, we observed increased interaction between microglia and neuronal soma, which correlated with reduced neuronal hyperactivity. Interestingly, prolonged activation of microglial Gi-Dreadds by repeated doses over 3 days, arrested microglia in a less active, homeostatic-like state, which associated with increased neuronal loss after KA induced seizures. RNAseq analysis revealed that prolonged activation of Gi-Dreadd interferes with interferon β signaling and microglia proliferation. Thus, our findings highlight the importance of microglial activation not only during *status epilepticus* (SE) but also within later seizure induced pathology.

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Poster

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Topic: B.09. Glial Mechanisms

Support: NINDS, R01NS088627
NINDS, R35NS132326

Title: Microglial volume regulated anion channel (VRAC) in epilepsy

Authors: *A. S. BARATH[1], A. DHEER[2], D. BOSCO[2], K. HARUWAKA[2], R. SAH[3], L.-J. WU[2];
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Abstract: Microglia modulate neuronal activity during acute seizures as well as phagocytose injured neurons. They are thus attractive targets for anti-epileptic interventions. However, the mechanisms of their anti-epileptic effects are not completely understood. RNA sequencing data from our lab has shown upregulation of the volume regulated anion channel (VRAC) subunits in mice microglia at 3-7 days following seizures. The VRAC channel classically helps cells deal with hypoosmotic stress. However, it has multiple cell-type and context dependent functions. Therefore, we sought to study the role of microglial VRAC in the context of epilepsy. We used an inducible Cre-lox system to selectively delete SWELL1, the obligatory subunit of VRAC in microglia of adult mice. Acute status epilepticus was induced in these mice through an intra-

cerebroventricular injection of kainic acid (a glutamate agonist). Our preliminary results show a reduced CD68 expression in hippocampal microglia 3 days following seizures in SWELL1 cKO mice compared to their littermate controls. *In vitro* investigations revealed impaired phagocytosis of opsonized beads by SWELL1 cKO microglia. We functionally validated SWELL1 cKO *in vitro* by observing impaired microglial volume regulation and reduced taurine release in response to a hypotonic challenge. We continue to investigate the mechanism by which VRAC/ SWELL1 affects phagocytosis and its downstream effect on post seizure memory and learning.

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Poster

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Topic: B.09. Glial Mechanisms

Support: NIH Grant R35NS132326
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Title: Chemogenetic manipulation of CX3CR1^{+} cells transiently induces hypolocomotion independent of microglia

Authors: *S. ZHAO[1], J. ZHENG[1], L. WANG[1], A. D. UMPIERRE[1], S. PARUSEL[1], M. XIE[1], A. DHEER[1], K. AYASOUFI[1], A. J. JOHNSON[1], J. R. RICHARDSON[2], L.-J. WU[1]; [1]mayo clinic, rochester, MN; [2]florida international university, miami, FL

Abstract: Chemogenetic approaches using Designer Receptors Exclusively Activated by Designer Drugs (DREADD, a family of engineered GPCRs) were recently employed in microglia. Here, we used *Cx3cr1*$^{CreER/+}$*:R26*$^{hM4Di/+}$ mice to express Gi-DREADD (hM4Di) on CX3CR1^{+} cells, comprising microglia and some peripheral immune cells, and found that activation of hM4Di on long-lived CX3CR1^{+} cells induced hypolocomotion. Unexpectedly, Gi-DREADD-induced hypolocomotion was preserved when microglia were depleted. Consistently, specific activation of microglial hM4Di cannot induce hypolocomotion in *Tmem119*$^{CreER/+}$*:R26*$^{hM4Di/+}$ mice. Flow cytometric and histological analysis showed hM4Di expression in peripheral immune cells, which may be responsible for the hypolocomotion. Nevertheless, depletion of splenic macrophages, hepatic macrophages, or CD4^{+} T cells did not affect Gi-DREADD-induced hypolocomotion. Our study demonstrates that rigorous data analysis and interpretation are needed when using *Cx3cr1*$^{CreER/+}$ mouse line to manipulate microglia.

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Poster

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Topic: B.09. Glial Mechanisms

Support: NIH Grant R01NS088627
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Title: Microglia enhance neuronal activity through inhibitory shielding

Authors: ***K. HARUWAKA**[1], Y. YING[2], Y. LIANG[1], A. D. UMPIERRE[1], M.-H. YI[1], F. QI[1], V. KREMEN, Jr.[1], S. ZHAO[1], J. ZHENG[1], T. CHEN[1], T. XIE[3], Y. U. LIU[2], H. DONG[4], G. A. WORRELL[1], L.-J. WU[1];
[1]Mayo Clin., Rochester, MN; [2]South China Univ. of Technol., Guangzhou, China; [3]Univ. of Technol. Sydney, Sydney, Australia; [4]Fourth Military Med. Univ., Xi'an, China

Abstract: Microglia are resident immune cells of the central nervous system, constantly monitor the brain environment, and can regulate neuronal circuits through their interactions with neurons. Typical roles for microglia-synapse interactions include synaptic pruning and promoting spine formation, resulting in relatively persistent changes to neuronal circuits. Recently, several studies have demonstrated that microglia dampen neuronal hyperactivity in a P2Y12 receptor-dependent manner during acute brain injury and seizures. Previous studies reported that microglia increase their dynamic process surveillance and interact more closely with neurons depending on norepinephrine reduction during anesthesia. However, the functional significance of microglial process dynamics and neuronal interaction has remained unclear. Our hypothesis is that microglia promote neuronal activity to maintain homeostasis during hypoactive periods. To test this possibility, we investigated the role of microglia in regulating neuronal activity during recovery from general anesthesia. Using in vivo two-photon imaging, we discover that microglia enhance neuronal activity after the cessation of general anesthesia. We found that during anesthesia emergence, the excitatory neurons increased their spontaneous calcium activity, which correlated with enhanced sensory perception and animal locomotion. During anesthesia, microglial processes increased their contact with neuron somas and exhibited bulbous process endings at their contact sites. To study how microglial contacts may promote neuronal activity, we assessed excitatory and inhibitory synapses present on neuronal soma together with microglial processes with immunostaining. Hyperactive neuron somata are directly contacted by microglial processes, which specifically co-localize with GABAergic boutons rather than excitatory synapses. Three-dimensional electron microscopy-based synaptic reconstruction after two-photon imaging reveals that microglial processes enter the synaptic cleft to shield

GABAergic inputs. Two-photon live imaging further revealed that the interaction of microglial processes with GABAergic boutons was temporary and reversible, rather than phagocytosis. Microglial ablation or loss of microglial β2-adrenergic receptors prevents shielding GABAergic inputs and post-anesthesia neuronal hyperactivity. Together, our study demonstrates a previously unappreciated function of microglial process dynamics, which allow microglia to transiently boost neuronal activity by physically shielding axo-somatic inhibitory inputs.

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Poster

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Program #/Poster #: PSTR391.06/D22

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: ONR Grant 24
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Title: Alterations in microglia morphology and expression following in vitro traumatic brain injury

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Abstract: Neuroinflammation following traumatic brain injury (TBI) remains an understudied link between primary injury and neurodegenerative disease, with post-mortem analysis of TBI patients demonstrating a brain-wide immune response up to 18 years after injury. Microglia, the resident immune cells of the brain, are crucial in this process as their prolonged activation is associated with driving secondary injury, cognitive decline, and cell death. Microglia are sensitive to their microenvironment and undergo functional and morphological transitions in response to inflammatory signals, adopting either anti- or pro-inflammatory roles. Most research on microglia activation relies on using lipopolysaccharide (LPS), a bacterial membrane component that targets microglial-specific receptors, but the research investigating the response of microglia to mechanical impacts is lacking. Furthermore, most TBI studies are conducted *in vivo* and, although biologically complex, they preclude reliable application of strain with the accuracy required to determine a correlation between impact and inflammatory response at a single cell level. In this study, we utilize a novel and highly precise *in vitro* model of TBI to evaluate alterations in microglia morphology and cytokine expression in response to controlled mechanical stress. Microglia are isolated from primary glial cultures derived from P0/P1 Sprague Dawley rats and seeded onto a customized dogbone-shaped polydimethylsiloxane (PDMS)

substrate treated with poly-d-lysine (0.1 mg/mL) and laminin (0.4 mg/mL). The geometric design and substrate properties enable the use of high strain rates without disrupting the gripping points and allow the gauge area hosting the cells to experience a realistic and uniform strain field. The dogbone is adhered between two grippers that are contacted by a custom-built tension device that applies programmed uniaxial stress. Strain rates between 10 s^{-1} to 50 s^{-1} are evaluated at 30 % strain. Live-cell fluorescent imaging is utilized before, during, and after the strain is applied and is analyzed in conjunction with images of fixed samples stained for Iba-1 to examine changes in microglial morphology. Additionally, an ELISA panel measures changes in relevant pro- and anti-inflammatory cytokines. Our approach enables us to investigate the effect of precise and reproducible mechanical strain on microglia and better understand their response to injury magnitudes experienced during TBI. This work offers valuable insights into the mechanosensitive response of microglia and could improve our understanding of TBI-related neuroinflammation.

Disclosures: **E. Blick:** None. **C. Franck:** None. **A. Hai:** None.

Poster

PSTR391. Microglia-Neuron Interactions Health and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR391.07/D23

Topic: B.09. Glial Mechanisms

Title: Microglial NF-kB activation contributes to neuronal network and behavioral alterations

Authors: *P. HONMA[1,3], C. CASTAGNOLA[4], Y. HUANG[5], C. WANG[3], L. FAN[4], M. ZHAO[4], L. GAN[4], J. J. PALOP[3,2];
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Abstract: Alzheimer's Disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline, brain network alterations, and pathological changes, including extracellular amyloid-beta (Aβ) deposits and gliosis. AD-related network alterations include deficits in network hyperexcitability and gamma oscillatory (30-90 Hz) activity, which are canonically linked to alterations in interneuron activity. However, the contribution of non-neuronal cells to these disease-related network alterations is still unknown. Microglia have emerged as a potential mediator of neuronal network dynamics, as they respond to neuronal activity via activation of inflammatory pathways such as NF-κB. Previously, it has been shown that constitutive activation of microglial NF-κB contributes to tau spreading and toxicity in a mouse model of tauopathy. We built upon these findings to investigate the impact of microglial NF-κB activation on other cell types and behavior. To do this, we used mice with cre-dependent, constitutive activation of *ikbkb*—the upstream regulator of NF-κB—in microglia (*Cx3cr1*$^{CreERT2/+}$*;ikbkbCA*$^{F/F}$). To assess the functional impact of this manipulation, we performed *in vivo* LFP/single-unit recordings and

machine learning behavioral phenotyping (DeepLabCut and VAME) in mice with or without microglial NF-κB activation. Using these methods, we discovered state-dependent alterations in neuronal synchrony and behavioral deficits. Collectively, these findings elucidate a novel mechanism by which aberrant microglia NF-κB signaling contributes to AD-related network dysfunction and behavioral alterations.

Disclosures: **P. Honma:** None. **C. Castagnola:** None. **Y. Huang:** None. **C. Wang:** None. **L. Fan:** None. **M. Zhao:** None. **L. Gan:** None. **J.J. Palop:** None.

Poster

PSTR391. Microglia-Neuron Interactions Health and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR391.08/D24

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Supported by NIH grant R01AG074968 (B.H.S).
Autopsy materials used in this study were obtained from the University of Washington Neuropathology Core, which is supported by the Alzheimer's Disease Research Center (AG05136) and the Adult Changes in Thought Study (AG006781).
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This research was supported in part through computational resources and services provided by Advanced Research Computing (ARC), a division of Information and Technology Services (ITS) at the University of Michigan, Ann Arbor.
Library prep and next-generation sequencing was carried out in the Advanced Genomics Core at the University of Michigan.

Title: Sepsis increases complement pathway expression in the mouse and human brain and is associated with increased microglial synaptic pruning in amyloid-burdened mouse brains

Authors: *K. GIFFIN[1], A. C. BUSTAMANTE[2], P. K. CRANE[4], B. H. SINGER[3];
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Abstract: Sepsis is associated with both acute and long-term brain dysfunction, including cognitive deficits and mental health disorders, especially in the elderly. Multiple mechanisms drive sepsis-induced brain dysfunction and are not fully characterized. We previously used bulk RNA-sequencing (RNA-seq) to explore transcriptomic changes acutely caused by sepsis in the human brain. RNA-seq was completed on 12 sex-matched postmortem parietal cortex samples

from older patients from the Adult Changes in Thought (ACT) brain bank (Bustamante et al. 2020, DOI:10.1164/rccm.201909-1713LE). In bulk RNA-seq sepsis was correlated with several gene modules, including ones involving inflammatory response ($\rho = 0.51$, P = 0.01) and synaptic function ($\rho = 0.41$, P = 0.05). Here, we present single-nucleus RNA-seq, performed on a subset of 8 sex-matched brain samples used in the bulk RNA-seq study, to identify cell types driving the immune pathway changes. Cytokine and damage-associated molecular pattern signaling (e.g., *TNFRSF1A*, *TLR4*) and complement system (e.g., *C1QB, C1QC, C5AR1*) genes were differentially expressed by sepsis in astrocytes and microglia.
The complement system is a mediator for both neuroinflammation and synaptic pruning, as microglia phagocytose synapses tagged with complement proteins. To further investigate complement and microglia activity in the brain after sepsis, we turned to a mouse model of abdominal sepsis, cecal ligation and puncture (CLP). We investigated complement expression via whole-brain bulk RNA-seq and microglia-mediated synaptic phagocytosis as measured by proportion of microglia ($CD11b^{+}/CD45^{mid}$) positive for synaptic protein (SNAP25) by flow cytometry. To investigate the potential synergistic interaction of sepsis and amyloid pathology, we assessed complement expression and synaptic phagocytosis in sex-matched wild-type and 5xFAD mice. *C1QA-C* gene expression was elevated in the brain after sepsis acutely and long term (5- and 25-days post CLP). We did not find changes in microglial synaptic phagocytosis in wild-type mice. However, 5xFAD sepsis survivor mice had increased levels of synaptic phagocytosis relative to 5xFAD controls acutely (5-days post CLP) that persisted 6 weeks after sepsis. We were underpowered to assess sex differences.
We found that sepsis increases the expression of complement system genes in the brains of both humans and mice. Furthermore, microglial phagocytosis of synapses was increased in 5xFAD sepsis survivors. In vulnerable patients with underlying amyloid pathology, complement-driven, microglial-mediated synaptic pruning may be a mechanism driving brain dysfunction after sepsis.

Disclosures: K. Giffin: None. **A.C. Bustamante:** None. **P.K. Crane:** None. **B.H. Singer:** None.

Poster

PSTR391. Microglia-Neuron Interactions Health and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR391.09/D25

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: This research was funded in part by Aligning Science Across Parkinson's [Grant ASAP-000525] through the Michael J. Fox Foundation for Parkinson's Research (MJFF)

Title: Parkin loss of function leads to increased LPS-induced microglial activation: evaluation of the impact on dopamine neurons

Authors: *I. NEDJAR;
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Abstract: Parkin loss of function leads to increased LPS-induced microglial activation: evaluation of the impact on dopamine neuronsAuthors *I. NEDJAR[1] , S. MUKHERJEE[1] , M. J. BOURQUE[1] , N. Giguere[1] , A. TCHUNG[1] , A. Fahmi[1] , M. DESJARDINS[1] , L. E. TRUDEAU[1] ; [1]Université de Montréal, Dept. Pharmacology and physiology, Dept. of neurosciences, Montreal, Quebec, Canada **DisclosuresI. Nedjar:** None. **S. Mukherjee:** None. **M. J. Bourque:** None. **N. Giguere**: None. **A. Tchung:** None. **A. Fahmi**: None. **M. Desjardins:** None. **L. E. Trudeau:** None.
Abstract It is likely that multiple mechanisms interact to cause the dysfunction and loss of substantia nigra and other vulnerable neurons in Parkinson's disease (PD). Growing evidence supports the hypothesis that activation of the immune system contributes to PD pathogenesis. In the brain, microglia are the main local actors of immune responses. These cells constantly probe their local microenvironment and interact with neurons through secreted factors. In PD postmortem tissue and in animal models of PD, evidence for microglial activation has been reported. Due to growing evidence suggesting the implication of Parkin in innate and adaptive immunity, we hypothesize that loss of function of this gene product could lead to increased activation of microglia following infections, with subsequent detrimental effects on dopamine neurons. Studying primary microglia obtained from neonatal Parkin KO mice, we observed that, as expected, these cells drastically change their morphology following exposure to the bacterial endotoxin LPS, switching from a polarized to an amoeboid shape, and show. increased expression of pro-inflammatory genes, such as MHC1, MHC2, iNOS and COX-2 . Interestingly, we observed that Parkin KO microglia show increased secretion of IL-6 and other cytokines and chemokines in response to LPS. They also show increased survival under culture conditions when compared to WT cells. Short-term co-culture of activated Parkin KO microglia with DA neurons did not induce loss of DA neurons. Functional studies are now required to examine their functionality. These initial results support the hypothesis of increased innate immune responses in the brain under conditions of Parkin loss of function in PD.

Disclosures: I. Nedjar: None.

Poster

PSTR391. Microglia-Neuron Interactions Health and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR391.10/D26

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Trem2 deficiency in microglia ameliorated neuroinflammation and photoreceptor loss at middle- and later-stages of retinitis pigmentosa disease

Authors: *R. LI[1,2], J. M. WU[2], J. Q. FAN[1], J. ZHANG[1], B. LIN[1,2];
[1]Sch. of Optometry, The Hong Kong Polytechnic Univ., Hong Kong, Hong Kong; [2]Ctr. for Eye and Vision Res., Hong Kong, Hong Kong

Abstract: Purpose: Neuroinflammation plays a critical role in various neurodegenerative diseases. Similarly, increasing evidence indicates the involvement of microglia-derived neuroinflammation in the pathogenesis of retinitis pigmentosa (RP). Triggering receptor expressed on myeloid cells 2 (TREM2) is specifically expressed by microglia in the brain. Previous studies have reported that loss-of-function mutations of TREM2 compromise microglial phagocytosis and result in chronic neurodegenerative diseases. However, the functional role of TREM2 in RP remains unclear. Here, we investigated the role of TREM2 in mediating neuroinflammation and photoreceptor degeneration in a mouse model of RP. **Methods:** To investigate the role of TREM2 in regulating neuroinflammation in RP, we crossed $TREM2^{-/-}$ mice with rd10 mice, a mouse model of autosomal recessive RP, to generate $TREM2^{-/-}$/rd10 mice. We evaluated temporal expression of TREM2, microglia activation and photoreceptor degeneration in the retina of $TREM2^{-/-}$/rd10 mice using immunohistochemistry, qPCR, western blot, flow cytometry and electroretinography (ERG) approaches. Moreover, we performed single-cell RNA sequencing to explore transcriptional changes in TREM2-deficient microglia in rd10 mice. **Results:** We found that TREM2 was significantly upregulated in the retina of rd10 mice after postnatal day 22 (P22), the middle-stage of the disease. Meanwhile, we found that most of TREM2- positive microglia were localized at the outer nuclear layer (ONL) and retinal pigment epithelium (RPE) layer of the retina. Moreover, we observed that TREM2 deficiency significantly reduced microglia activation and the release of proinflammatory cytokines, while preserved photoreceptor cells in rd10 retinas. In addition, we found that TREM2 deficiency accelerated cell apoptosis of activated microglia at p25 of rd10 retinas by flow cytometry analysis. Furthermore, we performed single-cell RNA sequencing of microglial cells and found that TREM2 deficiency significantly induced transcriptional alteration in microglia in rd10 retinas. Bioinformatics analysis revealed that one subtype of disease-associated microglia (DAM) expressed most of differential expressed genes (DEGs), in which inflammation-related DEGs were downregulated and apoptosis-associated DEGs were upregulated. **Conclusion:** We demonstrated that TREM2 participated in the pathogenesis of RP. TREM2 regulated neuroinflammation and photoreceptor loss at middle- and later-stages of RP. Targeting TREM2 signal could be a new therapeutic strategy for RP.

Disclosures: R. Li: None. **J.M. Wu:** None. **J.Q. Fan:** None. **J. Zhang:** None. **B. Lin:** None.

Poster

PSTR391. Microglia-Neuron Interactions Health and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR391.11/D27

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01AA025591
NIH Grant F32AA029928

Title: The impact of pharmacological depletion of microglia on alcohol-induced corticolimbic neurodegeneration in male rats

Authors: J. K. MELBOURNE, ***E. R. CARLSON**, K. NIXON;
Pharmacol. and Toxicology, The Univ. of Texas at Austin, Austin, TX

Abstract: Corticolimbic damage is a consequence of heavy drinking in individuals with an alcohol use disorder (AUD). Reactivity of microglia, the brain parenchymal macrophages, has been suggested as a mechanism underlying this damage, but causality has not been established. The aim of this study is to determine the role of microglia in alcohol-induced neurodegeneration in a rat model of alcohol dependence. We utilized the CSF1R inhibitor PLX-5622, an established tool for microglial depletion in mice, but infrequently used in rats. Adult male Sprague-Dawley rats were treated with PLX-5622 or vehicle (50mg/kg; MedChemExpress), by chow plus intraperitoneal injection or intragastric gavage (i.p., i.g. every 12 hours), for 7 days. For depletion plus alcohol exposure, rats received i.p. PLX-5622 or vehicle every 12 hours for 11 days, plus 4-day binge alcohol (25% (w/v) ethanol, i.g.) or isocaloric control diet every 8 hours in the final 4 days. Importantly, PLX-5622 did not significantly impact blood ethanol concentrations, which were 389.2 ± 23.98 mg/dl in the ethanol plus vehicle (Veh-EtOH) and 331.3 ± 42.49 mg/dl in the ethanol plus PLX-5622 (PLX-EtOH) groups. Rats were saline perfused, then brains were post-fixed in PFA and sectioned at 40 µm. Depletion was confirmed with immunohistochemistry for the microglial marker IBA1. Images were acquired in the hippocampus and peri/entorhinal cortex and counts performed to estimate % depletion. Histology and profile counts for FluoroJade B (FJB), a marker of dying neurons, were carried out in the hippocampus, peri/entorhinal and piriform cortices. Data were log transformed due to unequal group variance and analyzed by one-way ANOVA with Tukey's *post hoc*. 7 days of PLX-5622 i.p. plus chow resulted in ~90-94% microglial depletion, whereas there was no apparent depletion with PLX-5622 i.g. plus chow (n=2/group). In the alcohol experiment, 11 days of i.p. PLX depleted microglia ~97-98% (n=2/group). FJB+ cell counts were significantly increased for all rats that received ethanol diet (PLX-EtOH n=4; Veh-EtOH n=5) versus control diet (PLX-CON n=4; Veh-CON n=4) across all regions examined ($p<0.05$). In the peri/entorhinal cortex there were fewer FJB+ cells in the PLX-EtOH compared to Veh-EtOH group ($p<0.05$). While not statistically significant, fewer FJB+ cells were also noted in the other regions of PLX-EtOH rats. These data suggest that microglia may indeed contribute to ethanol-induced neurodegeneration. However, as neuronal death was observed in ethanol exposed rats depleted of >97% of their microglia, microglia-mediated mechanisms are unlikely to be the sole cause of corticolimbic damage in AUD.

Disclosures: J.K. Melbourne: None. **E.R. Carlson:** None. **K. Nixon:** None.

Poster

PSTR391. Microglia-Neuron Interactions Health and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR391.12/D28

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: VA Merit Award

Title: Microglial attenuate excitotoxicity in vitro by altering neuronal NMDA receptor subunit GluN2B expression independent of neuroprotection by an EP1 prostaglandin receptor antagonist

Authors: *N. G. CARLSON[1,3], L. SCHMIDT[2], B. WOOD[2], M. ROJAS[2], J. W. ROSE[4]; [1]Neurobio., [2]Neurovirology Lab., Univ. Utah, Salt Lake Cty, UT; [3]Grecc, VA SLCHCS, Salt Lake City, UT; [4]Neurol., VASLCHCS/University of Utah, Salt Lake Cty, UT

Abstract: Background: Microglia modulation of neuronal function is important in the pathogenesis of neurodegenerative diseases. Our previous study revealed that microglial cells co-cultured on transwells above neuronal cultures made neurons more resistant to NMDA-mediated excitotoxicity. Also, Neuroprotection mediated by an EP1 receptor antagonist was lost when neurons were co-cultured with microglia. Here we examine whether neuronal EP1 played a role in microglial-mediated neuroprotection and what changes in neuronal gene expression are induced by co-culturing neurons with microglia.
Methods: Cortical neurons were from E15 mouse embryos were cultured under conditions to produce nearly pure neuronal cultures. Microglia cultures were prepared from P1 post-natal mice and plated on transwells. After 1 week in culture, microglial transwells were placed above pure neuronal cultures for 48 hours. Neuronal viability was assessed following NMDA exposure to neuronal cultures in the presence or absence of microglial. In other experiments, neurons were transfected with EP1 shRNA to knock down expression of EP1. RNA was isolated from pure neurons grown with and without microglia and examined by transcriptome analyses for microglial induced changes in gene expression. Expression of GluN2B transcripts and protein was examined by real-time PCR and western blot respectively.
Results: To test whether the neuroprotective effect of microglia was mediated by altering the EP1 response in neurons, we knocked down neuronal EP1 expression by transfecting EP1 Sh RNA constructs into neurons and examined NMDA mediated excitotoxicity in the presence and absence of microglial trans-wells. Both control and EP1 knockdown neuronal cultures exhibited robust neuroprotection when co-cultured with microglial. As a positive control, protection by an EP1 antagonist in pure neuronal cultures was eliminated following Sh RNA mediated inhibition of EP1 expression. These results indicate that microglial-mediated neuroprotection occurs independent of neuronal EP1. To evaluate if microglia elicit neuroprotective effects through changing gene-expression in neurons, transcriptome analyses were conducted on neurons cultured in the presence and absence of microglia. The only difference in gene expression was GluN2B. A 1.5 fold decrease in expression of GluN2B was seen with RT-PCR and 2 fold change in expression by western blot analysis.
Conclusions: Our results showed that microglia can decrease neuronal expression of GluN2B. This in vitro system can facilitate future studies to examine microglial interactions that could play an important role in normal physiology and disease.

Disclosures: N.G. Carlson: None. **L. Schmidt:** None. **B. Wood:** None. **M. Rojas:** None. **J.W. Rose:** None.

Poster

PSTR391. Microglia-Neuron Interactions Health and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR391.13/D29

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Canadian Institutes of Health Research (PJT-162387 to S.L.)
Wings for Life Spinal Cord Research Foundation (WFL-CA-13/20 to S.L.)

Title: Understanding the contribution of microglia-derived IGF-1 to CNS injury and demyelination.

Authors: *J. FERRY[1], *J. FERRY[2], A. CASTELLANOS-MOLINA[1], N. VALLIÈRES[1], D. GOSSELIN[1], S. LACROIX[1];
[1]Univ. Laval, Québec, QC, Canada; [2]Dept. of Mol. Med. of the Fac. of Med., Neurosciences Axis of CHUL, Québec, QC, Canada

Abstract: Microglia are the immune resident cells of the central nervous system (CNS). They are known to be involved in phagocytosis of cellular and myelin debris, remyelination, and formation of a neuroprotective scar in the context of injury and disease. Insulin-like growth factor 1 (IGF-1) is known to activate various signaling pathways regulating cell proliferation, differentiation, migration, and survival. Since mechanisms by which microglia can repair CNS tissue remain unclear, we studied the effects of microglia-derived IGF-1 in different contexts such as spinal cord injury (SCI) and cuprizone-mediated brain demyelination. We hypothesize that IGF-1 is a key mediator for microglia to adopt a regenerative phenotype, thus contributing to CNS protection and repair. To test this hypothesis, we produced transgenic mice whose microglia are conditionally invalidated in IGF-1 after tamoxifen treatment, namely *Cx3cr1*^{CreER}::*Igf1*$^{fl/fl}$ mice. RNA-sequencing experiments revealed that genes (adj.p-value <0.05) and gene set supporting fibrotic scar formation are upregulated in conditional knockout mice at 7 days post-SCI, which corresponds to the peak of *Igf1* mRNA expression in wild-type (WT) littermates (GeneSet Enrichment Analysis with p-value <0.0001). Immunofluorescence data further showed that deletion of the *Igf1* gene in microglia slightly reduced the area occupied by Iba1^{+} signal outside of the primary lesion and was associated with changes in lesion morphology. In the cuprizone model, where microglia rather than peripheral macrophages are the predominant responders and key effectors of demyelination and myelin debris clearance, RT-qPCR analysis performed on dissected corpus callosum tissue samples revealed that *Igf1* mRNA levels are significantly decreased in *Cx3cr1*^{CreER}::*Igf1*$^{fl/fl}$ mice compared to WT littermates after tamoxifen and cuprizone treatment (p-value <0.005, n=3-4/group). Notably, reduced myelin staining was observed in the forebrain of *Cx3cr1*^{CreER}::*Igf1*$^{fl/fl}$ mice compared to WT mice at the peak of demyelination, i.e. after 5 weeks post-cuprizone feeding (p-value <0.05, n=5-7/group). In sum, our data reveal that microglia-derived IGF-1 could regulate formation of the glial and fibrotic

scars in SCI, while it may play a role in the activation/proliferation of microglia and clearance of myelin debris in areas of CNS demyelination.

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Poster

PSTR391. Microglia-Neuron Interactions Health and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR391.14/D30

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Sleep fragmentation alters microglial function leading to lethal neuroinflammation during sepsis-like inflammation

Authors: *A. WANI[1,2], A. RICKMAN[2], P. SHARMA[1], B. HECKMANN[2], D. R. GREEN[1]; [1]St. Jude Children's Res. Hosp., Memphis, TN; [2]USF Hlth. Byrd Alzheimer's Ctr. and Neurosci. Institute, Dept. of Mol. Medicine, Morsani Col. of Medicine, Tampa, FL, USA., Floida, FL

Abstract: It is well established that infection can promote slow wave sleep, but the functional importance of this phenomenon remains unclear. In this study, we utilized sleep fragmentation, i.e., intermittent disruption of sleep throughout the day, to explore the relationships between sleep and inflammatory disease. We found that sub-lethal injection of a bacterial lipopolysaccharide (LPS), prior to sleep fragmentation (SF) invariably resulted in dramatically increased mortality compared to LPS only or SF only controls, highlighting the importance of sleep following an inflammatory challenge. Further investigation revealed hyperactivation of microglia in the hypothalamus and other brain areas in mice subjected to LPS plus sleep fragmentation. As Toll-like receptor-4 (TLR4) is crucial for responses to LPS, we examined animals with microglia-specific deletion of TLR4 and found that it completely protected mice from the combination treatment. To further explore the role of sleep, we generated Dec2 (BHLHE41) mutant mice that display decreased sleep and found that these animals are protected from low dose LPS plus SF. We are currently extending our results to bacterial infection.

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Poster

PSTR391. Microglia-Neuron Interactions Health and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR391.15/D31

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01NS112308

Title: Microglial removal of dying cells after status epilepticus

Authors: *P. HEMAN BOZADAS, N. H. VARVEL, R. J. DINGLEDINE;
Pharmacol. and Chem. Biol., Emory Univ., Atlanta, GA

Abstract: Microglia are the resident immune cells of the Central Nervous System. In addition to responding to injuries and aiding in recovery, microglia also promote a healthy environment through a process known as efferocytosis, which is the clearance of apoptotic cells. If efferocytosis is not efficient, the cell membrane of an apoptotic cell can lose integrity, spilling intracellular content and leading to a secondary necrosis of the surrounding tissue. One important role of efferocytosis is to prevent inflammation by quickly removing apoptotic cells. Neurodegenerative diseases and stroke can result in inefficient clearance of apoptotic cells if the capacity of microglia is overloaded. However, in Status Epilepticus (SE) the role of efferocytosis in recovery has not been explored. The goal of this study is to identify the cells undergoing efferocytosis after SE. Male C57BL/6 mice experienced pilocarpine-induced SE that was interrupted by diazepam after 1 hr. Four days later, 35 micron frozen sections were prepared through hippocampus and immunohistochemistry was performed to identify specific cell types (NeuN for neurons; Iba1 for reactive microglia) in conjunction with TUNEL labeling to identify nuclei of dying cells. Z-stack confocal images were obtained from the hippocampus using an A1R HD Nikon Confocal Microscope and the images were processed using Imaris V10.0 software. NeuN and TUNEL positive cells were counted and the cell volume overlapped (i.e., engulfed) by microglia was analyzed. In the CA3 region a histogram of Tunel volume was bimodal, with peak means of 6.4 ± 7.0 and 112 ± 43 μm^3, suggesting two populations of Tunel+ objects: smaller debris and larger actively apoptosing cells. Of all TUNEL-positive cells 33% were neurons. For Tunel+ cells, 77% of neurons and 56% of non-neuronal cells were larger than 25 μm^3. Microglia had engulfed 35% (median) of neuron volume but only 1.6% of non-neuronal volume ($p=.0029$, Mann-Whitney test). By contrast, the more numerous neurons *not* undergoing apoptosis, lacking the Tunel stain, experienced only 1.06% (median) of engulfment volume. These data suggest that efferocytosis by microglia in the CA3 region is mainly restricted to neurons undergoing apoptosis, and the small neuron fragments that are Tunel negative are being cleared possibly because they already have passed through necrotic process. These data are also consistent with the notion that non-neuronal cells undergoing apoptosis might eventually be redirected to a necrotic pathway, increasing neuroinflammation.

Disclosures: P. Heman Bozadas: None. **N.H. Varvel:** None. **R.J. Dingledine:** None.

Poster

PSTR391. Microglia-Neuron Interactions Health and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR391.16/D32

Topic: D.02. Somatosensation – Pain

Support: NIH HEAL Grant RF1NS113840

Title: Microglia reactivity in sensory and affective brain regions after peripheral nerve injury

Authors: *R. A. CAZUZA[1], J. C. MORPHETT[2], M. LAOUMTZIS[2], M. J. LACAGNINA[1], A. R. GRIECO[1], S. M. ZAGRAI[1], M. HUTCHINSON[2], P. M. GRACE[1];
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Abstract: Microglia reactivity in sensory and affective brain regions after peripheral nerve injury Rafael A. Cazuza[1], Jane C. Morphett[2], Michail Laoumtzis[2], Michael J. Lacagnina[1], AnamariaGrieco[1], Sever M. Zagrai[1], Mark Hutchinson[2], Peter M. Grace[11] Laboratories of Neuroimmunology, Department of Symptom Research, University of Texas MDAnderson Cancer Center, Houston, TX 77030, USA.[2] Australian Research Council Centre of Excellence for Nanoscale BioPhotonics and AdelaideMedical School, University of Adelaide, Adelaide, South Australia 5005, Australia.Microglia are resident macrophages in the central nervous system, responsible for cellsurveillance, phagocytosis, and release of soluble factors. These cells can change theirmorphology from a ramified to the most unramified amoeboid phenotype once reactive. Inaddition, microglia are very heterogeneous and can display distinct phenotypes depending ontheir location in the central system. In the context of nerve injury, microglia become reactive andalter their morphology in the spinal cord, releasing inflammatory mediators which maintainneuropathic pain. However, the sensory inputs that are carried through the spinal cord constituteonly one dimension of pain. Pain is a multidimensional experience that is encoded by verycomplex brain circuitry. Thus far, microglial reactivity has been incompletely mapped in thebrain after a nerve injury.In this work we explored microglia reactivity across time in brain regions that are associatedwith pain processing, using both males and females rats. Chronic constriction injury (CCI) orsham surgery of the sciatic nerve was performed in rats (N=6/sex/group) and the whole brainwas collected at 7 or 28 days after the surgery. The entire brain was coronally cryosectioned at30µm and stained for CD11b using immunohistochemistry. An advanced software for automatedanalysis (HALO – Indica Labs) was used to assess microglia morphological changes after thenerve injury. The morphological assessment was compared to a densitometry method.The densitometry analysis showed that affective brain regions display sustained microgliareactivity while areas responsible for sensory processing have a transient response. The HALOdetection of reactive microglia is still under analysis and it could provide further informationhidden in the densitometry assessment. These results suggest a possible role of microglia in long-lasting disruptions in affective states in neuropathic pain.

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Poster

PSTR391. Microglia-Neuron Interactions Health and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR391.17/D33

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NMSS FAN-2106-37832
DOD/USAMRAA W81XWH2210819

Title: Mif nuclease contributes to neuronal death in a mouse model of neuroinflammation

Authors: *S. GADANI, J. MACE, D. GALLEGALIOUS, M. SMITH, T. DAWSON, V. DAWSON, P. CALABRESI;
Johns Hopkins Hosp., Baltimore, MD

Abstract: Neuronal loss in the central nervous system is an irreversible outcome of many neuroinflammatory illnesses, and while several cellular and humoral factors have been implicated, the final mechanism(s) of cell death remain unclear. Parthanatos is a novel form of programmed cell death that is triggered when excessive DNA damage, induced by various stimuli including ROS, leads to overactivation of the PARP1 enzyme. PARP1 catalyzes the addition of poly-ADP-ribose (PAR) to numerous proteins including macrophage migration inhibitory factor (MIF), which then traffics to the nucleus and cleaves DNA through endogenous nuclease activity. Parthanatos was recently found to mediate α-synuclein related neurodegeneration in Parkinson's disease, but it had not been studied in the context of inflammation. Here, we present evidence for an important role of Parthanatos in the mouse model of neuroinflammation, experimental autoimmune encephalomyelitis (EAE). We find high baseline expression of MIF in neurons and accumulation of PAR within the spinal cord after EAE corresponding with neuronal cell death. We also found preservation of spinal cord neurons and retinal ganglion cells in mice where MIF nuclease activity is inhibited through a point mutation of its catalytic domain (MIF-E22Q). MIF-E22Q mice develop less severe neurologic impairment in the chronic stage of EAE compared to wild-type mice, though importantly they have similar peak EAE severity and immune infiltration, supporting a specific role for this pathway in neuroprotection. Treatment with C831, a novel small molecule inhibitor of MIF-nuclease, also leads to increased neuronal survival in the retina and spinal cord after EAE. Future studies will be aimed at assessing markers of Parthanatos in human multiple sclerosis samples, cell type-specific alteration of the Parthanatos pathway, and defining optimal C831 treatment paradigms. This work is the first to explore Parthanatos as a mediator of inflammatory neuronal cell death, and it contributes to a growing understanding of the role of Parthanatos in neurodegeneration and neuroinflammation.

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Poster

PSTR391. Microglia-Neuron Interactions Health and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR391.18/D34

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: DOD/USAMRAA W81XWH2210819

Title: Inhibition of multifunctional MIF protein prevents inflammatory- and parthanatic-mediated neurodegeneration in the context of multiple sclerosis

Authors: *J. W. MACE[1], S. P. GADANI[2], D. GALLEGUILLOS[2], M. D. SMITH[2], B. KANG[3], T. GARTON[1], M. GHARAGOZLOO[2], V. L. DAWSON[3], T. M. DAWSON[3], P. A. CALABRESI[2];
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Abstract: Nearly three million people worldwide are living with multiple sclerosis (MS), an autoimmune condition characterized by peripheral immune cell infiltration into the central nervous system (CNS) and reactive gliosis, demyelination, and neuroaxonal degeneration. Existing therapies target adaptive immune cells and treat relapsing MS, but are not effective in halting neurodegeneration in progressive MS. Numerous studies have identified prominent dysregulation of macrophage migration inhibitory factor (MIF), a multifunctional protein with cytokine and enzyme activity, in MS. MIF has two discrete enzymatic functions, as a tautomerase and a nuclease, that have not been well studied in MS. Our lab has shown that MIF nuclease acts as the final executioner of the caspase-independent cell death pathway, parthanatos, that is triggered upon DNA damage and contributes to neuronal loss in Parkinson's disease and stroke. Because neuroinflammation and parthanatos-inducing conditions, such as high ROS concentrations, are pathologically prevalent across many neurological diseases including MS, we hypothesized that MIF contributes to neurodegeneration in MS. Our lab used a commercially available mouse line (P2G) with its MIF tautomerase activity selectively ablated and created a mouse line (E22Q) with its MIF nuclease activity selectively ablated. We have also synthesized a compound (PAANIB1) that specifically inhibits MIF nuclease. These tools were used to assess the therapeutic efficacy of blocking MIF activity in an experimental autoimmune encephalomyelitis (EAE) mouse model of MS. Our data showed that P2G-EAE and E22Q-EAE mice had a decreased clinical disease severity over time. P2G-EAE mice had less peripheral immune cell infiltration into the CNS, whereas E22Q-EAE mice did not undergo a change in peripheral immune cell infiltration into the CNS. We further determined that E22Q-EAE mice, P2G-EAE mice, and PAANIB1-treated EAE mice were protected against retinal ganglion cell death. Lumbar spinal cord neuroprotection was also evident in E22Q-EAE mice and PAANIB-treated EAE mice. Both enzymatic activities of MIF appear to act in concert in EAE, as MIF global knockout EAE mice had the most protection from clinical disease severity and neurodegeneration, while E22Q-EAE and P2G-EAE mice were only partially protected. Altogether, if neurons degenerate by MIF-mediated inflammatory or parthanatic processes in the context of MS as indicated by our data, this research could establish a pharmacological target to treat the ongoing loss of grey matter in patients with this disease.

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Poster

PSTR392. Disease Mechanisms and Therapeutics for Glioblastoma

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR392.01/D35

Topic: B.11. Neuro-Oncology

Title: Feasibility of prolyl oligopeptidase (PREP) as tumor biomarker in glioblastoma patients: a pilot study

Authors: *E. ESPOSITO[1], G. CASILI[1], M. CAFFO[2], S. SCUDERI[1], R. BASILOTTA[1], M. LANZA[1], A. FILIPPONE[1], I. PATERNITI[1], M. CAMPOLO[1], S. CUZZOCREA[1];
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Abstract: Prolyl oligopeptidase (PREP) is a serine protease that hydrolyzes biologically active oligopeptides on their carboxyl side. PREP has been connected with multiple physiological and pathological conditions. Increased PREP activities have been found in tumors, although its involvement in the modulation of cancer mechanism is still unclear. Thus, the identification of PREP and clarification of its clinical significance are important for improving the diagnosis and treatment of brain tumors. The purpose of this study was to analyze the expression of PREP and its diagnostic and prognostic value in patients with glioblastoma (GBM). In this study, GBM patients and controls were enrolled and clinical data and PREP expression, from serum and biopsies, were analyzed. Moreover, the relationships between PREP, Protein phosphatase 2A (PP2A), signal transducer and activator of transcription (STAT3) and Transforming growth factor-β (TGF- β) were also examined. Our study demonstrated that PREP was significantly upregulated in both serum and in biopsy from GBM patients compared with controls. This upregulation was negatively correlated with survival, but significantly correlated with age and Mindbomb Homolog-1 Index (MIB1). Also, PREP expression was inversely correlated with tumor suppressor PP2A in biopsy from GBM patients, while PREP protein levels resulted to be positively correlated with STAT3 and TGF-β protein expression. The detection of PREP in serum and biopsies have the potential to serve as diagnostic and prognostic biomarker in GBM patient. The findings may provide diagnostic and therapeutic target for GBM.

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Poster

PSTR392. Disease Mechanisms and Therapeutics for Glioblastoma

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR392.02/D37

Topic: B.11. Neuro-Oncology

Support: NIH NS114221
VA RX003865

Title: Reprogramming of Astroglioma Cells for Redirecting the Tumor Cell Fate as a Potential Anti-Cancer Gene Therapy

Authors: *T. ESTABA[1], M. Q. JIANG[1,3], S. DHARANENDRA[1], X. GU[1,3], A. WU[1], K. BERGLUND[2,3], S. YU[1,3], L. WEI[1];
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Abstract: Gliomas are primary central nervous system tumors derived from glial cells. Glioblastoma (a type of glioma derived from astrocytes) is considered a grade 4 astrocytoma and is the most common type of adult brain malignancy that can be very aggressive with a very poor prognosis. Because of their highly vascularized nature, along with their predilection for widespread invasions of brain tissues, glioblastomas present a uniquely life-limiting illness. Direct reprogramming or direct conversion of glial cells to non-glial cells such as neurons has provided a genetic tool to manipulate cell fate as a potential therapy for neurological diseases. NeuroD1 (ND1) is a master transcription factor for neurogenesis with critical roles in regulating neuronal differentiation and maturation during nervous system development. In the present study, we tested the hypothesis that overexpression of the master transcription factor ND1 can redirect the fate of malignant glioma cells to postmitotic neuronal cells for slowing down or ceasing glioblastoma tumor progression. Our in vitro experiments used cell cultures of human glioblastoma cell lines LN229 and U87 as temozolomide (TMZ)-sensitive cells and T98G as TMZ-insensitive cells. ND1-initiated cell reprogramming was induced by adding an AAV carrying ND1 and mCherry. Three to 14 days after reprogramming, ND1-treated cells showed evidence of successful reprogramming and neuronal lineage conversion with significantly increased MAP2, TUJ1, and BDNF expression. TUNEL staining identified increased apoptotic cells co-labeled with ND1 overexpression in U87 and T98G cultures. Western blot analysis confirmed restoration of anti-tumor and cell cycle regulatory protein p53 in reprogrammed cells. Consistently, cultures with overexpressed ND1 had significantly fewer BrdU+ cells, indicating decreased cell proliferation. In an in vivo experiment, ND1-treated human U373 glioma cells were orthotopically transplanted into the fornix of cyclosporine-induced immunocompromised C57 mice. Immunohistochemical staining revealed increased neuronal markers such as TUJ1 expression of transplanted cells in the tumor area. The transplantation of ND1-converted cells also resulted in much-reduced tumor formation in the animal. The study provides evidence of successful reprogramming and conversion of glioblastoma tumor cells into postmitotic neuron lineage cells, suggesting that the direct reprogramming strategy using ND1 is an effective treatment for an extremely deadly cancer.

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Poster

PSTR392. Disease Mechanisms and Therapeutics for Glioblastoma

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR392.03/D38

Topic: B.11. Neuro-Oncology

Title: Inhibition of WNT and TBK1 induces changes in cell cycle and cellular viability in human glioblastoma cells

Authors: *G. CONTRERAS CHÁVEZ[1], J. ESTRADA[2], L. ZAPI-COLÍN[2], I. CONTRERAS[2]; [1]Univ. Autonoma del Estado de Mexico, Metepec, Mexico; [2]Univ. Autonoma del Estado de Mexico, Toluca, Mexico

Abstract: Glioblastoma multiforme (GBM) remains difficult to treat, with current therapeutic options failing to significantly improve long-term life expectancy in patients. GBM is frequently accompanied by alterations in signaling pathways involved in inflammation, cellular proliferation, angiogenesis and survival. Hence, pharmacological modulation of cell signaling is commonly used as a therapeutic alternative for different types of cancer.WNT and TBK1 signaling pathways are involved in essential cellular processes, such as regulation of cell proliferation and apoptosis. The objective of this study is to determine the effects of pharmacological blockage of WNT and TBK1 signaling pathways on cell cycle progression and cell viability in U-87 MG human glioblastoma-derived cells. U-87 MG cells were cultured and supplemented with WNT (IWP12) and TBK1 (BX795) inhibitors at 0.1, 1, 5 or 10 μM concentrations, for 24, 48 and 72 hrs. Changes in cell cycle progression and cellular viability were evaluated by flow cytometry and crystal violet assays.Addition of BX795 to cell cultures increased cell cycle progression in U-87 MG cells, with the 10 μM concentration nearly doubling the frequency of cells at G2/M phase after 24 and 48 hours, compared to untreated controls (55.16% vs. 29,97%, respectively). However, after 72 hours, this effect disappeared, causing treated cells to behave like untreated controls. Treatment with IWP12 did not appear to influence cell cycle progression, regardless of dose and exposure time.Concerning cell viability, our results show reductions in viability using either inhibitor at all concentrations and exposure times, with the greatest effect observed being a 13.92% reduction in viability using BX795 at 10 μM concentration for 72 hours, compared to untreated cells.Our results suggest that pharmacological inhibition of TBK1 signaling using BX795 initially promotes proliferation of U-87 MG cells, but this effect becomes reduced after 72 hours of exposure, leading to increased apoptosis of treated cells in a dose-dependent manner.Further analyses to determine the mechanisms underlying these observations are currently underway.

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Poster

PSTR392. Disease Mechanisms and Therapeutics for Glioblastoma

Location: WCC Halls A-C

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Program #/Poster #: PSTR392.04/D39

Topic: B.11. Neuro-Oncology

Support: Mayo Clinic-Koch Institute Cancer Solutions Team Grant
R01 NS103212
GI Innovations, Inc.

Title: Extended half-life IL-2 synergizes with α-PD-1 for treatment of preclinical murine glioblastoma models

Authors: *C. OWENS[1], Z. P. TRITZ[1], K. AYASOUFI[2], D. WOLF[1], M. A. MAYNES[1], J. FANG[1], C. MALO[1], B. HIMES[3], C. FAIN[1], E. GODDERY[1], L. YOKANOVICH[1], I. PARNEY[1], M. HANSEN[1], C. WANG[4], K. MOYNIHAN[4], D. IRVINE[4], D. WITTRUP[4], R. DIAZ MERCANO[1], R. VILE[1], J. CAMPIAN[1], A. J. JOHNSON[1];
[1]Mayo Clin., Rochester, MN; [2]Duke Univ., Durham, NC; [3]Albert Einstein, New York, NY; [4]MIT, Cambridge, MA

Abstract: With more than 13,400 adults diagnosed in the United States in 2022, Glioblastoma (GBM) is responsible for 49% of all primary malignant brain tumors. Despite decades of research, the median life expectancy for patients who undergo the current standard of care is between 12 and 16 months. α-PD-1 checkpoint blockade is a promising immunotherapeutic approach for many solid tumors; however, this strategy is minimally effective for GBM patients as a monotherapy. A potential explanation of why α-PD-1 checkpoint blockade is ineffective for GBM is the tumor microenvironment and systemic derangements of the immune system prevalent in these patients. We have successfully modeled peripheral immune suppression in experimental murine GBM models. These include low peripheral blood CD4 T cell counts, reduced MHC class II expression on monocytes, and atrophy of primary immune organs in animals harboring gliomas. We therefore hypothesized that extended half-life IL-2, a potent cytokine which promotes the proliferation, differentiation, and killing activity of T cells, could overcome immune derangements and potentially synergize with α-PD-1 checkpoint blockade inhibitor. In cohorts of C57Bl/6 mice, we supplemented α-PD-1 checkpoint blockade with multiple bio-engineered extended half-life IL-2 molecules to treat GL261-Luc and CT2A orthotopic glioma models, which are historically resistant to monotherapy. We demonstrate a statistically significant extended survival in GL261-bearing animals receiving the combination therapy of an extended half-life IL-2 reagent and αPD-1 relative to control-treated mice. Some animals receiving this combination therapy had a reduction in tumor burden to levels below detection. These long-term survivors underwent a rechallenge experiment with a second inoculation of GL261-Luc and effectively cleared tumor without further therapeutic intervention. This synergistic intervention causes a significant shift in the immune profile, including the formation of mature T cell responses, as well as restoring the diminished peripheral immune cell

counts in GL261-bearing animals. Finally, combining extended half-life IL-2 with α-PD-1 checkpoint blockade is effective independently of the CD8 T cell responses. Together, these data suggest that combination immunotherapies employing extended half-life IL-2 fusion proteins are translational to the clinic for GBM treatment and do not require the identification of tumor specific antigens for efficacy.

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Poster

PSTR392. Disease Mechanisms and Therapeutics for Glioblastoma

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR392.05/D40

Topic: B.11. Neuro-Oncology

Support: NIH R35NS132326 (LJW)
Support from the Center for Biomedical Discovery and the Mayo Clinic Cancer Center
R01 NS122174 (AJJ)
K99 NS1177992 (KA)

Title: Trem2 mediates mhcii-associated cd4^{+} t cell response against gliomas

Authors: *J. ZHENG[1], L. WANG[1], S. ZHAO[1], W. ZHANG[1], H. DONG[2], L.-J. WU[3]; [1]Mayo Clin. Grad. Sch. of Biomed. Sci., ROCHESTER, MN; [2]Immunol., [3]Neurol., Mayo Clin., ROCHESTER, MN

Abstract: Myeloid cells comprise up to 50% of the total tumor mass in glioblastoma (GBM) and have been implicated in promoting tumor progression and immunosuppression. Therefore, modulating the response of myeloid cells to the tumor has emerged as a promising new approach for cancer treatment. In this regard, we focus on the Triggering Receptor Expressed on Myeloid cells 2(TREM2), which has recently emerged as a novel immune modulator in peripheral tumors. We showed that brain tumors exhibit significantly higher levels of TREM2 expression, predominantly in tumor-associated myeloid cells. In a pre-clinical model of glioma, TREM2 deficiency did not have a beneficial effect, but instead accelerated glioma progression. Through

the integration of in vivo two-photon imaging, spectrum flow cytometry, quantitative real-timepolymerase chain reaction (qRT-PCR), and immunofluorescence staining, we discovered that TREM2 deficiency impair tumor-myeloid phagocytosis and MHCII expression. Furthermore, we found that deficiency of TREM2 significantly reduced CD4 + T cells in tumor hemispheres. Our results revealed a previously unrecognized protective role of tumor-myeloid TREM2 in promoting MHCII-associated CD4 + T cell response against gliomas.

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Poster

PSTR392. Disease Mechanisms and Therapeutics for Glioblastoma

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Topic: B.11. Neuro-Oncology

Support: Valencian Council for Innovation, Universities Science and Digital Society (PROMETEO/2019/075
Asociación Española Contra el Cáncer (PRDVA222457MORA)

Title: Breast Carcinoma-Amplified Sequence1 defines a highly proliferative population in gliomas.

Authors: *M. J. ULLOA NAVAS[1], R. MORALES-GALLEL[3], V. CAPILLA-GONZALEZ[4], P. SUAREZ-MEADE[2], P. GARCIA-TARRAGA[3], J. FERRER-LOZANO[5], A. QUINONES-HINOJOSA[2], J. GARCIA-VERDUGO[3];
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Abstract: Glial-derived tumors account for most of the central nervous system (CNS) tumors. The initiating cell of glial-derived tumors is still elusive. However, state-of-the-art studies point towards immature oligodendrocytes as a possible source of gliomagenesis. Thus, studying markers related to oligodendrocyte precursors has become of great interest. Breast carcinoma amplified sequence 1 (BCAS1) has emerged as a novel marker that defines an immature oligodendrocyte population in the human CNS. The expression of this protein has been described as a key marker for tumorigenesis in non-CNS tumors, such as prostate cancer. However, its role and expression in gliomas is still underexplored. In this study, we analyzed the expression of BCAS1 in a series of oligodendrogliomas (n = 17), astrocytomas (n = 8) and glioblastomas (n = 60) surgically removed from patients, as well as four primary cell lines derived from patient tumors. The distribution, microenvironment, genetic alterations, and proliferative status of this cell subpopulation were analyzed, and further stereological quantifications were performed. Additionally, $BCAS1^{+}$ cells ultrastructure was studied by immunoelectron microscopy. Our

results show that BCAS1$^+$ cells constitute a morphologically heterogeneously distributed population in glial-derived tumors. BCAS1$^+$ cells exhibit stellate or spherical morphology. Stellate and spherical cells were detected in all studied gliomas as isolated cells. Interestingly, only stellate cells proliferate and form tightly packaged nodules in oligodendrogliomas, but not in glioblastomas or astrocytomas. We did not detect any BCAS1 gene amplification in any of the studied tumors. In conclusion, our findings suggest that BCAS1 defines a specific subpopulation within glial-derived tumors, which could correspond to a transient amplifying neoplastic cell state due to the proliferative features of BCAS1$^+$ stellate cells, thus contributing to tumor malignancy.

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Poster

PSTR392. Disease Mechanisms and Therapeutics for Glioblastoma

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Program #/Poster #: PSTR392.07/D42

Topic: B.11. Neuro-Oncology

Support: 5R01CA203861-05
WashU Cognitive, Computational and Systems Neuroscience Pathway (CCSN) Fellowship

Title: Optimizing functional neuroimaging analysis in glioma patients

Authors: ***K. PARK**[1], J. SHIMONY[2], E. C. LEUTHARDT[3], A. Z. SNYDER[4];
[1]Washington Univ. in St. Louis Neurosci. PhD Program, SAINT LOUIS, MO; [2]Washington Univ. Sch. of Med., Saint Louis, MO; [3]Washington Univ. Sch. of Med., St. Louis, MO; [4]Radiol Dept, Washington Univ. Sch. Med., Saint Louis, MO

Abstract: Resting-state fMRI is becoming increasingly used to study the effects of gliomas on the functional organization of the brain. A variety of image processing and functional connectivity (FC) analysis techniques is represented in the literature but, to date, there has been no systematic examination of how this diversity affects observed results. To this end, we evaluated the impact of atlas registration option, parcellation granularity, and parcellation scheme in contrasting FC between patients and age-matched reference subjects. We analyzed data representing 59 glioma patients and 163 age-matched reference subjects. Differences between the two groups strongly depended on the methodologic choices. Our key findings are as follows: (1) Nonlinear atlas registration is required to compensate for anatomical distortions in glioma-bearing brains. Use of affine atlas registration leads to the false appearance of FC abnormalities. Theoretically, tumor masking could impact the results. However, in our data, the impact of tumor masking was not significant. (2) Functional parcellation schemes, as opposed to

anatomical parcellation schemes (AAL/Brainnetome), maximize sensitivity for the detection of glioma-induced FC abnormalities. (3) FC variability in normal subjects must be considered when identifying FC abnormalities in glioma patients. FC abnormalities in glioma patients are most evident in analyses based on fine parcellations. (4) Much prior work evaluates FC in glioma patients using graph-theoretic measures. As previously noted, not all such measures are equally sensitive to FC abnormalities in glioma patients. In particular, modularity appears to be unchanged in glioma patients. Importantly, the differences between patients and the reference subjects as assessed with graph-theoretic measures were only evident at fine parcellation granularities.

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Poster

PSTR392. Disease Mechanisms and Therapeutics for Glioblastoma

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Topic: B.11. Neuro-Oncology

Support: NIH Director's Pioneer Award (DP1NS111132) to M.M.
Neurosurgery Research and Education Foundation to M.B.K.

Title: Investigating the evolution of neuron-glioma circuit dynamics using an in vivo imaging method

Authors: *K. SHAMARDANI[1], M. B. KEOUGH[1,2], M. MONJE[1,3];
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Abstract: Pediatric high-grade gliomas (pHGG) are aggressive primary brain neoplasms with a dismal prognosis, making them the leading cause of brain tumor-related deaths in children. pHGGs occur in specific anatomical locations at specific ages underscoring their origin in neurodevelopment and the critical importance of the brain tumor microenvironment. Activity-regulated mechanisms such as neurotransmitter, neurotrophin, and calcium-mediated signaling are major regulators of neural development and plasticity. Many pHGGs originate from oligodendroglial precursor cells (OPC) and, like OPCs, neuronal activity promotes pHGG proliferation. Recent work has demonstrated that pHGG cells form calcium-permeable AMPAR-mediated synapses with neurons analogous to the axo-glial synapses that form between neurons

and OPCs. Neuronal activity drives pHGG growth through the secretion of activity-regulated mitogens and through electrochemical communication with pHGG cells that integrate into neural circuits. In turn, pHGG increases neuronal excitability and remodels functional neural circuits. The interconnected network of glioma cells and neurons is fundamental to pHGG progression. However, how these neuron-glioma networks evolve over time remains to be fully understood. We hypothesize that as gliomas progress, the neuron-glioma malignant circuitry evolves chiefly through synaptogenesis to promote activity that fosters glioma progression. Thus, we developed a two-color in vivo imaging method to study neuron-glioma cell interactions over time in freely behaving mice enabling us to record the frequency, pattern, and synchronicity of calcium transients in neurons, glioma cells, and their coactivity giving us a comprehensive view of the changes in neuron-glioma circuit dynamics over time. We have observed increasing neuronal activity throughout the disease, consistent with increasing neural hyperexcitability over time, and have observed that different types of gliomas exhibit unique patterns of calcium transients (rise time, peak amplitude, decay time, and half-width). Our imaging paradigm offers a unique ability to study neuron-glioma interactions at the systems level and will allow us to study how pharmacological inhibitors and neuronal experience shape neuron-glioma malignant circuit dynamics.

Disclosures: K. Shamardani: None. **M.B. Keough:** None. **M. Monje:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Family holds equity in MapLight Therapeutics. F. Consulting Fees (e.g., advisory boards); Scientific advisory board for TippingPoint Biosciences..

Poster

PSTR392. Disease Mechanisms and Therapeutics for Glioblastoma

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR392.09/D44

Topic: B.11. Neuro-Oncology

Support: PhRMA Foundation Postdoctoral Fellowship
Sullivan Brain Cancer Fund
NIH Grant K08NS110919
NIH Grant P50CA097257
Robert Wood Johnson Foundation Grant 74259
UCSF LoGlio Collective
Resonance Philanthropies

Title: Glioblastoma-neuronal circuit integration is modulated by interleukin-6

Authors: *A. G. S. DANIEL, S. KRISHNA, S. L. HERVEY-JUMPER;
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Abstract: The pleiotropic cytokine interleukin-6 (IL-6) is known to be involved in both pro- and anti-inflammatory signaling cascades. In glioblastoma studies using animal and human models, IL-6 promotes immunosuppression and tumor progression and is negatively associated with survival. Intriguingly, IL-6 also contributes to increased synaptogenesis during development and following focal brain injury. Glioblastoma remodeling of neuronal circuits influences patient survival; therefore, it is possible that IL-6 has mechanistic significance. Here, we investigated IL-6 as a driver of activity-dependent glioblastoma proliferation using patient-derived xenograft (PDX) mouse and human glioblastoma models. We assessed neuronal activity by microelectrode arrays and in vitro calcium imaging using weighted mean firing rate (WMFR), network burst frequency (NBF), and synchrony index. Next, we used bulk and single-cell RNA sequencing on 13,731 cells from ten tumors including pair-matched samples to identify differentially expressed genes in intratumoral regions with elevated functional connectivity and found IL6 to be highly upregulated. Mouse embryonic cortical neurons and cerebral organoids co-cultured with primary patient-derived glioblastoma cells demonstrated concentration-dependent neuronal hyperexcitability (increased WMFR and NBF) in IL-6-overexpressing conditions compared to the neuron-only condition. Pharmacological inhibition using the humanized IL-6 receptor antibody tocilizumab reduced network synchrony across models. These findings suggest that IL-6-induced glioma-neuronal hyperexcitability may be inhibited by the FDA-approved tocilizumab, thereby providing preclinical support for its use to treat activity-dependent mechanisms of glioblastoma proliferation. Future studies are needed to uncover mechanisms and clinical efficacy.

Disclosures: A.G.S. Daniel: None. **S. Krishna:** None. **S.L. Hervey-Jumper:** None.

Poster

PSTR393. Consequences of Diet, Exercise or Environment

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR393.01/Web Only

Topic: C.01. Brain Wellness and Aging

Title: Sodium pump activity in the cerebral cortex of rats fed with a high-calorie diet.

Authors: *R. MERCADO[1], O. GUZMAN-QUEVEDO[2], R. MANHÃES-DE-CASTRO[3], M. FLORES, Jr[4];
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Abstract: The central nervous system coordinates all the functions of the organism and its main function is the transmission of the nerve impulse, with the help of neurotransmitters. On the other hand, the Na+/K+-ATPase (sodium pump) is a membrane-bound enzyme responsible for maintaining the ionic gradients across the membrane, also it is part of the cellular signaling pathway so we know that is stimulated by serotonin. In Mexico and the world, metabolic related

diseases are a public health problem, especially diabetes mellitus, a disease related to a high-calorie intake and which is part of the metabolic syndrome. To this day it is unknown if a high-calorie intake would alter the sodium pump activity and its effect in the metabolic of lipids and carbohydrates, therefore our main goal is to determinate the effect of a high-calorie intake in the lipid profile, the tolerance of glucose and the activity of the sodium pump. A diet model of high carbohydrates and high lipids intake was established in male Wistar rats and its effect was compared against a control group. Meanwhile this model was implemented, body weight and blood glucose levels were recorded, also oral glucose tolerance test and insulin resistance test were executed. Lipid profile and activity of the sodium pump were determined. Results show an increase in body weight in animals with high-calorie diet, also an increase in blood lipids level was observed. Fasting blood glucose levels were not different between groups, meanwhile oral glucose tolerance curves and insulin resistance test in fact shows hints about an impaired glucose tolerance in rats with high-calories diet. In relation with the activity of the sodium pump, we observed that there was a statistically significant decrease in the experimental group as compared with the control group. These results suggest us that a high-calorie diet decreased sodium pump activity although without signals of hyperglycemia, altered lipid profile.Acknowledgements: The present work was partially supported by CIC-UMSNH, ICTI-Michoacán.

Disclosures: R. Mercado: None. **O. Guzman-Quevedo:** None. **R. Manhães-de-Castro:** None. **M. Flores:** None.

Poster

PSTR393. Consequences of Diet, Exercise or Environment

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR393.02/D45

Topic: C.01. Brain Wellness and Aging

Support: Florida Department of Health Ed and Ethel Moore Alzheimer's Disease Research program 21A12

Title: Transient exercise-induced changes of circulating factors in the mouse muscle-brain axis

Authors: *J. JUERGENSMEYER[1], C. DESAI[2], S. MORET[2], J. MURRAY[2], E. KANG[2], S. VILLARINO[2], K. ALVIÑA[2];
[2]Neurosci., [1]Univ. of Florida, Gainesville, FL

Abstract: Exercise promotes healthy cognitive aging and could be an effective non-pharmacological alternative to prevent or delay pathology associated with neurodegenerative disorders such as Alzheimer's disease (AD). However, mechanisms underlying such positive effects are not fully understood. Irisin, a myokine that is secreted from skeletal muscle during exercise, is linked to mechanisms that are dysfunctional in AD, such as glucose metabolism and hippocampal neurogenesis. Nevertheless, there have been contradictory reports on changes in irisin levels following exercise. Such inconsistencies might be a result of variability in

experimental conditions, such as duration of exercise protocols (e.g., acute or chronic) and/or timing of tissue collection (e.g. immediately after or hours later).
To determine the precise timespan of exercise-induced changes in circulating irisin, we designed exercise protocols that tested the effect of these experimental variables. We hypothesized that changes in circulating irisin concentration occur during or immediately after exercise and return to baseline shortly thereafter. Moreover, we predict the irisin-mediated benefits of exercise will not be represented by changes in baseline levels. To test these hypotheses, we subjected adult male and female mice to swimming or running exercise for 20 min and measured post-exercise serum irisin concentration (via ELISA) at four different time points: 0, 30, 60, or 120 min after. Compared to sedentary controls, we observed a ~20% increase in male mice immediately after either exercise protocol, which decreased to baseline levels within 60 min. No changes were detected in female mice. Next, we performed a chronic exercise protocol (swimming for 20min/day for 21 days) with a separate group of male and female mice, and measured serum irisin 24 h after the last exercise session. As expected, our results show no difference between the exercise group and the sedentary controls. Our results suggest that irisin is released during exercise and circulating levels return to baseline within an hour. Therefore, the timing of sample collection should be carefully considered to reliably detect exercise-induced changes in circulating irisin. In addition, we observed robust sex-dependent differences that should be further explored, including the potential relationship between Irisin release and stress.

Disclosures: J. Juergensmeyer: None. **C. Desai:** None. **S. Moret:** None. **J. Murray:** None. **E. Kang:** None. **S. Villarino:** None. **K. Alviña:** None.

Poster

PSTR393. Consequences of Diet, Exercise or Environment

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR393.03/D46

Topic: C.01. Brain Wellness and Aging

Support: NCBiotech
NIH grant R25GM077634

Title: Evaluating dietary and synthesized agents associated with modulation of the autophagy-lysosomal pathway for improving synaptic resilience and reducing aging-related cognitive deficits

Authors: M. F. ALMEIDA[1,2], K. N. ADAMS[1], R. HICKS[1], M. GREENE[1], S. T. KINSEY[2], R. DAVIDSON[1], J. ENRIQUEZ[1], M. VIANA[1], K. L. G. FARIZATTO[3,1], *B. A. BAHR[4];
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Abstract: Cognitive health is a major research focus for pharmaceutical and nutraceutical studies. Cognitive decline is strongly associated with aging, and it can be attenuated by physical exercise and a healthy diet which were recently associated with the modulation of proteostasis. Neuronal compromise signifies vulnerability that fosters cognitive impairment, whether facilitated by aging, excitotoxicity, cholinergic crises, or traumatic events. These risks to cognition negatively affect proteostasis and synaptic integrity, and they are known to compromise the autophagy-lysosomal protein clearance pathway. Natural products have been linked to cognitive health, thus a range of agents were tested for the ability to amplify cathepsin B (CatB), a lysosomal hydrolase that degrades pathogenic proteins and is synaptoprotective. After three daily infusions into hippocampal explants, extracts of bacopa and the ginseng *P. quinquefolius* enhanced the CatB-30 active form by over 3-fold; several other natural products were ineffective. Only *P. quinquefolius* produced correlative enhancements of CatB-30, LC3-II, and synaptic markers. Interestingly, this ginseng and the exercise mimetic β-GPA both protected synapses in cultured explants subjected to age-related stress induced by a lysosomal inhibitor. From subsequent translational work, *in vivo* studies identified cognitive defects in middle-aged rodents, and the measured cognitive decline was offset by the two different treatments. Currently, chemoproteomics techniques are being utilized for protein target discovery to decipher the potential mechanistic pathway for the lysosomal enhancement produced by unique compounds that are sensitive to brefeldin A with the apparent involvement of the AMPK/SIRT1 axis. Note that disruption of lysosomal function has been linked to neurodegenerative disorders (Wang et al. 2018 *Curr Opin Neurobiol* 48:52), while the polyphenol-type compounds that upregulate/promote CatB maturation provide protective enhancement of protein clearance capacity in models of AD, PD, and MCI (Viswanathan et al. 2012 *ACS Med Chem Lett* 3:920; Hwang et al. 2019 *International J Mol Sci* 20:4432). We are particularly interested in whether targets of the protective compounds are also compromised by organophosphate toxicity (Farizatto et al. 2019 *Scientific Reports* 9:6532), seizure induction, and/or primary blast exposures (Almeida et al. 2021 *Brain Pathology* 31:e12936), all of which cause dementia-related synaptopathy. These studies suggest that dietary and other agents act via a similar pathway as does exercise, promoting protein clearance activation and corresponding synaptic protection.

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Poster

PSTR393. Consequences of Diet, Exercise or Environment

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR393.04/D47

Topic: C.01. Brain Wellness and Aging

Support: CAPES Grant 88887.716851/2022-00

Title: Exercise Modalities Improve Motor Coordination and Balance Skills in Aged Rats

Authors: *I. SIQUEIRA[1], L. MEIRELES-MORAES[1], L. C. RECK[1], F. GALVÃO JÚNIOR[1], L. HOLT[2], P. MEWS[2], E. J. NESTLER[2];
[1]Univ. Federal Do Rio Grande Do Sul, Porto Alegre, Brazil; [2]Nash Family Dept. of Neurosci. and Friedman Brain Inst., Icahn Sch. Med. At Mount Sinai, New York, NY

Abstract: Although several lines of evidence suggest that exercise has neuroprotective effects, there are few reports comparing different exercise modalities in an aged model. In addition, the prefrontal cortex (PFC), which is involved in cognitive control including motor functions, may have a central role in exercise-induced improvements on brain functions. Our aim was to investigate the protective effects of different exercise modalities on age-related declines in cognitive skills, specifically on motor function. The Local Ethics Committee at UFRGS approved all handling and experimental conditions (CEUA-UFRGS, no. 29818). Adult and aged male Wistar rats (2 and 22 months old) were subjected to aerobic, acrobatic, resistance, or combined exercise modalities for 20 min, 3 times a week, for 12 weeks. Beam balance and rotarod tests were used to investigate motor function. All rats were decapitated 1 h after the last exercise session. The PFC was quickly isolated by punch dissection, immediately snap-frozen in liquid nitrogen, and then stored at −80°C until the nuclear-FACS procedure. PFC nuclei were isolated and sorted into NeuN+ and the remaining NeuN- fractions by fluorescence activated cell sorting (FACS). Total RNA was isolated, cDNA libraries were constructed, and RNA-seq was performed. Aged rats had worse motor performance. All exercise modalities, except acrobatic, showed a trend to improve rotarod index. Sedentary aged rats were unable to stay on the rotarod apparatus even at a relatively slow speed (10 rpm), while all modalities improved rotarod performance at this speed. Aerobic, acrobatic, and combined exercise modalities improved beam balance performance. All exercise modalities improved postural stability and balance and represent a potential approach to reduce the incidence and severity of falls in older people. Moreover, our evolving RNA-seq data will provide insight into the molecular mechanisms in PFC by which exercise improves motor performance.

Disclosures: I. Siqueira: None. **L. Meireles-Moraes:** None. **L.C. Reck:** None. **F. Galvão Júnior:** None. **L. Holt:** None. **P. Mews:** None. **E.J. Nestler:** None.

Poster

PSTR393. Consequences of Diet, Exercise or Environment

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR393.05/D48

Topic: C.01. Brain Wellness and Aging

Support: NIEHS IZIAES090057-24

Title: Anabolic effects of dexamethasone on hippocampal lipidome

Authors: *F. I. AGUAYO[1], J. KUHN[2], J. CIDLOWSKI[2];
[1]Signal Transduction Lab., [2]Mass Spectrometry Res. and Support group, NIEHS, Durham, NC

Abstract: Background: Dexamethasone (DEX) is a synthetic glucocorticoid broadly used for treatment of inflammatory affections, such as rheumatic problems, allergies, asthma, chronic obstructive lung diseases, brain swelling, among others. However, its chronic use has been related to the development of mood disorders, including depression and anxiety; and less commonly with seizures, euphoria, sleep disorders and psychosis. This drug exerts its actions by binding to the glucocorticoid receptor (GR), a member of the nuclear receptor family of ligand-activated transcription factors that modulate the expression of numerous genes either by repressing or increasing their transcription. These genes mostly regulate inflammation process and catabolism of carbohydrates and lipids. In a recent analysis of transcriptomic data from mice depleted from hippocampal GR, our laboratory has shown a decrease in mRNAs related to the biosynthesis of phosphoinositides, diacylglycerol, fatty acids, ethanolamines, and sphingolipids. **Hypothesis:** Dexamethasone has an anabolic effect on the hippocampal lipidome **Methodologies:** HT-22 mice hippocampal cell line was incubated with DEX 10 nM, RU-486 100 nM (RU, a GR antagonist), the co-incubation with DEX and RU, and the vehicle solution by 48 hours. Then lipids were isolated by the Bligh and Dyer method, and semi-quantified by non-targeted lipidomic. Additionally, adrenalectomized male mice were administrated with DEX 2.5 mg/L in the drinking water. After 8 weeks of drug administration, mice were euthanized and hippocampi were dissected, followed by lipid extraction the Bligh and Dyer method, and semi-quantified by non-targeted lipidomic. After initial data collection, PCA and hierarchical clustering analysis were used as a non-supervised classification method. Differential lipid levels were determined by using Partek suite software, and lipid enrichment analysis was performed in LION database. **Results:** Dexamethasone incubation of HT-22 cells induce a general increase in levels of phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol, sphingomyelin, and ceramide. These effects are blunted by GR antagonist co-incubation. On the other hand, the hippocampi from DEX treated mice showed increases and decreases in phosphatidylethanolamine, phosphatidylcholine and phosphatidylserine, along with a decrease in sphingomyelin and triglycerides. **Conclusions:** Dexamethasone presents an anabolic effect on lipidome in the *in vitro* mice hippocampal model. However, this drug displayed both anabolic and catabolic effects on lipids from our *in vivo* model.

Disclosures: F.I. Aguayo: None. **J. Kuhn:** None. **J. Cidlowski:** None.

Poster

PSTR393. Consequences of Diet, Exercise or Environment

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR393.06/D49

Topic: C.01. Brain Wellness and Aging

Support: BrightFocus grant A2021046S
NIH grant U19 AG69701
NIH grant RF1 AG046205

Title: The multifaceted impact of APOE genotype on the benefits of calorie restriction during aging

Authors: *W. QIAO[1], Y. CHEN[2], Y. REN[3], T. IKEZU[2], S. JOHNSON[2], F. LI[2], K. CHEN[2], M. PAN[6], A. MENESES[2], A. KURTI[4], J. CHEN[5], X. HAN[7], G. BU[2], N. ZHAO[8];
[1]Neurosci., [3]Hlth. Sci. Res., [4]Neurosci. Res. Dept., [2]Mayo Clin., Jacksonville, FL; [5]Mayo Clin., Rochester, MN; [6]Med., [7]Univ. of Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX; [8]Mayo Clin. Jacksonville, Jacksonville, FL

Abstract: Introduction: Calorie restriction (CR) is known to promote healthy aging and extend lifespan. However, the influence of different apolipoprotein E (*APOE*) genotypes on the outcomes of CR intervention remains unclear. **Methods:** To address this question, we conducted an 8-month CR study in APOE-targeted replacement (TR) mice expressing human *APOE2*, *APOE3*, or *APOE4* gene. The CR treatment involved a 30% reduction in caloric intake starting at 12 months of age until 19.5 months, while control littermates received a normal diet *ad libitum* (AL). We also examined the effects of CR in a separate cohort of mice with a history of 3.5-month high-fat diet (HFD) exposure. We conducted a battery of behavioral tests to assess the anxiety, working memory, and associative memories. We then harvested brains and blood and collected fecal samples from these animals and performed multi-omics profiling, including transcriptomics, lipidomics, and microbiomics. **Results:** We found that CR reduced anxiety and improved associative memory in *APOE3* and *APOE4* mice but not *APOE2* mice compared to the AL group. Transcriptome analysis of the brain identified an activation of the cholesterol synthesis pathway and predicted changes in myelination through the activation of oligodendrocyte precursor cell differentiation in *APOE3* and *APOE4* mice upon CR. CR also had significant effects on brain and blood lipid metabolism, with APOE genotype-dependent alterations mainly observed in the blood lipidome, particularly in APOE2 mice. Microbiota profiling showed that CR increased the abundance of Bifidobacteriaceae species in *APOE3* and *APOE4* mice, correlating with memory improvement, while APOE2 mice exhibited increased abundance of Lactobacillus species without memory-related correlations. These effects of CR were also observed in mice with prior HFD exposure and were specific to *APOE3* and *APOE4* mice. **Conclusions:** Our study demonstrates that *APOE* genotypes play a differential role in mediating the multifaceted effects of CR on behavior, gene expression, lipid metabolism, and microbiome diversity, providing important implications for early prevention strategies targeting aging and aging-related disorders.

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Poster

PSTR393. Consequences of Diet, Exercise or Environment

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR393.07/D50

Topic: C.01. Brain Wellness and Aging

Support: National Institute of General Medical Sciences / National Institutes of Health, grant R01GM128183.

Title: Propofol attenuates surgery induced cognitive impairment in aged mice via α5-$GABA_A$ receptors

Authors: *R. NAGARAJAN[1], J. LYU[2], M. KAMBALI[1], M. WANG[2], R. A. PEARCE[3], U. RUDOLPH[1];
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Abstract: Postoperative neurocognitive disorder (poNCD), i.e., cognitive deficits present more than four weeks after surgery and anesthesia that may lead to increased morbidity and mortality, are relatively common in aging human patients. Propofol, the most commonly used intravenous anesthetic, exerts its anesthetic actions primarily via $GABA_A$ receptors. However, it has also been shown to have neuroprotective effects in aged mice and in a mouse model of Alzheimer's disease. We wanted to test the hypothesis that chronic intermittent propofol prevents or reverses postoperative cognitive deficits in aged mice via α5-$GABA_A$ receptors. Abdominal surgery was performed under isoflurane anesthesia in 21-24 months-old wild-type mice and global α5 knockout mice. Animals received either chronic intermittent propofol (CIP, 75 mg/kg i.p.) or vehicle (Intralipid^R) every 5th day throughout the experiment. CIP led to a sustained redistribution of the $GABA_A$ receptor α5 subunit to the surface membranes. Laparotomy performed on day 17 impaired learning and memory functions, as determined using a behavioral test battery that included Y maze alternation, novel object recognition, water maze learning, reversal learning, and cued and contextual fear conditioning, compared to no surgery controls. Strikingly, CIP improved cognitive function postoperatively and attenuated surgery-induced mitochondrial dysfunction and the oxidative stress marker nitrite in the hippocampus. Western blots on hippocampal tissues showed that CIP attenuated the surgery-induced apoptotic cell death and microglial activation in the aged mice. Importantly, these protective effects of propofol were absent in aged global α5 knockout mice, indicating that α5-$GABA_A$ receptors are involved in mediating the cognitive benefits of propofol. The current study suggests that perioperative intermittent propofol administration might prevent or attenuate surgery-induced cognitive dysfunction and thus potentially reduce the liability for undesired long-term outcomes. Our results reveal that chronic intermittent propofol
administration increases the surface expression of α5-$GABA_A$ receptors, improving cognitive function in aged mice after surgery.

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Poster

PSTR393. Consequences of Diet, Exercise or Environment

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR393.08/D51

Topic: C.01. Brain Wellness and Aging

Title: Ipsc-derived mononuclear phagocytes rescue cognitive impairment and improve neural health in aging mice

Authors: *V. MOSER[1], L. J. DIMAS-HARMS[2], J. INZALACO[2], S. BELL[2], R. M. LIPMAN[4], H. S. GOODRIDGE[3], C. SVENDSEN[3];
[1]Regenerative Med. Inst., Cedars-Sinai Med. Ctr., West Hollywood, CA; [2]Regenerative Med. Inst., [3]Regenerative Med. Ctr., Cedars-Sinai Med. Ctr., Los Angeles, CA; [4]Univ. of Maryland, College Park, MD

Abstract: A number of studies have demonstrated the ability of young blood or plasma to improve cognitive function in aged animals, and we have previously shown that bone marrow transplanted from young to aging mice has beneficial effects on cognition and neural health in aging recipients. In the current study, we sought to evaluate the cell type that might be responsible for the observed effects, focusing on mononuclear macrophages as these cells are known to become dysfunctional in aging. We used human induced pluripotent stem cells (iPSCs) to generate mononuclear macrophages (iMPs), as this enables the generation of a renewable and scalable cell therapy. iMPs were administered to aging mouse models via tail vein injection. Aging-associated cognitive deficits were significantly improved in iMP-treated mice. Moreover, relative to vehicle-treated aging mice, aging mice treated with iMPs showed improvements in several neural health outcomes, including increased synaptic marker expression, and reduced microglial activation. A proteomic analysis of plasma revealed that several proteins increased in aging plasma but were restored to levels observed in young animals after iMP treatment. Interestingly, several of these same pathways were altered with aging and after iMP treatment in hippocampal single nucleus RNA sequencing (snRNA-seq) data, suggesting potential targets through which iMPs may be acting. iMPs present a promising novel therapeutic for aging-associated declines in cognition and neural health.

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Poster

PSTR393. Consequences of Diet, Exercise or Environment

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR393.09/D52

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant RF1AG028271
NIH Grant T32NS105864

Title: Tlr4 mediates development of persistent post-operative cognitive dysfunction following short-term high-fat diet consumption in male rats

Authors: *S. MUSCAT, M. BUTLER, M. BETTES, J. DEMARSH, R. BARRIENTOS;
Inst. for Behavioral Med. Res., The Ohio State Univ., Columbus, OH

Abstract: Background: Gradual declines in cognition are associated with normal aging but can turn precipitous following peripheral immune insults. Post-operative cognitive dysfunction (POCD) is an abrupt decline in neurocognitive function - difficulty with executive functions, memory impairment, confusion - experienced following surgery, lasting days-to-months after surgery. Importantly, longer-lasting POCD can develop into dementia. Advanced age and obesity & other comorbidities linked to high-fat diet (HFD) consumption are identified as risk factors for POCD, although underlying mechanisms remain unclear. We previously showed that just 3-days of HFD can evoke neuroinflammation sufficient to cause memory deficits in aged rats. Therefore, we hypothesized that HFD consumption before surgery would potentiate the neuroinflammatory response & memory deficits via activation of the innate immune receptor TLR4, which both insults are known to stimulate. **Methods:** Young-adult (3mo) & aged (24mo) rats were fed chow or HFD for 3-days immediately before sham surgery or laparotomy. 2-weeks later, rats underwent contextual fear conditioning to assess hippocampus- & amygdala-dependent long-term memory. Expression of neuroinflammatory molecules was assessed. In a separate experiment, rats were administered the TLR4-specific antagonist LPS-RS immediately before HFD onset; surgery & memory tests were performed as before. Additionally, the efficacy of DHA supplementation to mitigate HFD and surgery-induced neuroinflammation and memory deficits was assessed. **Results:** Aged rats who received both HFD & laparotomy had impaired hippocampus-dependent memory compared to all other groups, and both aged & young-adult rats fed HFD before surgery experienced amygdala-dependent impairment. Interestingly, protein expression of the pro-inflammatory cytokines IL-1β, IL-6, & TNF was significantly increased in aged rats who received both HFD & surgery in the hippocampus, but not amygdala, 3-weeks after surgery. The HFD+surgery-induced memory impairments are dependent on activation of TLR4, as LPS-RS pre-treatment completely prevented this effect. Additionally, pre-operative DHA supplementation effectively prevented HFD and surgery-induced neuroinflammation and memory impairments. **Conclusions:** These findings suggest that HFD may 1) increase risk of persistent POCD-associated memory impairments following surgery in rats in 2) a TLR4-dependent manner, which 3) can be targeted by DHA supplementation to mitigate development of persistent POCD.

Disclosures: S. Muscat: None. **M. Butler:** None. **M. Bettes:** None. **J. DeMarsh:** None. **R. Barrientos:** None.

Poster

PSTR393. Consequences of Diet, Exercise or Environment

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR393.10/D53

Topic: C.01. Brain Wellness and Aging

Title: Efficacy of microalgae extract from Phaeodactylum tricornutum on cognitive function in elderly

Authors: *J. MAURY[1], C. SEXTON[2], V. BARBOSA[2], G. DUNNGALVIN[2], T. G. DINAN[3], R. PRADELLES[1];
[1]Res. & Develop., Microphyt, Baillargues, France; [2]Atlantia Food Clin. Trials, Cork, Ireland; [3]APC Microbiome Ireland, Univ. Col. Cork, Cork, Ireland

Abstract: In developed countries, a rapidly ageing population is being observed due to declining birth rates and increased life expectancy, and it has a direct impact on public health policies from on a societal and economic perspectives. One of the critical health and societal issues facing an ageing population is the maintenance of cognitive function. In this regard, some pharmacological, behavioural and dietary preventive approaches have been evaluated to improve age related decline in the cognitive function. Microalgae is a promising approach as they are able to produce bioactive molecules, such as pigments, fatty acids, peptides and sterols. Fucoxanthin is one of the major carotenoid found in microalgae as *Phaeodactylum tricornutum* (PT), well known for its neuroprotective effect. There are no human studies to our knowledge, however, results from an *in vivo* study showed a significant improvement in learning, short term and spacial memory, a decrease in brain inflammation and oxidative stress following microalgae extract from PT in a mice model of ageing (article submission in progress). The objective of the present double-blind, placebo-controlled study was to evaluate in healthy older adults the effect of daily supplementation of microalgae extract *PT* during a 24-week period on cognitive function of healthy older adults with age-related mild cognitive decline (NCT04832412). In total, 66 males and females, aged between 55 and 75 years old were randomly assigned to ingest placebo (PL) or 550mg of *PT* extract (including 0.8% of fucoxanthin, Brainphyt™). The definition of age-related mild cognitive impairment was a score of ≥24 on the Mini Mental State Examination (MMSE) and a score of ≥25 on the Memory Assessment Clinic-Q (MAC-Q). Participants performed a battery of cognitive tests using Computerised Mental Performance Assessment System (COMPASS) to measure spatial, working, episodic, long term memory, attention and vigilance and executive function. Sleep quality, mood and stress states were also evaluated using validated questionnaires. In addition, blood samples were collected to evaluate the effect of supplementation on neuro-inflammatory markers (e.g. BDNF, CCL2, IL6, IL18, TNFa, VEGF) and identify their potential mechanism of actions. All endpoints were collected at baseline, week 12 and week 24, and the interventional phase has been completed. This study is now in the statistical analysis and interpretation phase, which is expected to be completed in Q3 2023.

Disclosures: **J. Maury:** A. Employment/Salary (full or part-time):; Study sponsor employee. **C. Sexton:** 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.; Contract research organization member. **V. Barbosa:** 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.; Contract research organization member. **G. DunnGalvin:** 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.; Contract research organization member. **T.G. Dinan:** 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.; Study PI. **R. Pradelles:** A. Employment/Salary (full or part-time):; Study sponsor employee.

Poster

PSTR393. Consequences of Diet, Exercise or Environment

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR393.11/D54

Topic: C.01. Brain Wellness and Aging

Support: Brazilian National Council for Scientific and Technological Development - CNPq
Foundation for the Support and Promotion of Science, Technology and Innovation of RN - FAPERN

Title: Tamsulosin impairs object recognition without affecting anxiety-related behaviors in mice

Authors: *C. D. MOURA[1], V. A. HOLANDA[2], M. C. OLIVEIRA[1], C. I. TORRES[1], E. D. JUNIOR[1], E. C. GAVIOLI[1];
[1]Dept. of Biophysics and Pharmacol., Federal Univ. of Rio Grande do Norte, Natal, Brazil; [2]Neurosurg. Departament, Johns Hopkins Univ., Baltimore, MD

Abstract: Tamsulosin is an uroselective α1-adrenoceptor blocker used to treat benign prostatic hyperplasia. This drug displays high affinity for α1A and α1D-receptors subtypes, which are also expressed in the brain. Dementia symptoms after administration of tamsulosin in humans have been reported, but studies on its brain-derived effects are still lacking. On that account, this study aimed to investigate the effects of tamsulosin on cognitive performance and anxiety, by using object recognition task (ORT) and elevated plus-maze (EPM) test in mice. For this, tamsulosin (0.001-0.01 mg/kg) was orally administrated in male Swiss mice (12-16 weeks old) at three separate time points: 60 min before training session (TS), immediately after TS and 60 min before test session (TES). Biperiden (BPD; 2-4 mg/kg, ip) was used as an amnesic standard. The elevated plus-maze (LCE) was conducted after oral administration of tamsulosin (0.0001-0.1 mg/kg, 60 min prior to the test). Diazepam (DZP; 1 mg/kg, ip) was used as an anxiolytic standard. BPD impaired object recognition when injected both pre- (Recognition index, RI - interaction between treatment and sessions: $F(1,26)=11.8$, $p<0.05$, 2-way ANOVA, Bonferroni's test) and post-training (time: $F(1,28)=13.2$, $p<0,05$, 2-way ANOVA, Bonferroni's test). Tamsulosin 0.01 mg/kg impaired object recognition at all times measured, while at the dose of

0.001 mg/kg did not affect mouse performance in the ORT (RI-pre-training: $F(2,42)=36.4$; post-training: $F(2,42)=43.5$; for both $p<0.05$, 2-way ANOVA, Bonferroni's test). In the EPM, DZP was able to increase the percentage of time spent in the open arms compared to the control ($F(5,40)=5.7$, $p<0.05$, 1-way ANOVA, Bonferroni's test). However, tamsulosin did not affect mouse behavior at any dose tested ($p>0.05$). The sample size was 7-8 animals per group. In conclusion, tamsulosin impaired memory performance by affecting acquisition and consolidation of memory in mice. The cognitive impairment of tamsulosin is not related to anxiety. Previously, we reported that tamsulosin (0.01 mg/kg) increases susceptibility to depressive-like behaviors in mice (Holanda et al. 2022). Altogether, behavioral effects of tamsulosin contribute to understand its potential side effects, and it can also add to the role played by α1-adrenoceptor in the etiology of psychopathologies.

Disclosures: C.D. Moura: None. **V.A. Holanda:** None. **M.C. Oliveira:** None. **C.I. Torres:** None. **E.D. Junior:** None. **E.C. Gavioli:** None.

Poster

PSTR393. Consequences of Diet, Exercise or Environment

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR393.12/D55

Topic: C.01. Brain Wellness and Aging

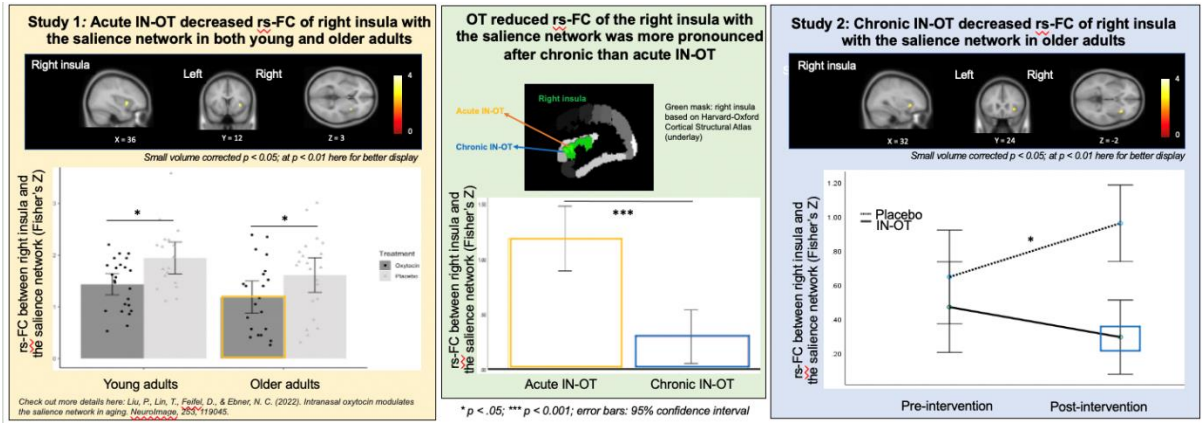
Support: NIH/NCATS UL1 TR000064
NIH/NIA R24 AG039350
ARG DTD 03-26-2008
R01AG059809
T32AG020499
N00014-21-1-2201

Title: Effects of acute and chronic administration of intranasal oxytocin on large-scale brain networks in older adults

Authors: *P. LIU[1], T. LIN[2], R. POLK[2], H. FISCHER[3], D. FEIFEL[4], N. C. EBNER[2,5,6,7]; [1]Dept. of Psychology, Univ. of Florida, GAINESVILLE, FL; [2]Dept. of Psychology, Univ. of Florida, Gainesville, FL; [3]Psychology, Stockholm Univ., Stockholm, Sweden; [4]Kadima Neuropsychiatry Inst., La Jolla, CA; [5]Inst. on Aging, Gainesville, FL; [6]Dept. of Clin. and Hlth. Psychology, Ctr. for Cognitive Aging and Memory, Gainesville, FL; [7]Florida Inst. for Cybersecurity Res., Gainesville, FL

Abstract: Intranasal oxytocin (IN-OT) is a key modulator of social-cognitive capacities such as emotion identification (*Horta et al., 2019)*, social memory (*Tse et al., 2018)*, animacy perception (*Valdes-Hernandez et al, 2021)*. IN-OT has also been shown to modulate resting-state functional connectivity (rs-FC) between key nodes of the salience network (e.g., insula and amygdala; *Ebner et al., 2016; Brodmann et al., 2017*), known to gate attention (*Uddin, L. Q., 2016).*

However, not well understood yet are IN-OT's effects on large-scale brain networks: (i) among older adults, despite robust evidence of age-related differences in brain network function and increasing evidence of age-differential effect of IN-OT on both brain and socioemotional behavior (*Ebner et al., 2013; Horta et al., 2020);* (ii) for chronic (i.e., repeated) in addition to acute (i.e., single-dose) administration regimens, to determine OT's therapeutic potential (*Horta et al., 2020)*. Given that, the research aim in the present study is to determine the extent to which IN-OT (relative to placebo) modulates rs-FC between the salience network and its key nodes in the aging brain both after acute (*Study 1*) and chronic (*Study 2*) IN-OT. The results were shown that (i) acute IN-OT decreased rs-FC of right insula with the salience network in both young and older adults; (ii) chronic IN-OT decreased rs-FC of right insula with the salience network in older adults; (iii) OT reduced rs-FC of the right insula with the salience network was more pronounced after chronic than acute IN-OT.



Disclosures: P. Liu: None. **T. Lin:** None. **R. Polk:** None. **H. Fischer:** None. **D. Feifel:** None. **N.C. Ebner:** None.

Poster

PSTR393. Consequences of Diet, Exercise or Environment

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR393.13/D56

Topic: C.01. Brain Wellness and Aging

Support: NRF-2022R1A4A1018963
2022R1A2C1003878

Title: Genkwa Daphne flower extract (GFE) and the major compounds restored neuroprotective functions of disease-associated senescent microglia.

Authors: S. PARK[1], D. GUPTA[1], Y.-S. LEE[1], ***G. SONG**[2,1];
[1]Translational Brain Res. Ctr., Intl. St. Mary's Hospital, Catholic Kwandong Univ., Incheon, Korea, Republic of; [2]Dept. of Med., Catholic Kwandong Univ., Incheon, Korea, Republic of

Abstract: The population is facing an era of super-aging, and the number of patients with degenerative brain diseases such as dementia is rapidly increasing. Neuroinflammation, commonly observed in degenerative brain disorders, is closely related to microglial dysfunction. Microglia are the innate immune cells in the central nervous system that play a crucial role in neuroprotection by releasing neurotrophic factors and removing pathogens through phagocytosis and regulating brain homeostasis. The extract from Daphne Genkwa plants were reported to have neuroprotective effects in animal models of neuroinflammation. This study aims to study the anti-inflammatory and neuroprotective effect of GFE and major compounds in disease-associated senescent microglia (DASM). First, we developed in vitro model of DASM. GFE inhibited LPS-induced inflammatory factors in DASM, supporting the anti-inflammatory effect of GFE. Arg1 and BDNF mRNA was increased in primary microglia treated with GFE, supporting the neuroprotective effect of microglia. In vivo effects of GFE were also found in mouse brain as oral administration of GFE significantly inhibited the LPS induced neuronal loss and inflammatory activation of microglia. This study suggests that oral GFE may be a promising approach that may promote neuroprotection, and anti-inflammatory effects can be a preventive measure for neurodegenerative diseases via promoting microglial neuroprotective effects.

Disclosures: **S. Park:** None. **D. Gupta:** None. **Y. Lee:** None. **G. Song:** None.

Poster

PSTR393. Consequences of Diet, Exercise or Environment

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR393.14/D57

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Anonymous Foundation Grant to Saint Vincent College

Title: Presentation and loss of environmental enrichment toys: effects on stress and affective behaviors in male and female mice

Authors: *M. RHODES[1], M. L. JENKINS-ANDREWS[1], M. R. BISIGNANI[1], C. M. MONROY[1], S. A. LIVELSBERGER[1], G. C. NOEL[1], R. T. RUBIN[2];
[1]Biol. Sci., St. Vincent Col., Latrobe, PA; [2]David Geffen Sch. of Med. at UCLA, Oxnard, CA

Abstract: Structural aspects of housing and husbandry conditions can influence the physiology and behavior of laboratory animals. We previously demonstrated that housing jugular vein-cannulated (JVC) rats with commonly used environmental enrichment (EE) toys resulted in lower hypothalamic-pituitary-adrenal (HPA) axis activity before and after mild stress. Other studies indicated that removal of EE in these animals resulted in sexually diergic HPA activity before and after restraint stress: male HPA responses increased, and female HPA responses decreased. The goal of the present study was to extend these studies in male and female mice by determining the influences of presenting and removing EE toys on adrenal hormone responses and on anxiety and depressive behaviors, as an animal model of stress and mental health changes

following significant loss in humans.
The EE toys, Nesting Sheets™, Gummy Bones, and Mouse Igloos® and Arches™, were chosen because of their potential to enhance nesting, gnawing, playing, and sheltering behaviors. Following a one week acclimation period, the mice were presented with EE for two weeks. At the end of the two weeks, EE was removed randomly from selected cages to form two experimental groups: 1) male and female mice that remained housed with EE, and 2) male and female mice that had lost EE. Control mice were housed without EE under standard husbandry conditions throughout. Each week, marble-burying and sucrose preference tests were performed, and fecal samples were collected. Five days after EE removal, the mice in each group were restrained for 10 min, followed by blood collection. Fecal and serum corticosterone (CORT) concentrations were determined by highly specific immunoassays.
EE increased, and EE removal decreased, anxiety and depressive behaviors in female mice. In contrast, EE decreased, and EE removal increased, anxiety and depressive behaviors in male mice. Consistent with our previous findings, removal of EE resulted in sexually diergic CORT concentrations in fecal samples as well as CORT responses after restraint stress: male CORT concentrations increased and female CORT concentrations decreased.
These data indicate that sudden changes to the housing environment of laboratory mice can affect their physiology and behavior in sexually diergic ways: Housing male mice with EE may lower their stress and affective behaviors, whereas housing female mice with EE may increase their stress and behavioral responses. In contrast, removal of EE may lower stress and affective behaviors in female mice, while increasing them in male mice.

Disclosures: M. Rhodes: None. **M.L. Jenkins-Andrews:** None. **M.R. Bisignani:** None. **C.M. Monroy:** None. **S.A. Livelsberger:** None. **G.C. Noel:** None. **R.T. Rubin:** None.

Poster

PSTR393. Consequences of Diet, Exercise or Environment

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR393.15/D58

Topic: F.04. Neuroimmunology

Support: NSERC
Canada Research Chair

Title: Running to remember: The effects of exercise on perineuronal nets, microglia, and vascularization in female and male mice

Authors: *M. G. MAHEU[1], N. JAMES[1], Z. CLARKE[2], A. YANG[1], S. M. BEAUDETTE[1], R. PATEL[3], R. E. K. MACPHERSON[1], P. DUARTE-GUTERMAN[4];
[1]Hlth. Sci., [2]Psychology, [3]Neurosci., [4]Psychology, Ctr. for Neurosci., Brock Univ., St Catharines, ON, Canada

Abstract: Exercise is accepted as a positive health behaviour; however, the mechanisms underlying its role in neuroprotection and cognitive health are less well understood. The purpose of this investigation was to explore the neurobiological benefits of treadmill exercise in female and male mice through its role in regulating microglia (Iba-1), cerebral vascularization (CD-31), and perineuronal net (PNN, with WFA) expression. We aimed to examine how these neurobiological changes relate to spatial memory outcomes. C57BL/6J mice were assigned to a sedentary (12F/12M) or exercise group (11F/12M). Mice were treadmill-trained for an hour per day, five days per week, at increasing speeds and incline for eight weeks. During the final week of the exercise intervention, all mice were trained on a spatial memory task (Barnes Maze) for five consecutive days. A probe trial was conducted, after which brains were perfused for immunohistochemical analysis. Exercised mice incurred fewer errors than sedentary mice during the first two days of training as well as the probe. Females, regardless of exercise regimen, made fewer errors during training, had shorter latencies, and had a greater frequency of spatial strategy use compared to males. Exercised mice, regardless of sex, expressed fewer PNNs in the dentate gyrus compared to sedentary controls. Dorsal PNNs were positively correlated with greater errors on the first day of training, and this effect was strongest among females. PNNs were negatively correlated with microglia in the ventral dentate gyrus, and this effect was greatest among the exercised females. Microglia count and cerebral vascularization were not affected by exercise. Our study suggests that exercise improves spatial memory and decreases PNNs in the dentate gyrus, but may pose greater cognitive benefits to females compared to males.

Disclosures: **M.G. Maheu:** None. **N. James:** None. **Z. Clarke:** None. **A. Yang:** None. **S.M. Beaudette:** None. **R. Patel:** None. **R.E.K. MacPherson:** None. **P. Duarte-Guterman:** None.

Poster

PSTR393. Consequences of Diet, Exercise or Environment

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR393.16/D59

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH R01MH109471

Title: Glutamatergic synapse properties are rapidly modulated by ERα but not ERβ or GPER1 activation in female rat nucleus accumbens core medium spiny neurons

Authors: A. KRENTZEL, C. MILLER, ***J. MEITZEN**;
North Carolina State Univ., Raleigh, NC

Abstract: Sex steroid hormones such as 17β-estradiol (estradiol) modulate the nucleus accumbens core (NAcc), a sexually differentiated brain region integral for motivated behaviors and addiction and mental health disorders. Estradiol action upon the NAcc is sex-specific, with increased sensitivity exhibited by adult females compared to males. One estradiol action in adult female rat NAcc is rapid modulation of excitatory synapse properties of medium spiny neurons

(MSNs), the predominant neuron type of the NAcc. Topical estradiol application acutely and rapidly decreases the frequency of MSN miniature excitatory postsynaptic currents (mEPSCs), mimicking natural fluctuations in mEPSC properties observed across estrous cycle phases. We tested whether activation of estrogen receptor α (ER), ERβ, or GPER1 was sufficient for inducing changes in mEPSC properties assessed via whole-cell patch clamp in acute brain slices of adult female rat NAcc. The ERα agonist PPT induced robust decreases in mEPSC frequency, while the ERβ agonist DPN and the GPER1 agonist G1 did not. These data indicate that activation of ERα is sufficient for inducing changes in mEPSC frequency, implicating this receptor as a primary target for future research.

Disclosures: **A. Krentzel:** None. **C. Miller:** None. **J. Meitzen:** None.

Poster

PSTR393. Consequences of Diet, Exercise or Environment

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR393.17/D60

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant DK090823
NIH Grant GM133510
Miami University Committee Faculty Research Award

Title: Impact of estrogen on gut microbiome and their metabolites and cognitive function in young adult rats following adolescent stress

Authors: M. XU[1], K. KROLICK[1], J. ZHU[2], *H. SHI[1];
[1]Biol., Miami Univ., Oxford, OH; [2]Human Sciences; James Comprehensive Cancer Ctr., The Ohio State Univ., Columbus, OH

Abstract: Adolescence is a period with hormonal changes and continuing brain maturation. Chronic stress with elevated glucocorticoids due to disruption of the hypothalamic-pituitary-adrenal (HPA) axis during this developmental stage increases the risk for psychological and cognitive maladies. The brain-gut axis serves as two-way communication connecting the central nervous system (CNS) that regulates behaviors and the gastrointestinal tract where a diverse population of microorganisms resides. The gut microorganisms constantly produce metabolites that provide feedback to the CNS. Both rodents and humans display estrogen-dependent stress responses. It is unclear how estrogen affects gut microbial population and their metabolites and cognitive behavior following stress. Female rats at 4 weeks of age, sham or ovariectomy (OVX), with or without estradiol (E2) replacement, underwent 13 days 1 hour/day restraint stress. Plasma corticosterone (CORT) levels during stress were measured using ELISA. After chronic stress or no-stress in 6-week-old rats, a complex-paradigm reward-nose-poke design known as reversal learning behavioral test was used to assess cognitive flexibility, which represents the ability of behavioral adjustment in new environmental challenges and is associated with

increased resilience to negative life events such as stress. The gut microbial population and their metabolites were characterized in cecum fecal samples using integrated genomic analysis and mass spectrometry (MS)-based metabolic profiling. Stress increased CORT levels of sham and OVX+E2 rats compared to their respective no-stress rats at the beginning of the stress period, but such increase disappeared at the end of the stress period. When estrogen level was reduced in OVX+Oil rats, the HPA axis stress response persisted, indicated by increased CORT levels throughout the entire stress period. Stress OVX+E2 rats, but not OVX+Oil rats, showed an increased rate of reversal learning compared to their no-stress counterparts, suggesting that E2 enhances coping following stress. Beta diversity of microbial community indicated distinctly separated clusters between no-stress and stress rats in sham and OVX+E2 groups, but not OVX+Oil group. Microbial metabolic analysis revealed a similar attribute, with differences in major metabolic pathways between stress and no-stress rats of sham and OVX+E2 groups, but not OVX+Oil group. The findings suggest that estrogen may benefit brain-gut homeostasis and improve cognitive functioning, at least partially via relieving prolonged stress response involving the HPA axis and maintaining changes in gut microbiota and their metabolites.

Disclosures: **M. Xu:** None. **K. Krolick:** None. **J. Zhu:** None. **H. Shi:** None.

Poster

PSTR393. Consequences of Diet, Exercise or Environment

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR393.18

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH R01 DK056731

Title: Lepr-pirt neurons of the hypothalamic arcuate nucleus promote pituitary reproductive function

Authors: Y. WANG[1], A. TOMLINSON[1], J. G. SANTINGA[1], Q. WEIWEI[1], A. C. RUPP[1], C. F. ELIAS[2], S. M. MOENTER[3], *M. MYERS, Jr[1];
[2]Mol. and Integrative Physiol., [3]Mol. and Integrative Physiology, Intrnl. Medicine, and Obstetrics and Gyne, [1]Univ. of Michigan, Ann Arbor, MI

Abstract: Secreted by adipocytes, leptin acts on leptin receptor (LEPR) neurons in the central nervous system to signal the repletion of body fat/energy stores. In addition to decreasing appetite and food intake, adequate leptin concentrations permit the expenditure of energy on a host of energy-intensive processes, including basal metabolism, immune function, and the neuroendocrine reproductive and growth axes. The specific Lepr cell types that mediate each effect have yet to be defined, however. Our recent single nucleus RNA sequencing analysis identified several novel populations of hypothalamic Lepr neurons, including an arcuate nucleus (ARC) populations marked by the expression of Pirt (LeprPirt neurons). While LeprPirt neurons reside in the ARC, they are distinct from other well-known populations of ARC Lepr neurons,

including those that contain POMC, AgRP, or kisspeptin/dynorphin. While these other populations of ARC neurons mainly project into the hypothalamus, ARC Pirt neurons (a surrogate for LeprPirt cells) project mainly into the median eminence, suggesting their potential role in the control of pituitary function. We used the stereotaxic injection of cre-dependent AAVs to express Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) in ARC Pirt cells to examine their function. Activating these cells did not alter food intake or energy balance. In contrast, this stimulus increased circulating concentrations of luteinizing hormone (LH) and follicle stimulating hormone (FSH) while decreasing growth hormone (GH); ACTH, TSH, and Prolactin were unaltered. Consistently, the activation of ARC Pirt neurons in diestrous female mice promoted entry into estrus as measured by vaginal cytology. These data are consistent with the notion that ARC Pirt neurons project into the median eminence to activate the pituitary reproductive axis, linking energy balance and other signals to the neuroendocrine control of reproductive function.

Disclosures: Y. Wang: None. **A. Tomlinson:** None. **J.G. Santinga:** None. **Q. Weiwei:** None. **A.C. Rupp:** None. **C.F. Elias:** None. **S.M. Moenter:** None. **M. Myers:** 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.; AstraZeneca, Novo Nordisk, Eli Lilly and Company.

Poster

PSTR393. Consequences of Diet, Exercise or Environment

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR393.19/D61

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIMH IRP Funding

Title: Presynaptic Dopamine Synthesis Capacity during the Menstrual Cycle in Healthy Women Studied with Positron Emission Tomography

Authors: *C. RECTO[1,2], S.-M. WEI[2,3], D. P. EISENBERG[2], P. D. KOHN[2], M. D. GREGORY[2], J. B. CZARAPATA[2], M. N. GOLDBERG[2], I. M. WILDER[2], P. J. SCHMIDT[3], K. F. BERMAN[2];
[2]Section on Integrative Neuroimaging, Clin. & Translational Neurosci. Br., [3]Behavioral Endocrinol. Br., [1]Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Background: Sex differences in the course and prevalence of dopamine-related neuropsychiatric disorders such as schizophrenia and Parkinson's disease have raised the possibility of clinically important effects of sex hormones on the central dopamine system. Preclinical experiments have identified a number of sex hormone-dopamine interactions, including rodent studies finding that estradiol may facilitate presynaptic dopamine function, though human data are limited. In menstruating women, we hypothesized that presynaptic

dopamine synthesis capacity would be increased in the follicular phase of the menstrual cycle, when estradiol levels are both higher and unopposed by progesterone compared to the luteal phase.
Methods: To directly measure presynaptic dopamine synthesis capacity, [^{18}F]-FDOPA PET scans were obtained for thirty-one healthy, regularly menstruating women. A separately-collected T1-weighted anatomical MRI scan was segmented to provide a cerebellar reference region and was co-registered to each participant's realigned, attenuation-corrected PET data. Using the Gjedde-Patlak method, extracted cerebellar time activity curves were used to calculate the specific uptake constant K_i. Voxel-wise analyses ($p=0.005$, uncorrected) were performed using SPM within the striatum to compare eleven women studied during the follicular phase (days 4-11 of menstrual cycle; mean age=39.0±11.5 years) to twenty women studied during the luteal phase (days 18-39; mean age=35.0±9.0 years).
Results: We observed greater [^{18}F]-FDOPA K_i in the follicular relative to luteal phase in bilateral striatum, specifically within the left putamen, right caudate, and right ventral striatum (peak $p=0.001$). No effects were observed in the opposite direction ($p<0.05$).
Conclusions: Compared with the luteal phase, greater [^{18}F]-FDOPA uptake was found in women assessed during the follicular phase, which is associated with relatively higher estradiol levels. Our results align with preclinical evidence suggesting estradiol's modulatory role within the dopamine system, and, if confirmed longitudinally, may provide impetus for more directed studies in clinical populations. Future work will additionally explore associations between the menstrual cycle and other aspects of the dopamine system, such as dopamine receptor density.

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Poster

PSTR393. Consequences of Diet, Exercise or Environment

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR393.20/D62

Topic: F.04. Neuroimmunology

Support: NCI Grant K99CA273424
NCI Grant R21CA276027
NCI Grant R01CA194924
NIGMS P20GM121322
NIGMS S10OD028605

Title: Social enrichment alters the response of brain leukocytes to chemotherapy and tumor development in aged mice

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Abstract: Age is a risk factor for the development of breast cancer. Breast cancer patients frequently report alterations in behavior and cognition. Foundational science studies have supported associations among neuroinflammation, breast cancer, and chemotherapy, but to date, these associations are based on studies using young adult rodents. The current study examined the neuroinflammatory effects of chemotherapy in aged, tumor-naïve and tumor-bearing mice with or without social enrichment. We hypothesized that chemotherapy increases neuroinflammation via increased peripheral immune cell trafficking within the brain and predicted this effect would be modulated by social enrichment. Mice received two intravenous injections of doxorubicin (A; 9mg/kg) and cyclophosphamide (C; 90mg/kg) separated by two weeks. Brain immune cells were enriched and assessed via flow cytometry seven days following the second chemotherapy injection. Contrary to our hypothesis, social enrichment enhanced peripheral immune cell trafficking in aged tumor-naive mice treated with AC. However, group-housed aged tumor bearing mice receiving AC displayed a reduced percentage of IL-6+ monocytes and granulocytes relative to their singly- housed counterparts, demonstrating the ability of social enrichment to attenuate some aspects of neuroinflammation in aged mice. There was no effect on tumor growth when aged social enrichment partners were used. In contrast, social enrichment with young mice reduced TNF^{+} monocytes, tumor volume, and tumor mass in aged tumor bearing mice relative to their singly-housed counterparts. These data add to the literature detailing the beneficial effects of social enrichment and are the first to demonstrate that social support with young housing partners reduces tumor growth in aged mice.

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Poster

PSTR394. Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR394.01/D63

Topic: C.01. Brain Wellness and Aging

Support: National Research Foundation of Korea (NRF) Grant 2023R1A2C1004955
Ministry of Trade, Indstry & Energy (MOTIE, Korea) Grant 20009707

Title: Mitochondrial fission inhibition by isoliquiritigenin ameliorates LPS-induced Inflammation in BV-2 microglial cells

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Abstract: Excessive microglial cell activation in the brain can lead to the production of various neurotoxic factors (e.g., pro-inflammatory cytokines, nitric oxide) which can, in turn, initiate neurodegenerative processes. Recent research has been reported that mitochondrial dynamics regulate the inflammatory response of lipopolysaccharide (LPS). Isoliquiritigenin (ISL) is a compound found in Glycyrrhizae radix with anti-inflammatory and antioxidant properties. In this study, we investigated the function of ISL on the LPS-induced pro-inflammatory response in BV-2 microglial cells. We showed that ISL reduced the LPS-induced increase in pro-inflammatory mediators (e.g., nitric oxide and pro-inflammatory cytokines) via the inhibition of ERK/p38/NF-κB activation and the generation of reactive oxygen species (ROS). Furthermore, ISL inhibited the excessive mitochondrial fission induced by LPS, regulating mitochondrial ROS generation and pro-inflammatory response by suppressing the calcium/calcineurin pathway to dephosphorylate Drp1 at the serine 637 residue. Interestingly, the ISL pretreatment reduced the number of apoptotic cells and levels of cleaved caspase3/PARP, compared to LPS-treated cells. Our findings suggested that ISL ameliorated the pro-inflammatory response of microglia by inhibiting dephosphorylation of Drp1 (Ser637)-dependent mitochondrial fission.

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Poster

PSTR394. Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR394.02/D64

Topic: C.01. Brain Wellness and Aging

Support: National Research Foundation of Korea (NRF) Grant 2023R1A2C1004955
Ministry of Trade, Indstry & Energy (MOTIE, Korea) Grant 20009707

Title: Amyloid beta oligomers intensify ER stress-associated apoptotic cell death by upregulating of Parkin expression levels.

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Abstract: Recently, Parkin has been reported to induce endoplasmic reticulum (ER) stress. In addition, amyloid beta oligomers (AβO), hallmarks of Alzheimer's disease (AD), also increase ER stress in neurons. Because a mutation in the Parkin gene is a well-known predominant cause of familial Parkinson's disease (PD), Parkin has been well studied in PD but has not been well researched in AD. In this study, we investigated the role of AβO-mediated Parkin associated with ER stress in AD. For AD-based research, we used AβO treatments in mouse hippocampus derived HT-22 cells. We stably expressed Parkin in HT-22 cells to confirm the hypothesis and used siParkin for downregulation of Parkin expression. Moreover, using hippocampi from amyloid precursor protein/presenilin 1/Tau triple transgenic mice (3xTg-AD mice), which are used for AD models, we confirmed the relationship between ER stress and Parkin in vivo. We observed that ATF4 upregulated AβO-increases in Parkin. Parkin overexpression aggravated ER stress in AβO-treated HT-22 cells and the hippocampi of 3xTg-AD mice. Parkin downregulation led to no significant change when compared to AβO-treated cells. Moreover, Parkin-mediated ER stress was not related to oxidative stress. Our study indicates that AβO-induced ATF4 upregulated Parkin levels and that Parkin increases ER stress as a positive feedback loop. Through this study, our findings provide a foundation for future studies on the specific mechanisms related to the role of Parkin in AD.

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Poster

PSTR394. Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR394.03/D65

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Support: National Research Foundation of Korea (NRF) Grant 2023R1A2C1004955
Ministry of Trade, Indstry & Energy (MOTIE, Korea) Grant 20009707

Title: Peroxiredoxin 2 relives amyloid-beta oligomer associated autophagy through ROS-NRF2-p62 pathway in N2a-APP Swedish cells

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Abstract: In Alzheimer's disease, reactive oxygen species (ROS) are generated by the deposition of amyloid-beta oligomers (AβOs), which represent one of the important causes of neuronal cell death. Additionally, AβOs are known to induce autophagy via ROS induction. Previous studies have shown that autophagy upregulation aggravates neuronal cell death. In this study, the effects of peroxiredoxin 2 (Prx2), a member of the peroxidase family of antioxidant enzymes, on regulating AβO-mediated autophagy were investigated. Prx2 decreased AβO-mediated oxidative stress and autophagy in N2a-APPswe cells. Further, we examined the relationship between the neuronal protective effect of Prx2 and a decrease in autophagy. Similar to the effects of N-acetyl cysteine, Prx2 decreased AβO-induced ROS and inhibited p62 protein expression levels by downregulating the activation of NRF2 and its translocation to the nucleus. In addition, treatment with 3-methyladenine, an autophagy inhibitor, ameliorates neuronal cell death. Overall, these results demonstrate that the Prx2-induced decrease in autophagy was associated with the inhibition of ROS via the ROS-NRF2-p62 pathway in N2a-APPswe cells. Therefore, our results revealed that Prx2 is a potential therapeutic target in anti-Alzheimer therapy.

Disclosures: G. Kim: A. Employment/Salary (full or part-time):; School of Life Sciences, BK21 FOUR KNU Creative BioResearch Group, Kyungpook National University, Daegu 41566, Republic of Korea. **D. Lee:** A. Employment/Salary (full or part-time):; School of Life Sciences, BK21 FOUR KNU Creative BioResearch Group, Kyungpook National University, Daegu 41566, Republic of Korea.

Poster

PSTR394. Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR394.04/D66

Topic: C.01. Brain Wellness and Aging

Support: Intramural Research Program at NIDA, NIH

Title: Expression and activity of rat esterases in response to a high fat diet

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Abstract: The endoplasmic reticulum (ER) contains the highest level of intracellular calcium. Calcium is imperative to proper protein folding, modification, processing, and secretion. Perturbations to ER calcium have previously been shown to activate the unfolded protein response (UPR) and elicit a mass departure of ER resident proteins in a phenomenon called exodosis. Exodosis has been associated with increased extracellular esterase activity, mediated by ER-resident carboxylesterase (Ces) proteins. Ces proteins metabolize ester containing compounds and drugs. Previous work from our lab showed that a high-fat diet (HFD) causes increased blood levels of Ces activity. To test whether the HFD only increases the secretion of esterases or alters the expression of Ces1 in the liver and brain, we measured mRNA expression of the different Ces1 isoforms from rats on HFD compared to the standard chow diet. Consistent with previous results, we found that HFD increased esterase activity in the plasma. Liver and brain tissue were analyzed for Ces1 mRNA expression using digital polymerase chain reactions (dPCR). Using multiplex dPCR assays that we developed to measure multiple Ces1 isoforms and other genes related to ER stress, we found that expression of Ces1 isoforms and ASNS, a gene associated with the unfolded protein response (UPR) increased in rat tissue that underwent HFD. Studying how HFD can influence esterase activity may advance our understanding of medicinal and illicit drug metabolism and pharmacology.

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Poster

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Topic: C.01. Brain Wellness and Aging

Support: NINDS BINP R01 NS117754
T32 AG052375

Title: Does Xanthine Oxidase accelerate Alzheimer's Disease-related pathologies in a high fat and fructose diet?

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Abstract: Approximately 42% of individuals in the United States suffer from obesity, widely caused by low activity and poor diet. This percentage is only predicted to grow and leaves countless Americans at risk for other serious comorbidities, including an increased risk for

Alzheimer's Disease (AD). The main hallmarks of AD are cognitive decline, neuroinflammation, and oxidative stress that worsen as the disease progresses. Nonalcoholic steatohepatitis (NASH), another comorbidity of obesity, is characterized by an irreversible fat build-up in the liver and the increased presence of reactive oxygen species. We hypothesized that oxidative stress driven by xanthine oxidase (XO) is a main factor for the accelerated accumulation of AD-related pathologies caused by a NASH diet. In order to test this theory, we used a hepatic xanthine dehydrogenase knockout (XDH-KO), floxed control (FLX), and wild-type control (WT) mouse model and induced obesity through a NASH diet. We administered a 40% fat and 25% fructose diet for 9 months to the XDH-KO and FLX groups, while the WT group was given normal chow. At 12 months of age, the mice were euthanized, and whole brain samples were collected. These samples were homogenized and processed for protein quantification using western blot analysis and detection by fluorescence. Tau had a 74% increase in the FLX NASH diet group compared to the WT control diet groups. However, Tau was elevated only by 13% in the XDH-KO NASH diet compared to the WT control diet. Additionally, significantly higher levels of amyloid beta were found in the FLX NASH diet compared to the XDH-KO NASH diet ($p<0.05$). Tau and amyloid beta are two major proteins associated with AD, and our study supports the previous literature that has shown the upregulation of these compounds within a high fat and fructose diet. However, our data show that knocking out XO production hepatically in mice on a NASH diet decreases the levels of these two major products of AD. This data suggests that XO is a driving factor that leads to the accumulation of AD-related pathologies within high fat and fructose diets.

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Poster

PSTR394. Metabolism, Oxidative Stress, and Cellular Mechanisms

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Topic: C.01. Brain Wellness and Aging

Support: NIH R01NS064263
NSF 1755019

Title: Molecular analysis of neural mechanisms controlling organismal responses to oxidative stress

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Abstract: Organisms have evolved protective strategies that are geared toward limiting cellular damage and enhancing organismal survival under environmental stresses. There is considerable emerging evidence for neuronal regulation of organismal stress responses, but the underlying

molecular mechanisms remain unclear. Reactive Oxygen Species (ROS) such as superoxide and peroxides can cause oxidative stress and cellular damage by reacting with biomolecules. We are exploring neuronal mechanisms for initiating or regulating responses to oxidative stress, using the nematode *Caenorhabditis elegans* as a model. The neurotransmitters and signaling mechanisms used by *C. elegans* neurons are highly conserved. From a survey of six neurotransmitter systems (glutamate, acetylcholine, GABA, serotonin, dopamine and octopamine), we found that cholinergic and glutamatergic transmission have significant roles in promoting antioxidant defenses. Specifically, we observed that animals carrying null or hypomorphic mutations in genes related to vesicular loading of these two transmitters have increased susceptibility to the toxic effects of prolonged exposure to the ROS generator paraquat (PQ). Importantly, these mutations have no effects on lifespan in the absence of PQ, suggesting that glutamate and acetylcholine neurotransmission are important for coordinating organismal protective responses. To determine how specific receptor types may be involved in antioxidant signalling, we measured the survival of *C. elegans* strains carrying mutations in candidate genes encoding various receptor subunits during prolonged oxidative stress. We found that mutations in the metabotropic glutamate receptor genes *mgl-1* and *mgl-2* increased susceptibility to oxidative damage while mutation of genes encoding ionotropic AMPA- and NMDA-type glutamate receptor subunits had little effect. Null mutations that affected the metabotropic acetylcholine receptor *gar-3* also increased susceptibility to PQ. In contrast, a null mutation in the *ric-3* gene that encodes a chaperone broadly required for assembly of ionotropic nicotinic acetylcholine receptors had little effect. Survival assays following cell specific inactivation of the vesicular acetylcholine transporter gene *unc-17* suggested cholinergic motor neurons are particularly important for protection from oxidative damage. We are now using RNA sequencing to investigate how loss of cholinergic and glutamatergic neurotransmission affect transcriptional activation of oxidative stress response pathways and are excited to explore downstream signalling mechanisms in neural regulation of these pathways.

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Poster

PSTR394. Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR394.07/D69

Topic: C.01. Brain Wellness and Aging

Title: Are Glx and GABA levels changed in type 2 diabetes patients?

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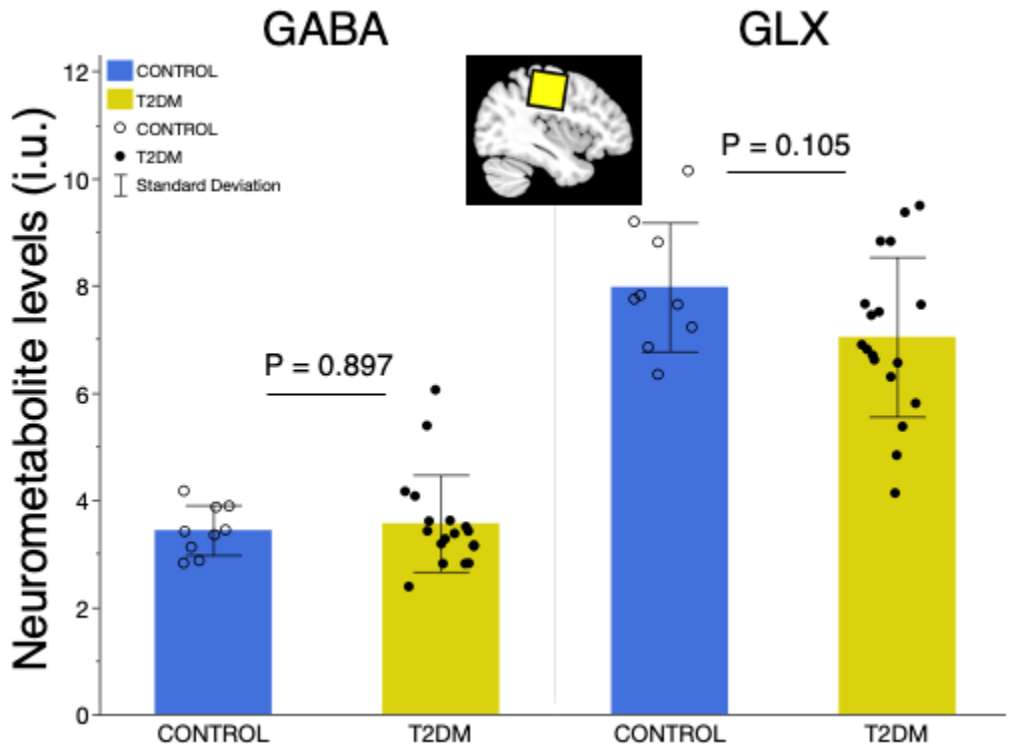
Abstract: Background: Next to the cardiovascular complications, type 2 diabetes mellitus (T2DM) also affects brain function, structure and metabolism. Mild cognitive impairment (MCI) is prevalent in around 45% of all T2DM patients (Chakraborty et al., 2021), and T2DM is causally related to the development of unspecified or vascular dementia (Benn et al., 2020). Recent studies reported significant unfavourable changes in the brain of T2DM patients, such as a decreased regional grey matter volume, and altered intrinsic activity mainly in the default mode network (Li et al., 2022). Additionally, there is evidence that the brain's neurometabolites change dramatically, suggesting disturbances in metabolism and neurotransmission (Zhao et al., 2018). Hence, T2DM patients are at high risk for brain complications. **Aim:** To determine differences in neurometabolite levels between T2DM patients and healthy controls using HERCULES (Oeltzschner et al., 2019), a novel magnetic resonance spectroscopy (MRS) sequence. This may help to determine better how T2DM mechanistically contributes to brain disorders, such as Alzheimer's disease and MCI. **Methods:** The data were derived from 18 T2DM patients and 9 healthy controls, with a male to female ratio of 3 to 1 and an age range between 51 and 73 years. By means of a 3T MRI scanner, we compared neurometabolite levels in the posterior cingulate cortex (PCC), the left primary sensorimotor cortex (LSM), and the medial prefrontal cortex (mPFC) between patients and controls. Although a full spectrum analysis was performed, we focussed on glutamine/glutamate (Glx) and GABA. **Results:** The data was analysed using the GANNET pipeline (Edden et al., 2014). Visual inspection of the data revealed that the spectral quality and fit was insufficient for data-analysis in the PCC and mPFC. For LSM the data quality was sufficient, however, there were no significant group differences nor for GABA (P = 0.897) neither for Glx (P = 0.105) levels (Figure 1). **Conclusion:** Our results suggest that GABA and Glx levels in the LSM do not differ between T2DM patients and healthy controls.



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Poster

PSTR394. Metabolism, Oxidative Stress, and Cellular Mechanisms

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Topic: C.01. Brain Wellness and Aging

Support: PAPIIT-IN201621

Title: Prolactin regulate the antioxidant response of astrocytes through the activation of STAT3 signaling pathway.

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Abstract: Astrocytes exert a wide variety of functions in health and disease, including regulating defense against oxidative stress. Agents that improve astrocytes antioxidant defenses could be potential therapies for brain pathologies associated with oxidative stress The STAT3 signaling is a key pathway through which astrocytes regulate their antioxidant response. In this regard, prolactin (PRL) displays antioxidant and cytoprotective effects in retinal pigmented epithelial cells and JAK/STATs are an important pathway associated with prolactin receptor (PRLR) signaling. This study sought to determine the protective effect of PRL against hydrogen peroxide (H_2O_2)-induced cytotoxicity in primary rat cortical astrocytes in culture. PRL treatment led to increased expression of the long PRLR isoform, and this response was associated with upregulation of STAT3 phosphorylation/activation, increased antioxidant capacity, and both the transcriptional upregulation and increased enzymatic activity of the antioxidant enzymes: superoxide dismutases, glutathione peroxidase and peroxiredoxins. H_2O_2 induced cell death, increased ROS generation, lipid peroxidation and protein oxidation. Preincubation with PRL protected astrocytes against H_2O_2-induced cell death and increased lipids and proteins oxidative damage. Pharmacological blockade of STAT3 (S31-201) or genetic deletion of the long PRLR isoform ($Prlr^{-/-}$) suppressed the protective effect of PRL against H_2O_2-induced cell death and oxidative damage, by decreasing both antioxidant capacity and the activity of antioxidant enzymes. Our data suggest that PRL might represent a promising strategy for the treatment of brain pathologies associated with oxidative stress.
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Poster

PSTR394. Metabolism, Oxidative Stress, and Cellular Mechanisms

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Topic: C.01. Brain Wellness and Aging

Support: NIH Grant 1R21AG081763-01
NIH Grant R01 AG062509
NIH Grant R01 AG070761

Title: Selective impairment of interaction of ADP and ATP synthase by anesthetics

Authors: *F. LIANG[1,2], K. KURMI[3], S. JOSHI[3], M. HAIGIS[3], Y. GUO[4], Z. XIE[2]; [1]Massachusetts Gen. Hosp., Charlestown, MA; [2]Dept. of Anesthesia, Critical Care and Pain Med., Massachusetts Gen. Hosp. and Harvard Med. Sch., Charlestown, MA; [3]Dept. of Cell Biology, Blavatnik Institute, Harvard Med. Sch., Boston, MA; [4]Swansea Univ., Swansea, United Kingdom

Abstract: Introduction: General anesthesia has been reported to be associated with a higher risk of developing dementia in patients and cognitive impairment in animals. Our research has demonstrated that anesthetic isoflurane, but not desflurane, can induce apoptosis and promote the generation of Aβ by impairing mitochondrial function. However, the underlying mechanism remains largely unknown. We, thus, employed nanobeam technology and other methodologies to investigate the potential differences between isoflurane and desflurane on the interaction between ATP synthase and ADP at single molecular level, and the associated changes in mitochondrial function. **Method**: Human H4-APP cells were subjected to 2% isoflurane or 12% desflurane treatment for 6 hours. LC-MS based metabolomics analysis were used to differentiate the effects of isoflurane and desflurane. Mitochondrial respiration was assessed using a Seahorse XFp Extracellular Flux Analyzer. Nanobeam technology was used to investigate the interaction between ADP and ATP synthase at the single molecular level. Additionally, molecular dynamics simulation was employed to identify the specific amino acids involved in the binding of isoflurane or desflurane with ATP synthase. Through the use of CRISPR/Cas9 technology, we mutated the identified amino acid and conducted rescue studies using the mutant cells and peptides. **Results**: Isoflurane and desflurane exhibit distinct metabolic profiles, as desflurane only increased the levels of four metabolites, whereas isoflurane increased the levels of 13 metabolites and decreased 10 compared to the control. Moreover, isoflurane, but not desflurane, reduced mitochondrial metabolic function. Nanobeam technology demonstrated the impairment of the interaction between ATP synthase and ADP at the single molecular level by isoflurane, but not desflurane. Molecular dynamics simulation identified the 549th amino acid residue, alanine, of ATP synthase as the site of action for isoflurane. Finally, the mutation of alanine 549 residues of ATP synthase attenuated the effects of isoflurane. **Conclusion**: Our data provide compelling evidence of the distinct effects of isoflurane and desflurane on the alterations linked to the pathogenesis of Alzheimer's disease. The findings indicate that the mutation of a specific amino

acids residue, alanine 549 of ATP synthase, to cysteine or serine, can attenuate the impairment caused by isoflurane in the interaction between ATP synthase and ADP, as well as mitochondrial function. These significant findings serve to promote further investigation into the relationship between anesthesia and the pathogenesis of Alzheimer's disease.

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Poster

PSTR394. Metabolism, Oxidative Stress, and Cellular Mechanisms

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Topic: C.01. Brain Wellness and Aging

Support: BBRF NARSAD Young Investigator Award

Title: MICAL2: Bridging synaptic function and the antioxidant system in bipolar disorder

Authors: *K. KRUTH[1], P. SURYAVANSHI[2], J. B. MEHR[3], A. KARKI[4], A. WILLIAMS[1]; [1]Psychiatry, Univ. of Iowa, Iowa City, IA; [2]Pediatrics, Univ. of Iowa, Iowa city, IA; [3]Rutgers Brain Hlth. Inst., Piscataway, NJ; [4]Rutgers Univ. Behavioral and Neural Sci., Edison, NJ

Abstract: Bipolar disorder remains one of the most severe and least treatable of all neuropsychiatric illnesses, but new treatments have remained elusive due to poor understanding of its etiology. Lithium remains the gold standard of treatment, but its use comes with many caveats, including numerous side effects, a narrow therapeutic window, and the potential for life-threatening toxicity. Better treatments are desperately needed, but the mechanism behind lithium's effectiveness remains unclear, limiting our ability to improve upon its action. In 2014, Beech et al. identified MICAL2 in a screen as a top hit for genes associated with lithium responsiveness in bipolar patients, suggesting it may play a crucial role in bipolar disorder treatment. MICAL2 is an NADPH-dependent enzyme that reversibly oxidizes actin, leading to rapid cytoskeleton collapse. Intriguingly, its reliance on NADPH, the primary electron donor of the antioxidant system, ties its function to the cell's redox balance, which has been shown to be altered in patients with bipolar disorder. However, to our knowledge, MICAL2 has never before been studied in brain, and its role in neuronal function—and bipolar disorder—remains completely unknown. Here, we have begun to characterize the role of MICAL2 in human stem cell-derived neurons in an effort to ascertain whether it may be a viable new treatment target in bipolar disorder. Via immunofluorescence, MICAL2 appeared to precisely colocalize with synapsin 1, suggesting its role may be primarily synaptic. Western blots on synaptosomes isolated from adult mouse brain confirmed this result, showing that MICAL2 is present in both synaptic and nuclear/cytosolic cellular fractions, though the banding pattern suggests there may be synapse-specific isoforms of MICAL2. We then knocked down MICAL2 in human iPSC-derived neurons and analyzed structural changes via super resolution microscopy and functional

changes through calcium imaging and patch clamp. Our results suggest that MICAL2 may play an important role in synaptic function, and further research is warranted to ascertain whether it may be a viable treatment target for bipolar disorder.

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Poster

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Topic: C.01. Brain Wellness and Aging

Support: NSTC-109-2314-B-195-015-MY3
MMH-E-111-02

Title: Impact Of Mitochondrial G13513A Heteroplasmic Mutation On Mitochondrial Function

Authors: *D. LIN, T.-Y. WU, T.-H. LEE, Y.-W. HUANG, Z.-D. HUANG, C.-S. HO; Mackay Mem. Hosp., Taipei, Taiwan

Abstract: Mitochondria play a vital role in cellular energy production through oxidative phosphorylation (OXPHOS) in the respiratory complexes. Mitochondrial DNA alterations have the potential to disrupt mitochondrial function and contribute to the development of heterogeneous diseases. Leigh syndrome (LS) is a progressive neurodegenerative disorder commonly observed in infants and children, characterized by bilateral symmetric necrosis of the basal ganglia and/or brain stem. The G13513A mutation in mitochondrial DNA is a frequent cause of LS with isolated complex I deficiency, although its impact on mitochondrial function remains poorly understood. In this study, we determined the heteroplasmy of the mitochondrial G13513A variant in leukocytes, buccal mucosa, hairs, urine sediments, and dermal fibroblasts derived from two LS patients (LS1: 2 years and 5 months old boy and LS2: 1-year-old boy). We investigated various mitochondrial functions, including bioenergetics, ATP production, reactive oxidative species (ROS) generation, and mitochondrial membrane potential (MMP), in fibroblasts. The heteroplasmy of the mt G13513A variant (M1/M2) was found to be 36%/47%, 43%/58%, 31%/58%, 70%/82%, and 67%/33% in leukocytes, buccal mucosa, hairs, urine sediments, and dermal fibroblasts, respectively. Bioenergetic profiles, measured by real-time oxygen consumption rate (OCR) using a Seahorse Extracellular Flux Analyzer, revealed significant differences in basal, ATP-production, maximal respiration, and spare respiratory capacity between control and LS fibroblasts (C/M1/M2). The corresponding values for these parameters were 9.9/4.2/6.4, 7.8/1.7/5.3, 15.7/5.3/9.6, and 5.8/1.1/3.2 pmol/min/protein, respectively. ATP production in C/M1/M2 fibroblasts was 33.7/29/31 nmol/mg protein. MMP and ROS levels in C/M1/M2 fibroblasts were measured as 1/0.88/0.72 and 1/1.49/0.98 fold of the normal, respectively, using flow cytometry and fluorescent dyes TMRE and DCFDA. Our

results demonstrate that complex I deficiency caused by mt G13513A mutation leads to impaired mitochondrial bioenergetics, elevated ROS levels, reduced MMP, and decreased ATP production. Furthermore, heteroplasmy of mt G13513A above 60% exhibited a more pronounced impact on mitochondrial function. These findings validate the pathogenic role of mt G13513A variants in LS and provide insights into the pathological mechanisms involved, using primary fibroblasts derived from LS patient

Disclosures: **D. Lin:** None. **T. Wu:** None. **T. Lee:** None. **Y. Huang:** None. **Z. Huang:** None. **C. Ho:** None.

Poster

PSTR394. Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR394.12/E4

Topic: C.01. Brain Wellness and Aging

Support: R01EY028917
Prevention of Blindness

Title: Pgc-1α Repression dysregulates lipid metabolism and induces lipid droplet accumulation in retinal pigment epithelium

Authors: S. ZHOU, ***K. TASKINTUNA**, J. HUM, J. GULATI, S. OLAYA, J. STEINMAN, N. GOLESTANEH;
Ophthalmology, Georgetown Univ., Washington, DC

Abstract: The purpose of this study is to investigate the role of peroxisome-proliferator-activated receptor-γ coactivator 1α (PGC-1α) in lipid metabolism in the retinal pigment epithelium (RPE). As the leading cause of blindness in the elderly, age-related macular degeneration (AMD) is a debilitating late-onset neurodegenerative disease which results in loss of central vision. Drusen are a hallmark characteristic of AMD and lipid droplets are reported in RPE from AMD donors. However, the mechanisms underlying these disease phenotypes remain elusive. Previously, we showed that PGC-1α repression, combined with a high-fat diet, induces AMD-like phenotypes in mice. We also reported increased PGC-1α acetylation and thus deactivation in the RPE derived from AMD donor eyes. Here, through a series of *in vivo* and *in vitro* experiments we show that PGC-1α plays an important role in RPE and retinal lipid metabolism and functions. For *in vivo* experiments, we used the whole-body *Pgc-1α* heterozygous and knockout mice on a C57BL/6J background. We performed electroretinography to test retinal function and fundus photography to assess drusen-like deposits. For *in* vitro experiments, CRISPR-Cas9 gene editing was used to generate the ARPE19-*PGC1A* KO cell line. qPCR, Western Blotting, cell viability experiments, various assays to measure mitochondrial activity, fatty acid oxidation (FAO), and lipid peroxidation assays were performed. *In vivo* analyses showed increased accumulation of drusen-like deposits as well as impaired

photoreceptor and RPE function in both heterozygous and knockout mice compared to wild-type controls. In addition, the drusen-like deposits seemed to be bigger in size in KO compared to heterozygous. *In vitro* inhibition of PGC-1α by CRISPR-Cas9 gene editing in human RPE (ARPE19- *PGC1A* KO) reduced expression of genes responsible for lipid metabolism, fatty acid β-oxidation (FAO), and lipids biosynthesis. Furthermore, inhibition of *PGC-1α* in the RPE cells caused lipid droplets accumulation and increased lipid peroxidation. ARPE19-*PGC-1A* KO cells also showed reduced mitochondrial biosynthesis, mitochondrial dynamics and activity, FAO, mitochondrial membrane potential, loss of cardiolipin, and increased susceptibility to oxidative stress. Our data reveal an important role for PGC-1α in regulating lipid metabolism in the RPE, and shed light on mechanisms involved in lipid and drusen accumulation in the RPE and retina during aging and AMD.

Disclosures: S. Zhou: None. **K. Taskintuna:** None. **J. Hum:** None. **J. Gulati:** None. **S. Olaya:** None. **J. Steinman:** None. **N. Golestaneh:** None.

Poster

PSTR394. Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR394.13/E5

Topic: C.01. Brain Wellness and Aging

Title: Reversibly oxidized thiols on metabolic enzymes support compartmentalization of redox homeostasis in the brain and expose a transient reductive stress following global ischemia

Authors: *T. FOLEY, W. HUANG, E. PETSCHE, E. FLEMING, J. HORNICKLE;
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Abstract: Perturbations in metabolism and associated redox homeostasis have been implicated in brain aging and disease but fundamental tenets of redox biology operating in the brain, including the relevance of reversible oxidations of protein thiols, which can function as metabolic regulatory switches, remain to be established. Using redox phenylarsine oxide-affinity chromatography to capture proteins from rat brain forming reducible (i.e., potential regulatory) disulfide bonds, we report that creatine kinase B, alpha-enolase, and glyceraldehyde-3-phosphate dehydrogenase contained thiols that were selectively oxidized compared to other metabolic enzymes of interest, including neuron-specific (gamma) enolase, under experimental conditions designed to trap *in vivo* protein thiol redox states. In addition, short-term delays to tissue freezing, to allow perturbations in metabolism triggered by decapitation-induced global ischemia, exposed an early reductive shift marked by transient decreases in the extents of oxidizations of protein thiols. The results of this study (i) establish metabolic enzymes containing thiols that are preferred targets of reversible oxidations in the healthy brain, (ii) support the largely unrecognized view that neurons and astrocytes may operate at distinct protein thiol-linked redox potentials, *in vivo*, and (iii) suggest that reversals of thiol oxidations on metabolic

enzymes may occur during the earliest stages of ischemic injury, promoting rapid increases in anaerobic ATP production from glucose and creatine phosphate stores.

Disclosures: **T. Foley:** None. **W. Huang:** None. **E. Petsche:** None. **E. Fleming:** None. **J. Hornickle:** None.

Poster

PSTR394. Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR394.14/E6

Topic: C.01. Brain Wellness and Aging

Support: MQRF Postdoctoral Fellowship
Skipper Foundation Award

Title: Novel therapeutic strategies to enhance DNA repair in neurodegenerative diseases

Authors: *S. SHADFAR, F. FARZANA, M. VIDAL, C. JAGARAJ, S. PARAKH, A. S. LAIRD, J. D. ATKIN;
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Abstract: Background: DNA damage and defects in DNA repair are increasingly implicated in neurodegenerative conditions. However, there are no therapeutic approaches to prevent DNA damage in these disorders. Double-stranded DNA breaks (DSBs) are the most toxic type of damage, particularly for post-mitotic neurons because they rely entirely on the error prone non-homologous end joining (NHEJ) mechanism of repair. Previous studies in our group have established that protein disulphide isomerase (PDI) is protective against dysfunction to proteostasis induced by multiple mutant proteins *in vitro* in ALS. However, it remains unclear if PDI is also protective against DNA damage.
Objectives: In this study, we aimed to examine whether PDI is protective against DNA damage both *in vivo* and *in vitro*, to determine if it has potential as a novel therapeutic approach in ALS and/or other neurodegenerative diseases.
Methods: Two cell lines, NSC-34 and Neuro-2a, were transfected with PDI or PDI with ALS-associated mutant, TDP-43^{M337V}. Immunocytochemistry was performed to detect DNA damage induction. For *in vivo* studies, zebrafish embryos were microinjected with either RNA encoding PDI WT or a PDI mutant lacking redox activity (QUAD). At 24 hours post fertilization, embryos were incubated with 3mM H_2O_2 for 24 hours, then lysed to perform western blotting analysis.
Results: PDI was protective against DNA damage induced by etoposide and H_2O_2 in both Neuro-2a and NSC-34 cells, detected by a widely used marker, the formation of γH2AX foci. Moreover, PDI was also protective in cells expressing mutant TDP-43^{M337V}, implying it is protective against DNA damage induced during neurodegeneration. This was also confirmed using 53BP1, a specific marker for NHEJ. PDI also translocated from its normal location in the endoplasmic reticulum into the nucleus following etoposide treatment, where it was recruited

directly to sites of DNA damage, implying that it has a direct role in DNA repair. In addition, PDI was also protective *in vivo* against DNA damage induced by H_2O_2 in zebrafish. We also identified that the redox activity of PDI mediates these protective functions. From these findings, we then developed novel therapeutic peptides based on the redox activity of PDI that prevented key behavioural phenotypes in two mouse models of ALS. Hence, these data reveal that PDI and our novel therapeutic peptides are protective against DNA damage *in vivo* and *in vitro*. **Conclusion:** These results demonstrate the protective role of PDI against DNA damage both *in vivo* and *in vitro*. This study therefore has implications for future therapeutic studies based on preventing induction of DNA damage in neurodegenerative diseases.

Disclosures: S. Shadfar: None. **F. Farzana:** None. **M. Vidal:** None. **C. Jagaraj:** None. **S. Parakh:** None. **A.S. Laird:** None. **J.D. Atkin:** None.

Poster

PSTR394. Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR394.15/E7

Topic: C.01. Brain Wellness and Aging

Title: Mitochondrial responses to estrogen withdrawal in human neural cells

Authors: *P. SINCLAIR, N. LEBERT, N. KABBANI;
Neurosci., George Mason Univ., Fairfax, VA

Abstract: Estrogen is an important steroid hormone mainly produced in the ovaries but also in non-reproductive tissue including the brain and is important for the development and function. At the cellular level, estrogen signaling has been shown to regulate molecular signaling through various organelles including the mitochondria and nucleus. While there are several forms of estrogen in mammals, E2 is the most dominant form in premenopausal human females and is involved in synaptic function and brain neuroprotection. In order to explore the role of estrogen decline in neuronal health, I utilized an in vitro method that models E2 loss within the human SH-SY5Y neuronal cell line. Based on published evidence that estrogen decline is associated with mitochondrial oxidative damage. I examined how estrogen reduction impacts the mitochondrial proteome. Cells were treated with E2 for 3 days and then E2 was continued (E2+) or withdrawn (E2#) for another 3 days. Matching control cells did not receive E2 (E2-) throughout the 6-day period. Mitochondrial fractionation was used to obtain the mitochondrial enriched fraction (MEF) from cells under the 3 different treatment conditions. An LC-ESI MS/MS analysis of the MEF was used to identify proteomic changes in response to E2. The results indicate a significant effect of E2# on mitochondrial protein expression and function including increased reactive oxygen species (ROS) and cardiolipin (CL) levels. Our results also show an effect of E2 on estrogen receptor expression within the MEF. Taken together, the data reveal modifications to mitochondria in response to estrogen decline supporting the involvement of mitochondria in age-related neurodegeneration.

Disclosures: **P. Sinclair:** None. **N. Lebert:** None. **N. Kabbani:** None.

Poster

PSTR394. Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR394.16/E8

Topic: C.01. Brain Wellness and Aging

Support: Novartis Foundation for Medical Research 18C143
Swiss National Science Foundation 31003A-179294

Title: Preservation of an aging-associated mitochondrial signature in advanced human neuronal models: iPSCs derived neurons and directly converted neurons from human fibroblasts

Authors: *N. VARGHESE[1,2], L. SZABO[1,2], A. GRIMM[1,2], Z. CADER[3], I. LEJRI[1,2], A. ECKERT[1,2];
[1]Res. Cluster Mol. and Cognitive Neurosciences, Univ. of Basel, Basel, Switzerland; [2]Neurobio. Lab. for Brain Aging and Mental Hlth., Univ. Psychiatric Clinics Basel, Basel, Switzerland; [3]Nuffield Dept. of Clin. Neurosci., Univ. of Oxford, Oxford, United Kingdom

Abstract: Background information: Aging is a risk factor for several of the world's most prevalent diseases, including Alzheimer's disease (AD). Understanding the aging process in the human brain, particularly the role of mitochondria, is still poorly investigated. The main challenge is to find suitable human models to study brain aging. Animal models, brain biopsies, or carcinomic cell lines are indispensable but subject to transferability limitations, inaccessibility, and ethical constraints. Therefore, the advanced neuronal in-vitro models are of interest, including iPSCs-derived neurons (iPSCsN) or directly induced neurons (iNs) from human fibroblasts. By transiting an embryo-like state, the iPSCsN are suspected of undergoing a rejuvenation leading to a reset of the aging-associated phenotype, whereas directly converted neurons seem to retain an aging-associated donor signature. This study aimed to investigate how well the advanced neuronal models (iPSCsN and iNs) are preserving the aging donor signature, focusing on the neuronal powerhouse, the mitochondria. Methods: The bioenergetic profile of iPSCsN and iNs, both derived from the same young (n=3, Age_{mean} = 29 years) vs. aged (n=3, Age_{mean}= 67 years) donors respectively were compared by measuring the total ATP level, mitochondrial membrane potential (MMP), reactive oxygen species (ROS), mitochondrial respiration, glycolysis, mitochondrial mass, mitochondrial morphology, and mitophagy. Results: Our preliminary findings, showed that both the iNs and the iPSCsN, could maintain an aging-associated phenotype by comparing to the corresponding young neurons. With this, a decline in ATP, MMP, and mitochondrial respiration and a rise in ROS, glycolysis, and mitochondrial mass were measured in aged neurons compared to young neurons. The mitochondrial dynamics were characterized by a more fragmented mitochondrial morphology and a lower mitophagy rate in age. Conclusion: Our preliminary findings showed that the iNs preserve an aging-associated phenotype. More surprisingly, the iPSCsN also showed an aging-associated phenotype contrary

to the idea that the embryonic state of the iPSCs would lead to a rejuvenation of iPSCsN from aged donors. Nevertheless, the extent to which the iPSCsN and iNs preserve this aging-associated signatures is currently under investigation. Overall, this study will deepen our understanding of how well ‘‘aged” iNs and iPSCsN represent human brain aging. It may help to establish remarkable tools for identifying pharmaceutical targets to improve human health during aging and in various aging-related diseases.

Disclosures: **N. Varghese:** None. **L. Szabo:** None. **A. Grimm:** None. **Z. Cader:** None. **I. Lejri:** None. **A. Eckert:** None.

Poster

PSTR394. Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR394.17/E9

Topic: C.01. Brain Wellness and Aging

Title: Obesity related brain activation during physical activity under stress

Authors: *J. RHEE;
Envrn. and Occup. Hlth., Texas A&M Univ., College Station, TX

Abstract: Neural activation of older adults during muscular endurance task was measured using functional near infrared spectroscopy. Older adults older than 65 years old were recruited from the local community and categorized into two groups depending on their weight group (e.g. obese group and normal weight group)
During the motor control task, stress was provided using trier social stress task and stress related different neural activation.

Disclosures: **J. Rhee:** None.

Poster

PSTR394. Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR394.18/E10

Topic: C.01. Brain Wellness and Aging

Support: NINDS BINP R01 NS11754

Title: Altered brain microvascular bioenergetics by chronic stress: a pathway to cerebrovascular dysfunction and cognitive decline?

Authors: *A. A. HANSHEW[1], S. PRABHU[2], N. M. EMINHIZER[1], E. N. BURRAGE[3], J. CRAWFORD[1], D. THAPA[1], R. W. BRYNER[1], W. GELDENHUYS[4], P. CHANTLER[5]; [1]Exercise Physiol., [2]Pharmaceut. and Pharmacol. Sci., [4]WVU Sch. of Pharm., [3]West Virginia Univ., Morgantown, WV; [5]Exercise Physiol., WVU Robert C. Byrd Hlth. Sci. Ctr., Morgantown, WV

Abstract: Dementia is a progressive neurodegenerative disorder associated with oxidative stress. Chronic stress is linked to cognitive decline, but the role of the cerebrovasculature on the disease progression of cognitive decline has not been fully elucidated. Specifically, the role of mitochondrial and glycolytic energy pathways which are critical to cerebrovascular function is unknown. This study aimed to determine the impact of UCMS on cerebrovascular energetics. Four-month-old C57Bl/6 male and female mice were randomized into control (non-UCMS) and UCMS groups for 8 weeks. Control mice remained in home cages, while UCMS mice were exposed to chronic stress for 5 days/week for 7 Hrs/day for 8 weeks. At 6 months of age, mice were euthanized and the middle cerebral artery (MCA) was removed and positioned in a pressurize myobath and exposed to increasing concentrations of acetylcholine (ACh; 10^{-9}M to 10^{-4}M) to measure endothelial-dependent dilation (EDD). The remaining brain microvessels (BMVs) were homogenized to assess for hydrogen peroxide (H_20_2) production and bioenergetics using the coumarin boronic acid assay and Seahorse Bioflux Analyzer, respectively. Our data show that BMVs from UCMS mice had a significant ($p<0.05$) increase in H_20_2 production and a decrease in the rate of oxidative phosphorylation compared to control mice. This decrease corresponded to a decrease in ATP production ($p<0.05$) in mice experiencing UCMS vs. controls. This altered BMV bioenergetics coincided with a 57% reduction in the EDD of the MCA in UCMS vs. control mice ($p<0.01$). Taken together, our data suggest that the BMVs are susceptible to chronic stress with elevated vascular oxidative stress, and reduced vascular bioenergetics which affects the cerebrovascular functions and opens up avenues for therapeutic targeting of novel therapies to support the cerebrovascular function.

Disclosures: A.A. Hanshew: None. **S. Prabhu:** None. **N.M. Eminhizer:** None. **E.N. Burrage:** None. **J. Crawford:** None. **D. Thapa:** None. **R.W. Bryner:** None. **W. Geldenhuys:** None. **P. Chantler:** None.

Poster

PSTR394. Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR394.19/E11

Topic: C.01. Brain Wellness and Aging

Support: Ministerio de Ciencia e Innovación (PID2020-117422RB-C21)
Fundación Universitaria San Pablo-CEU (Adipobrain)

Title: Sexual dimorphism in the effect of high-fat diets on the sphingolipid composition of mouse hippocampus

Authors: *E. SANCHEZ-HITA[1], B. FERNANDEZ-REQUENA[2], C. GONZALEZ-RIANO[2], A. GARCIA[2], M. RUIZ-GAYO[1], B. MERINO[1], V. CANO[1], A. PLAZA[1];
[1]Hlth. and Pharmaceut. Sci., [2]Ctr. de Metabolómica y Bioanálisis (CEMBIO), Univ. San Pablo-CEU, CEU Universities, Madrid, Spain

Abstract: High-fat diets (HFD) promote the accumulation of ceramides and other sphingolipids in different organs, including the brain, a circumstance that has been related with lipotoxicity and cell aging. HFD also trigger memory deficits both in humans and rodents. Related with that, hippocampal synaptic plasticity has been shown to be impaired in the hippocampus of mice consuming HFD. Nevertheless, a relationship between cognition performance and the sphingolipid composition of the hippocampus has not been established. Otherwise, sexual differences in the effect of HFD have not been so far investigated. Our Aim was to analyse the effect of a regular intake of HFD enriched with either oleic acid (40% high-oleic sunflower oil; Unsaturated OiL-enriched Food, UOLF) or lauric/palmitic acids (40% palm kernel oil; Saturated OiL-enriched Food, SOLF) on the sphingolipid composition of the hippocampus. Methods: Five-week old male and female C57BL mice fed either SOLF, UOLF or standard diet for 8 weeks. Hippocampi were dissected and analysed using an Agilent 1290 Infinity II UHPLC system coupled to an Agilent 6546 quadrupole time-of-flight (QTOF) mass spectrometer in both MS and iterative-MS/MS modes using a reversed-phase lipidomics-based strategy [1]. Briefly, tissue was homogenized with MeOH:H2O 50% (v/v) and extracted with MeOH:methyl tert-butyl ether 4:1 (v/v)[2]. After data reprocessing using Agilent MassHunter Profinder B.10.0.2 and Lipid Annotator v1.0 (Agilent Technologies Inc., Santa Clara, CA, USA), 32 Cer, 20 SM and 45 HexCer lipid species were accurately annotated and statistically compared. Results: Compared to standard diets, SOLF increased the content of i) ceramides and dihydroceramides in males, and ii) cerebrosides in females, while UOLF i) diminished ceramide and dihydroceramide concentration in females, and ii) enhanced cerebrosides in males. Otherwise, the lauric acid-derived ceramide, Cer18:1/12:0, was specifically detected in animals that consumed SOLF (males 0.05±0.01 µg/g tissue; females 0.05±0.02 µg/g tissue; p&lt0.001). Conclusion: Our data show that both SOLF and UOLF differently affect male and female brain sphingolipid pattern.[1] C. Gonzalez-Riano et al. Journal of Chromatography Open, 1, 100018 (2021).[2] C. Gonzalez-Riano et al. Brain Structure and Function, 222, 2831-2853 (2017).

Disclosures: E. Sanchez-Hita: None. **B. Fernandez-Requena:** None. **C. Gonzalez-Riano:** None. **A. Garcia:** None. **M. Ruiz-Gayo:** None. **B. Merino:** None. **V. Cano:** None. **A. Plaza:** None.

Poster

PSTR394. Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR394.20/E12

Topic: C.01. Brain Wellness and Aging

Support: PO1AG078116

Title: Characterizing ATP:ADP using PercevalHR in mixed hippocampal cultures

Authors: *S. L. SIMS, S. L. CASE, R.-L. LIN, N. A. WRIGHT, J. RHINEHART, O. THIBAULT;
Pharmacol., Univ. of Kentucky, Lexington, KY

Abstract: Brain homeostatic equilibrium is a well-maintained and orchestrated metabolic process which, when lost, is associated with brain aging or neurodegenerative diseases, and is often detected as hypometabolism in aging and AD. It is, therefore, integral to maintain this homeostasis on a second-to-second basis and across multiple cell types. Major energetic processes can be addressed through the measurement of ATP levels within a single cell. In addition to the well-established role of glucose metabolism in the CNS, more recently, insulin has also been recognized to play an essential role in the regulation of cognitive function, particularly in the hippocampus, where it can ameliorate spatial memory recall. Using mixed primary hippocampal cultures (neurons and astrocytes), we tested the hypothesis that PercevalHR, an ATP:ADP biosensor, could reliably quantify bioenergetics with single cell resolution. Embryonic rat hippocampi (E17) were extracted and maintained for 12-16 days *in vitro* (DIV). Cultures were treated with lentivirus (Human Ubiquitin C promoter) containing the PercevalHR nanosensor. To control for PercevalHR's pH sensitivity, some experiments were conducted concomitantly with the intracellular pH sensor pHrodo. We attempted to normalize glucose transporter function following ~12 days in high glucose concentration (35 mM), by returning the cells to a serum-free 5.5 mM glucose media ~24 h prior to imaging. PercevalHR emission was filtered at 525 nm and pHrodo emission at 580 nm. After an initial baseline, cells were treated with one of several compounds (0.5 mM, 5.5 mM, and 10 mM glucose; 50 mM KCl; 20 µM glutamate; 10 nM insulin). Glutamate and KCl resulted in rapid decreases in ATP:ADP ratios. Insulin only demonstrated a slight drop in ATP:ADP. PercevalHR seems to reliably report on cell energetics in mammalian cultures and surprisingly, appears to indicate that neurons display higher baseline ATP:ADP compared to astrocytes. These data help evaluate bioenergetic status in two closely associated cell types that are known to share intermediates. Ongoing studies are investigating PercevalHR imaging in astrocytes using *in vivo* 2P microscopy in a mouse model of amyloidosis during ambulation (i.e., awake).

Disclosures: S.L. Sims: None. **S.L. Case:** None. **R. Lin:** None. **N.A. Wright:** None. **J. Rhinehart:** None. **O. Thibault:** None.

Poster

PSTR394. Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR394.21/E13

Topic: C.01. Brain Wellness and Aging

Support: Davidson Research Initiative Summer Fellowship

Title: Neuronal reactive oxygen species resistance in Caenorhabditis elegans mutants and its role in neurodegenerative disease

Authors: *N. PINZON[1,2], R. EL BEJJANI[1];
[1]Davidson Col. Neurosci. Program, Davidson, NC; [2]Davidson Col. Genomics Program, Davidson, NC

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease characterized by motor neuron (MN) degeneration that currently has no cure. Previous research has found morphological defects in patients' MNs to correspond with damage caused by oxidative stress mediated by Reactive Oxygen Species (ROS). ROS detoxification has remained a primary therapeutical target, leading the way for one of the only three FDA-approved treatments for ALS (Edaravone: a free radical scavenger molecule). Yet, oxidative stress has remained a challenging mechanism to study due to ROS's general and spreading nature. Previously in our lab, we developed an optogenetic tool (KillerRed) that allows for selective, non-invasive, and neuron-specific induction of ROS using light. We used forward genetic screens to isolate six neuronal ROS-resistant strains of Caenorhabditis elegans, which this work seeks to characterize fully. Utilizing Paraquat as a general ROS inducer, we confirmed one strain to be resistant and one to be hypersensitive, suggesting a mechanism through which these overcome ROS damage in all types of cells and specifically in neurons, respectively. We seek to further describe morphological neurodegeneration differences between our strains upon chemical and optogenetic-induced oxidative stress. Future work will focus on strain outcrossing and genomic sequencing to find single-gene mutations responsible for neuronal oxidative stress resistance. Through this research, we aim to identify therapeutically targetable pathways in ALS and further the understanding of ROS' role in neurodegenerative disease.

Disclosures: **N. Pinzon:** None. **R. El Bejjani:** None.

Poster

PSTR394. Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR394.22/E14

Topic: C.01. Brain Wellness and Aging

Title: Chaperon-mediated autophagy suppresses glioblastoma via degradation of E2F1

Authors: *W. TANG, K. KIANG, G. LEUNG;
Dept. of Surgery, The Univ. of Hong Kong, Hong Kong, China

Abstract: Chaperon-mediated autophagy (CMA) is a selective form of autophagy, which has been widely studied in neurodegenerative diseases like Alzheimer's disease and Parkinson's disease. However, the roles of CMA in brain tumors are still poorly understood. By using an orthotopic animal model, we showed that CMA blockade promotes glioblastoma progression, accompanied by the upregulation of glioma promoters, i.e., E2F1, and activated metabolic

pathways. Furthermore, we found that long-term Temozolomide treatment would upregulate the expression of LAMP2A, the indicator of CMA, both *in vitro* and *in vivo*. However, the inhibition of LAMP2A had no significant impact on the sensitivity of glioblastoma cells in response to Temozolomide. In summary, CMA plays a critical role in the regulation of glioblastoma growth via selective degradation of glioma-promoting proteins, and chemotherapy would affect the activity of CMA. These findings might broaden the knowledge of CMA and provide insights into how the modulation of CMA contributes to the treatment of brain diseases.

Disclosures: **W. Tang:** None. **K. Kiang:** None. **G. Leung:** None.

Poster

PSTR395. Human Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR395.01/E15

Topic: H.10. Human Learning and Cognition

Support: hochschulmedizin (hmz) flagship project STRESS
National Research Foundation, Prime Minister's Office, Singapore under its Campus for Research Excellence and Technological Enterprise (CREATE) programme (FHT)

Title: Self-regulating noradrenergic activity influences cortical excitability and neuroplasticity

Authors: *W. POTOK[1], S. MEISSNER[2], N. WENDEROTH[2,3];
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Abstract: The arousal state of the brain is determined by the neuromodulatory activity of several brainstem nuclei and, particularly, by the noradrenergic (NA) transmission from the locus coeruleus (LC). Activity of the LC-NA system causally influences the eye's pupil diameter. We have recently shown that pupil-based neurofeedback enables participants to acquire mental strategies for volitionally controlling LC activity. Pharmacological studies confirm that activating β- or α1-adrenoreceptors facilitates cortical excitability and neuroplastic effects. However, it is unknown whether self-regulation of noradrenergic activity has a similar effect. We use a multimodal approach combining (i) pupil diameter-based neurofeedback training that enables participants to control LC-activity, (ii) anodal transcranial direct current stimulation (a-tDCS) for inducing neuroplastic changes in the primary motor cortex, and (iii) single-pulse transcranial magnetic stimulation with electromyography for measuring cortical excitability using motor evoked potentials (MEP) as our main outcome parameter. We observed significant changes in cortical excitability when applying mental strategies to modulate the pupil size, reflected in 35±44% increase in MEP amplitude relative to rest during up-regulation and 2±12%

decrease in MEP amplitude relative to rest during down-regulation of the pupil size ($t_{(14)}$=3.2, p=0.006 (2-sided) d=0.8, N=15, 11 females). Moreover, our preliminary results indicate that applying plasticity-inducing a-tDCS to the primary motor cortex while participants up-regulate pupil diameter caused an increase in cortical excitability. Down-regulating pupil diameter, by contrast, reduced this a-tDCS effect on cortical excitability (d=0.5, N=5, 4 females, data collection in process). Together, we provide first evidence that pupil-based neurofeedback may be a new, non-invasive, and non-pharmacological tool for modulating cortical excitability, and potentially also neuroplasticity, most likely by changing the NA levels in the brain.

Disclosures: W. Potok: None. **S. Meissner:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MindMetrix. **N. Wenderoth:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MindMetrix.

Poster

PSTR395. Human Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR395.02/E16

Topic: H.10. Human Learning and Cognition

Title: The role of the intraparietal sulcus in numeracy: a review of parietal lesion cases

Authors: *E. DURICY, C. DURISKO, J. A. FIEZ;
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Abstract: We reviewed single case studies of acalculia following focal damage to examine the similarities and differences between cases and how they compare to the primary theory of numerical processing: the Triple Code Model (Dehaene, 1992). According to the Triple Code Model, different brain regions contribute to magnitude, verbal, and visual coding of number, with the intraparietal sulcus (IPS) supporting representations of magnitude that undergird an internal number line and meaningful calculation. Our goal was to examine numeracy-focused single case studies with parietal damage to test for causal relationships between damage to the IPS and impairments in different components of numeracy. We identified a set of 27 single case studies with parietal lesions and then assessed the role of the IPS across four key numeracy domains: Approximation, Calculation, Transcoding, and Ordinality/Cardinality. We compared individual-level published images by drawing a sphere at the estimated center of mass of each case's lesion on a standard template. These spheres were then overlapped with an IPS region-of-interest (ROI), and cases were grouped (IPS or Parietal) based on ROI overlap and original anatomical description. A Fisher's Exact Test was performed to compare behavioral performance on each numeracy domain between lesion groups. We then followed up these tests with an Activation Likelihood Estimation (ALE) analysis. We assessed the frequency of numeracy impairment between the IPS group and Parietal case groups and found that Approximation deficits were significantly more frequent in the IPS group. Further exploration using an ALE

analysis revealed that only Approximation deficit cases significantly overlapped with the IPS, while the other domains were localized to different regions of the parietal lobe. Based on the pattern shown across parietal-damage single case studies we conclude that damage to the left IPS is both necessary and sufficient to impair approximation ability, but not other components of numeracy. Thus, its contributions to numeracy seem more circumscribed than proposed in the Triple Code Model, and may be more compatible with models of dissociable circuits of numerical and verbal/visual processing in the parietal lobe.

Disclosures: E. Duricy: None. **C. Durisko:** None. **J.A. Fiez:** None.

Poster

PSTR395. Human Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR395.03/E17

Topic: G.08. Other Psychiatric Disorders

Title: Early sensory overstimulation leads to behavioral dysfunctions predominantly in male CD-1 mice: a mechanism for male vulnerability in ADHD?

Authors: *J. MERZ[1], T. FU[1], H. BACKHAUS[1], R. GUIMARAES-BACKHAUS[1], S. SCHWEIGER[2], A. STROH[3];
[1]Leibniz Inst. for Resilience Res., Mainz, Germany; [2]Inst. for Human Genet., Univ. of Mainz, Mainz, Germany; [3]Univ. Med. Ctr. Mainz, Mainz, Germany

Abstract: Sensory overstimulation (OS), caused by the increased consumption of audiovisual media usage, is a widespread phenomenon and runs through all areas of our society. The COVID-19 pandemic has further exacerbated this. OS is believed to be an important factor in the development of neuropsychiatric disorders such as attention deficit/hyperactivity disorder (ADHD) in children. Yet, the neuronal mechanisms and the temporal window of vulnerability linking ADHD and OS are largely unexplored. In this study, we used a mouse model of excessive sensory stimulation (ESS) to investigate the impact of OS on behavioral outcome and its underlying neuronal mechanisms. Male and female CD-1 mice at P10, prior to weaning, were overstimulated for 42 consecutive days, 6 hours per day. Subsequently, we investigated anxiety-like behavior in the Elevated Plus maze (EPM) and in the Light/Dark test (LDT), locomotor activity in the Open Field test (OFT), and cognitive functions in the 24-h Novel Object Recognition (24-h NORT) and Y-maze (YM). For assessing cortical microcircuit dynamics, we employed awake two-photon calcium imaging in the primary visual cortex (V1). ESS impacts anxiety-like and risk-taking behavior in OS mice. OS mice tend to explore the open arms of the EPM longer than the control group. Notably, we observed a gender-specific vulnerability in terms of the behavioral outcomes in the LDT, where male OS mice spent significantly more time in the brightly lit light chamber of the testing arena than male controls indicating an increase in risk-taking behavior in the OS male group. Female OS mice did not show this behavioral aberration. These findings reflect the human condition of ADHD, where male patients are more

likely to engage in risky behavior, which is associated with physical injuries, reckless driving, and substance abuse. Our data suggests that early sensory overstimulation leads to a durable shift in behavior, with a higher vulnerability in males.

Disclosures: **J. Merz:** None. **T. Fu:** None. **H. Backhaus:** None. **R. Guimaraes-Backhaus:** None. **S. Schweiger:** None. **A. Stroh:** None.

Poster

PSTR395. Human Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR395.04/E18

Topic: H.10. Human Learning and Cognition

Support: Puerto Rico's Board of Education

Title: Relationships among cognitive variables in Puerto Rican children using the Cognitive Assessment System 2 - Spanish Version

Authors: *A. L. BERRIOS-NEGRON, C. CENTENO-ROMAN, Y. PEREZ, J. MARTINEZ-TORO, G. CORDERO-ARROYO, M. MORENO-TORRES, M. BERMONTI-PEREZ; Clin. Psychology, Ponce Hlth. Sci. Univ., Ponce, PR

Abstract: The Cognitive Assessment System 2 – Spanish Version (CAS 2: SP) is a comprehensive test that assesses a range of cognitive variables, including planning, attention, simultaneous processing, and successive processing (PASS). Understanding the relationships among these variables can offer valuable insights into the interconnectedness of cognitive processes and their impact on overall cognitive functioning in children. However, there is a notable scarcity of research specifically examining these relationships in Puerto Rican children. Therefore, our study aims to fill this gap by exploring patterns, associations, and dependencies among different cognitive domains to deepen our understanding of how specific cognitive abilities influence one another and contribute to overall cognitive performance in children. To achieve this, we conducted a secondary data analysis with a sample of 34 children, ranging from first to fourth grade, representing 11 schools across the metropolitan and southern areas of Puerto Rico (IRB #1314-085). Employing bivariate correlation analysis, we examined the relationships between the PASS variables. The analysis revealed significant positive correlations between planning and attention ($r = 0.523$, $p < 0.001$), attention and successive processing ($r = 0.458$, $p = 0.003$), planning and successive processing ($r = 0.393$, $p = 0.011$), planning and simultaneous processing ($r = 0.387$, $p = 0.012$), and simultaneous processing and successive processing ($r = 0.330$, $p = 0.028$). However, no positive correlation was found between attention and simultaneous processing ($r = 0.282$, $p = 0.053$). By establishing these meaningful relationships, our study provides a more comprehensive understanding of the cognitive skills of Puerto Rican children. Ultimately, our research aims to contribute to enhancing the cognitive assessment and intervention practices specific to Puerto Rican children.

Disclosures: **A.L. Berrios-Negron:** None. **C. Centeno-Roman:** None. **Y. Perez:** None. **J. Martinez-Toro:** None. **G. Cordero-Arroyo:** None. **M. Moreno-Torres:** None. **M. Bermonti-Perez:** None.

Poster

PSTR395. Human Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR395.05/E19

Topic: H.06. Social Cognition

Title: Modeling the content of event representations along the cortical hierarchy during natural vision

Authors: *Y. CHEN[1], Z. ZADA[2], R. E. RICE[3], S. A. NASTASE[2];
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Abstract: How are multimodal event representations constructed in the brain from different stimulus features? This study explicitly modeled the content of neural event representations along the cortical processing hierarchy during natural vision. We developed a framework for combined voxelwise encoding models and event segmentation to quantify the contribution of different visual and linguistic features to event representations in brain activities. We employed an open fMRI dataset comprising 25 participants viewing the second half of the Grand Budapest Hotel in five 10-minute runs (Visconti di Oleggio Castello et al., 2020). fMRI data were preprocessed using fMRIPrep and organized according to a 1000-parcel cortical atlas (Kong et al., 2021). Three types of features were extracted from the stimulus: low-level visual motion energy, higher-level visual embeddings from VGG, and linguistic subtitle embeddings from GPT-2. Voxelwise encoding models were estimated for each feature space in order to predict BOLD time series in left-out runs. These model-predicted time series reflect components of the brain activity captured by the feature space of the respective encoding model. Event segmentation was then performed on both the original BOLD time series and the model-predicted time series for each encoding model. In line with prior work (Geerligs et al., 2022), we found a hierarchical distribution of events across brain regions, with higher-level brain regions having fewer and longer events than low-level brain regions. Model-predicted time series yielded more events than the original BOLD time series but with numerous matching event boundaries. Lower-level features (i.e., motion energy) yielded the most numerous model-predicted event boundaries, many of which matched the original event boundaries in early visual areas; higher-level features (i.e., VGG, GPT-2) yielded fewer event boundaries that better matched the original boundaries in higher-level visual and language areas. This suggests a hierarchical organization in brain responses to naturalistic stimuli, with different encoding models capturing different aspects of this hierarchy. Critically, this approach also allows us to compare model-predicted event representations (i.e., activity patterns) derived from a given feature space to event representations derived from the original BOLD data and quantify the relative contribution of each feature space

to the event representations in a given cortical area. This framework provides novel insights into the multidimensional structure of event representations and their hierarchical organization in the human brain.

Disclosures: **Y. Chen:** None. **Z. Zada:** None. **R.E. Rice:** None. **S.A. Nastase:** None.

Poster

PSTR395. Human Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR395.06/E20

Topic: H.10. Human Learning and Cognition

Support: 5R01AG057962

Title: Different cognitive domain induces different pattern of task-evoked negative BOLD response

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Abstract: Introduction:This study explores the overlooked negative blood-oxygen-level dependent (BOLD) responses (NBR) and their unique spatial pattern for each cognitive domains, advancing our understanding of the neurophysiological mechanisms underlying cognitive processes.
Method:Two separate cohorts (n=100, 30) of young participants from different institutes, and different scanners and pulse sequences were imaged with functional magnetic resonance imaging (fMRI) during 12 tasks targeting four cognitive domains (3 tasks per domain). We investigate variations in the spatial pattern of the NBR patterns. Our univariate first-level analysis employs multiple regression with task timing as the predictor, followed by voxel-wise one-sample t-tests at the second level to identify responsive voxels. Cluster-wise multiple comparison correction identifies patterns of deactivated regions. Dice overlap measures the similarity between each pair of task deactivated pattern, generating a 12x12 similarity matrix. Statistical significance is assessed using permutation tests to compare "within domain similarity" and "across domain similarity" of deactivation patterns.
Results:Fig. 1(a and b) presents a color-coded similarity matrix from the first and second cohorts, indicating that patterns of deactivated regions are similar within domains but less so between domains. The permutation test demonstrates significant differences between within-domain and across-domain similarity in both cohort data (∇=0.243/0.114, $p<0.001/0.002$). The data suggests task-specific patterns of deactivated regions, indicating active regulation rather than a biomechanical shutdown of the known default mode regions.
Conclusion: These findings enhance our understanding of neurophysiological signatures linked to cognition, uncovering interconnections and shared neural substrates across functions. They

underscore the significance of task-evoked NBR in studying cognitive domains and contribute to our knowledge of the neural underpinnings of cognition.

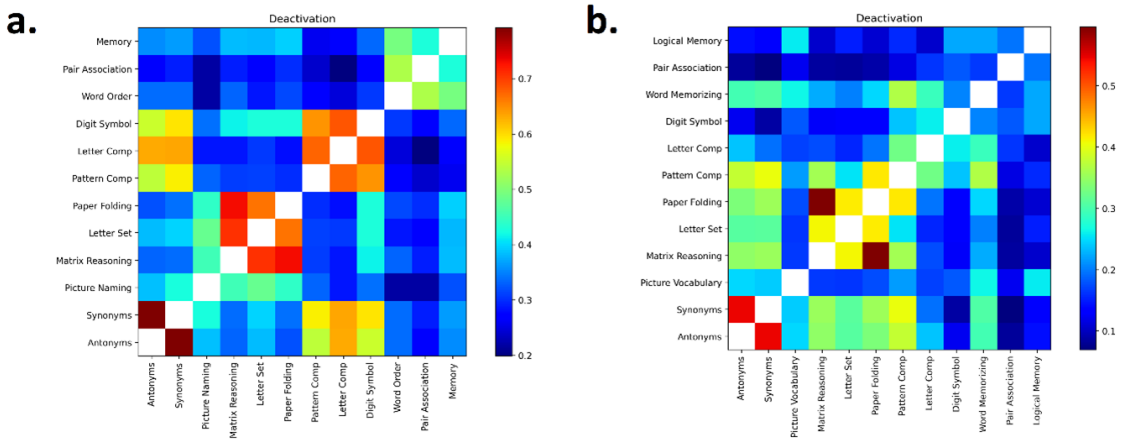

Figure 1

Disclosures: Q. Razlighi: None. **S. Gholipour Picha:** None.

Poster

PSTR395. Human Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR395.07/E21

Topic: H.01. Attention

Support: NIH Grant T32-DC011499
NIH Grant RO1-DC019126

Title: Selective Attention Modulates Cortical but not Subcortical Responses

Authors: *V. FIGAROLA[1], A. L. NOYCE[1], A. T. TIERNEY[3], R. K. MADDOX[4], F. DICK[5], B. SHINN-CUNNINGHAM[2];
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Abstract: Individuals often need to attend to one signal within a crowded acoustic scene. The phenomenon of attending to an important auditory source while ignoring other stimuli is known as the cocktail party effect. While attentional effects are well-documented in the auditory cortex, it is still unclear how the function of auditory subcortical nuclei is affected by attention.

This study combines two previous electroencephalography (EEG) paradigms to evaluate the effects of attention on brainstem and cortical physiological responses. The first paradigm replicated is by Dr. Adam Tierney (Birkbeck College, University College London) that requires top-down attention. Competing, interleaved melodies are played and the subjects are asked to attend to a high- or low-pitched melody, listening for 3-note repeats. To test attentional modulation in the brainstem, we modified the stimuli so that each complex tone evokes an auditory brainstem response (ABR) using a paradigm by Dr. Ross Maddox (University of Rochester). Specifically, we created two isochronous impulse trains (2Hz rate), one for the low and one for the high melody, offset in time by 250ms. Each was convolved with different narrowband tone complexes with different pitches (low and high).
We have recruited 19 subjects thus far (12F/7M) all with normal hearing thresholds. We extracted both subcortical ABRs and cortical evoked responses to each note, as well as the phase and inter-trial phase coherence (ITPC) at 2Hz. We found robust ABRs evoked by each tone pip, one per pitch period, within each note, especially wave V. However, there is little evidence suggesting attention modulates ABRs given that wave V latency and amplitude did not change while attending versus ignoring the distractor. On the other hand, each note onset evoked cortical activity, specifically enhancement of every other note depending on which stream was being attended. Additionally, the ITPC showed peaks at the within-melody repetition rate (2 Hz). Furthermore, the best attending listeners showed ~180 degree phase separation between conditions. By simultaneously recording cortical responses and ABRs, we are able to track attention-mediated changes in the neural signals in the cortex and brainstem, respectively.

Disclosures: V. Figarola: None. **A.L. Noyce:** None. **A.T. Tierney:** None. **R.K. Maddox:** None. **F. Dick:** None. **B. Shinn-Cunningham:** None.

Poster

PSTR395. Human Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR395.08/E22

Topic: H.06. Social Cognition

Support: NIH-R01MH110630
Iowa Initiative for Artificial Intelligence Pilot Project

Title: Brain responses to naturalistic videos predicted from audiovisual and semantic features.

Authors: A. L. VAN DE WATER[1,2], L. BYRGE[5,6], D. P. KENNEDY[7,8,9], R. GONZALEZ[1], J. Y. PETERS[1], D. KLIEMANN[1,2,3,4], J. A. TRAER[1,2,3];
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Abstract: Humans use complex multimodal information to build a rich dynamic interpretation of social environments. Multisensory integration may thus be key for effective social functioning. How are complex naturalistic features integrated and flexibly represented across brain networks? Which features are most relevant for social cognition? We used diverse naturalistic viewing paradigms with rich audiovisual social information [1] and fit encoding models to study how complex stimuli features (audio, visual, abstract semantic) predict brain activity. We used a functional magnetic resonance imaging (fMRI) dataset of healthy adults (n = 79, 23 females, mean(SD) age = 26.8(7.7) with six passive video watching scans (5-21 minutes) of different genres (black/white thriller, diverse movie trailers, animated family movie, TV sitcoms). MRI data processing (described in [1]) included rigorous quality control and parcellation of the cortex into 360 regions in 12 networks [2]. Semantic features (e.g., presence of speech, speaker identity, distance between interacting people) were denoted manually, object-level visual features were extracted with a deep neural network, and audio features were derived from sound statistics (e.g., spectral centroid, power). We fit cross-validated regularized linear regression models [3] using different subsets of features and tested which features best predicted held-out fMRI data. To address overfitting, we included null models (time reversed, block scrambled, within-block-scrambled features). We found robust encoding accuracy in language, visual, and auditory networks for most movies. Across movies, we found the lowest performance in the animated movie (which lacked speech). Across features, visual features alone often performed no better than null models, some of which yielded surprisingly high encoding performance, emphasizing the need for careful overfitting control. Interestingly, models using auditory features outperformed the null models not only in auditory and language networks, but also in visual and the posterior multimodal network across multiple movies. Our results indicate cross-modal integration of complex audiovisual information in semi-naturalistic movies beyond unimodal regions. Efforts to further characterize complex feature spaces and improve encoding models are currently underway with the aim to identify candidates which most reliably predict atypical social behavior.
[1] Byrge et al. Hum Brain Mapp 2022 43:2972-2991.[2] Glasser et al. Nature 2016 536:171-178.[3] Huth et al. Neuron 2012 76:1210-1224.

Disclosures: A.L. Van De Water: None. **L. Byrge:** None. **D.P. Kennedy:** None. **R. Gonzalez:** None. **J.Y. Peters:** None. **D. Kliemann:** None. **J.A. Traer:** None.

Poster

PSTR395. Human Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR395.09/E23

Topic: C.01. Brain Wellness and Aging

Support: NIH/NIA R61AG081723
Alzheimer's Association AARG-22-926139

Title: Automated estimation of participant engagement during computerized cognitive training from facial expressions in older adults at risk for dementia

Authors: *A. TURNBULL[1], Y. WANG[1], Y. XU[2], E. ADELI[3], F. V. LIN[4];
[1]Stanford Univ., Palo Alto, CA; [2]Univ. of Rochester, Stanford Univ., Rochester, NY; [3]Stanford Univ., Stanford Univ., Palo Alto, CA; [4]Stanford Univ., Stanford Univ., Sunnyvale, CA

Abstract: Computerized cognitive training is a frontline non-pharmacological intervention for slowing cognitive decline and brain aging in dementia. The effectiveness of computerized cognitive training in dementia is often limited by the engagement of participants. Monitoring at-risk older adult users' real-time engagement in the domains of attention, motivation, and affect is crucial to understanding and addressing heterogeneity in cognitive training outcomes. In the current project, we predicted engagement (measured using an established mental fatigue measure assessing perceived attention, motivation, and affect during computerized cognitive training sessions) in older adults with mild cognitive impairment (MCI), by monitoring their real-time video-recorded facial gestures in training sessions. Our novel Recurrent Video Transformer (RVT) model, which combines a clip-wise transformer encoder module and a session-wise Recurrent Neural Network (RNN) classifier, achieved the highest balanced accuracy, F1 score, and precision compared to other state-of-the-art models for both detecting mental fatigue/disengagement cases (binary classification) and rating the level of mental fatigue (multi-class classification). The fatigue feature derived from this model also significantly related to slower reaction times at the session level (B=.04, SE=.02, p=.016), providing further validation. By leveraging dynamic temporal information, the RVT model demonstrates the potential to accurately predict engagement among computerized cognitive training users, which lays the foundation for future work to modulate the level of engagement in computerized cognitive training interventions in MCI.

Disclosures: A. Turnbull: None. **Y. Wang:** None. **Y. Xu:** None. **E. Adeli:** None. **F.V. lin:** None.

Poster

PSTR395. Human Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR395.10/E24

Topic: G.08. Other Psychiatric Disorders

Title: Resolving interindividual propagation dynamics of slow wave events in human EEG/fMRI recordings

Authors: *M. ILHAN-BAYRAKCI[1], O. TÜSCHER[2], A. STROH[3];
[1]Leibniz Inst. for Resilience Res., Mainz, Germany; [2]Univ. Med. Ctr. Mainz, [3]Johannes Gutenberg-University Mainz, Univ. Med. Ctr. Mainz, Mainz, Germany

Abstract: The slow wave state is a brain state occurring in slow wave sleep and in certain anesthetic regimens, characterized by network quiescence interrupted by sudden bursts of activity or so-called slow wave events (SWEs). SWEs propagate as traveling waves of activity stereotypically along the anterior-to-posterior axis usually crossing mesial aspects of the cortex. SWE occurrence and propagation are highly susceptible to excitability changes and states of the neuronal network. Early stages of neurodegenerative and neuroimmunological disorders are marked by ensembles of hyperactive neurons and seem to alter the propagation of SWEs. This susceptibility makes SWEs an ideal target for studying brain (dys-)function across species and across diseases. In rats, we have assessed the relationship between SWEs and BOLD fMRI signals and have observed a cortex-wide BOLD pattern directly related to SWEs. In healthy humans, we have analyzed simultaneous EEG-fMRI data during non-REM sleep. SWEs individually detected in the EEG data were used as predictors in event-related fMRI analyses on single subject level to examine the relationship between SWEs and fMRI signals. For all subjects we identified significant changes in BOLD activity associated with SWEs covering substantial parts of the grey matter. We now ask whether we can resolve the interindividual differences in terms of the SWE propagation patterns in EEG-informed BOLD fMRI, a critical step towards SWE-based assessments of early network dysregulations in humans. For that, we analyzed two distinct datasets comprising simultaneous EEG-fMRI data of sleeping healthy humans (N = 29). We extracted the longest propagation path for each individual SWE based on the EEG data and considered only those events whose principal propagation direction was either along the anterior-to-posterior axis or the lateral axis for subsequent event-related fMRI analyses. Throughout the individual subjects we observed very consistently, significant and strong BOLD fMRI activations in the cingulate gyrus along with activations in the thalamus, the hippocampus, the cerebellum and the brainstem. These BOLD fMRI patterns clearly reflect the long-range coupling of activity between the cortex, the thalamus and the hippocampus and represent a key target to study SWE alterations.

Disclosures: **M. Ilhan-Bayrakci:** None. **O. Tüscher:** None. **A. Stroh:** None.

Poster

PSTR395. Human Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR395.11/E25

Topic: C.01. Brain Wellness and Aging

Support: NIH R21AG053193

Title: A Novel Computational Model of Risk-Taking Behavior in Older Adults

Authors: *Z. BARAKCHIAN, M. ANTHONY, A. G. TURNBULL, F. V. LIN;
Stanford Univ., Stanford, CA

Abstract: A Novel Computational Model of Risk-Taking Behavior in Older Adults
Functioning in daily life requires the ability to make risky decisions, for example during the managing of finances, an important activity of daily living that is affected by dementia. Understanding how risk-taking behavior changes into old age may provide a basis for understanding how and why risky decision-making is affected by cognitive decline. We introduce a novel computational model to examine decision-making under risk in older adults. Using the Balloon Analogue Risk Task (BART, a task encouraging risk-seeking) and the Iowa Gambling Task (IGT, a task encouraging risk-aversion), we explored the role of prior experience of value (average of previous rewards) and risk (standard deviation of previous rewards) in older adults' decision-making. We then performed correlations with the DOSPERT scale, a well-validated trait measure of risk tasking across multiple domains, to assess relationships between task-based decision-making metrics and trait-level risk-taking behavior. Results showed that in the BART model, both the expected value and the standard deviation of rewards significantly influenced decisions. Conversely, in the IGT model, these factors were significant for fewer participants, with the probability of winning having a more substantial impact on choices. We found trends for significant correlations of task-based risk sensitivity (standard deviation of previous rewards) with the DOSPERT for both the BART and IGT models, but in opposite directions that reflect the risk-seeking (BART: r=.3, p=.056) and risk-avoiding (IGT: r=-.3, p=.064) nature of these tasks. Our findings enhance our understanding of risk-taking behavior in older adults. We observed that individuals utilize different strategies on these two tasks. The standard deviation coefficient (risk sensitivity) of both models significantly correlated with a well-validated measure of trait-level risk taking, suggesting that how older adults evaluate recent experiences of reward during risk-taking may play a role in their trait-level risk taking behavior. This mechanism may represent an important future avenue for research into risk-taking behavior in older adults at risk for dementia.

Disclosures: Z. Barakchian: None. **M. Anthony:** None. **A.G. Turnbull:** None. **F.V. Lin:** None.

Poster

PSTR395. Human Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR395.12/E26

Topic: G.08. Other Psychiatric Disorders

Title: Disassociation between spontaneous and visually evoked cortical microcircuit activity in a mouse model of Anti-NMDA Receptor encephalitis

Authors: *S. ALTAHINI[1,2], J. DÖRING[4], H. BACKHAUS[5], R. GUIMARAES-BACKHAUS[5], H. PRÜSS[6], A. STROH[3];
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Abstract: Anti-NMDA receptor encephalitis (NMDARE) is an autoimmune brain inflammation induced by IgG antibodies that bind to the N-methyl-D-aspartate receptors. In addition to patients with acute anti-NMDARE, human anti-NMDAR antibodies were also detected in 1% of asymptomatic controls. This suggests that a considerable subgroup of pregnant women is at risk of transferring anti-NMDAR antibodies to the fetus during early stages of pregnancy. Here, we ask whether in utero exposure to anti-NMDAR antibodies (NMDAR-Ab) affects cortical microcircuit performance in adults. For that, we used a mouse model of maternofetally transferred anti-NMDA receptor antibodies. At P51, corresponding to adolescence in humans, we recorded cortical microcircuit activity with cellular resolution in layer I/II in visual cortex by two-photon calcium imaging in awake behaving mice. We found that microcircuits in NMDAR-Ab exposed mice exhibit a lower spontaneous activity signature compared to healthy control mice. Furthermore, antibody exposure led to a bursty neuronal firing with reduced temporal stability of network states. Notably, upon visual stimulation, neurons in NMDAR-Ab exposed mice had a higher orientation selectivity. In NMDAR-AB exposed mice, a large fraction of those neurons active upon visual stimulation did not fire spontaneously, and vice versa. This dissociation between spontaneous and stimulus-evoked activity is in sharp contrast to the operational principles in healthy networks, and indeed, in control mice, the majority of all neurons were co-active in both conditions. Overall, these findings suggest that in utero exposure to NMDAR-Ab shifts cortical microcircuits to a persistent maladaptive state, characterized by a disassociation between spontaneous and visually evoked activity. Such functional disassociation has been long hypothesized to play a role in the emergence of psychotic symptoms.

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Poster

PSTR395. Human Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR395.13/E27

Topic: H.10. Human Learning and Cognition

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HMZ Flagship Project STRESS
SNSF and Innosuisse BRIDGE Discovery Grant 40B2-0_203606
National Research Foundation, Prime Minister's Office, Singapore under its Campus for Research Excellence and Technological Enterprise (CREATE) programme

Title: Modulating arousal via pupil-based neurofeedback: what we’ve learned so far

Authors: *S. MEISSNER[1], M. BÄCHINGER[1], S. KIKKERT[1], J. IMHOF[1], S. MISSURA[1], M. CARRO DOMINGUEZ[1], N. WENDEROTH[1,2];
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Abstract: *Introduction:* The locus coeruleus (LC), the principal source of noradrenaline in the brain, is one of the key regulators of the brain's arousal level. Additionally, it modulates cardiovascular function via projections to the brainstem and spinal cord. Interestingly, previous research has provided ample evidence for a link between LC activity and non-luminance dependent changes in pupil size. Utilizing this link, we investigated in a series of experiments whether changes in pupil size (i) can be *volitionally* induced via pupil-based neurofeedback (pupil-NF) training, are associated with (ii) LC activity changes, and modulate (iii) electrophysiological and (iv) cardiovascular arousal markers. *Methods:* In experiment I, 54 healthy volunteers (19-47 years) received 3 days of pupil-NF training to learn to volitionally up- and downregulate pupil size. 28 control participants (19-40 years) received the same amount of training and instructions on mental strategies but no veridical pupil-NF. To explore the link between pupil size changes and LC activity, 25 pupil-NF participants were re-recruited for experiment II combining pupil-NF with 3T functional magnetic resonance imaging (fMRI) and pulse oximetry to measure BOLD responses and cardiovascular parameters. In experiment III, 23 participants (21-41 years) completed a slightly adapted pupil-NF training with simultaneous electroencephalography (EEG) and electrocardiography (ECG) recordings. *Results:* Participants of the pupil-NF group were able to successfully self-regulate their pupil size. Such self-regulation was significantly reduced in control participants (experiment I). fMRI, EEG and ECG analyses (experiment II and III) revealed that pupil self-regulation was linked to systematic activity changes in the LC and other arousal-regulating centers in the brainstem and to changes in electrophysiological and cardiovascular arousal markers: we found higher LC BOLD responses, shallower slopes of the EEG power spectrum from 30-40 Hz, higher heart rate, and lower heart rate variability during blocks of volitional pupil size *up-* than *downregulation. Conclusion:* We provide evidence that pupil-NF makes the brain's arousal system accessible to volitional control, a finding that has potential for translation to behavioral and clinical applications across various domains.

Disclosures: **S. Meissner:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MindMetrix. **M. Bächinger:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MindMetrix. **S. Kikkert:** None. **J. Imhof:** None. **S. Missura:** None. **M. Carro Dominguez:** None. **N. Wenderoth:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MindMetrix.

Poster

PSTR395. Human Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR395.14/E28

Topic: C.01. Brain Wellness and Aging

Support: Academy Professor project EmotionAI (grant 336116, 345122)
The University of Oulu & The Academy of Finland Profi 7 (grant 352788)

Title: Behind the mask: EEG as a gateway to distinguish induced and posed emotions

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Abstract: Facial expressions play a pivotal role in facilitating social interactions. However, the accuracy of facial expressions in reflecting genuine emotions is often questionable, and previous studies have predominantly relied on stereotypical posed facial expressions. This limitation hampers the true understanding of emotional expression perception. To address this challenge, our study aims to construct a comprehensive database by integrating facial expressions and corresponding physiological signals captured during both induced and posed emotional states. In the laboratory setting, participants' facial expressions were recorded along with a 32-channel electroencephalography (EEG) recording. Two distinct sessions were conducted: an emotional induction session and a posed emotion session. In the emotional induction session, participants were exposed to various film clips from standardized movie databases, aiming to elicit six types of emotional responses (i.e., happiness, sadness, fear, anger, disgust, surprise). Conversely, the posed emotion session involved the presentation of neutral movies, during which participants were instructed to display corresponding facial expressions in their own personal style. A total of 15 healthy participants (3 females, 12 males) were recruited for data collection, with a target sample size of 50 participants (the dataset will be publicly available for academic use upon completion). By analyzing the collected data, our study aims to uncover the underlying credibility of facial expressions from EEG signals for revealing true emotional states. For this purpose, we developed a deep neural network model based on one-dimensional convolutional operations to differentiate between induced and posed emotions in EEG signals. The model consists of a temporal layer that extracts multi-scale features of each EEG channel in the time dimension, and a spatial layer that further utilizes one-dimensional convolution across all EEG channels to extract global spatial information. Preliminary results from a 6-fold cross-validation experiment on the binary classification of induced and posed emotions showed a mean accuracy of 72.95% (SD: 7.4%) and a mean F1-score of 68.87% (SD: 9.07%). This result confirms our hypothesis that EEG signals can unveil emotions concealed by facial expressions, shedding light on the distinction between genuine and feigned emotional states using multimodal data. In the future, we will continue to explore the data and investigate the correlation of features from different modalities and their fusion to detect and recognize various types of emotions in both states.

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Poster

PSTR395. Human Cognition

Location: WCC Halls A-C

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Program #/Poster #: PSTR395.15/E29

Topic: H.10. Human Learning and Cognition

Support: ISCIII PI19/00298

Title: From the lab to everyday-life habits: the Impact of Striatal Hypoactivity on Habitual Behavior in Parkinson's Disease

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Abstract: Habitual behavior is crucial in every-life, covering a wide range of actions performed automatically and without conscious effort. The striatum plays a central role in the habitual system. In Parkinson's disease (PD), dopamine depletion initially affects caudal sensorimotor putamen and extends subsequently to more rostral regions. Dopaminergic putamen depletion is likely responsible for habits derangement observed in everyday-life activities in PD.Traditionally, the study of habits relied on use of lab-developed tasks. While valuable for understanding habit formation, these tasks may not fully capture the complexity of real-life behavior. To bridge this gap, we aimed to compare lab-developed and everyday-life tasks by means of behavioural and neural activations (fMRI) comparing newly diagnosed PD patients and healthy controls (HC). We designed a lab-developed task (Visuo-motor Association Task) to train associations followed by reversal phase with varying response preparation times. Also, an everyday-life task (handwriting of words) which highly automatic conditions (signature) to goal-directed ones (Greek). Patients were assessed behaviorally and inside the scanner while off medication. In the lab-developed habitual task, results revealed similar responses in both groups. However, PD exhibited fewer errors (habitual and non-habitual) than HC, specifically during the short preparation time interval. On the other hand, handwriting performance in PD patients showed reduced automatism across habitual and goal-directed conditions compared to HC. Posterior putamen activity was increased in HC during habitual conditions, while the caudate was active during goal-directed conditions in the lab task. In contrast, PD patients displayed a reduced posterior putamen activity for habitual trials. In the everyday-life task, HC also revealed posterior putamen activity during habitual conditions transitioning to anterior striatum as behavior required goal-directed efforts. Interestingly, PD patients exhibited reduced posterior putamen activity and a shift towards anterior regions during habitual behavior. This decrease in activity was more prominent when the affected side of patients matched their dominant hand.Altogether, PD patients reveal an enhanced ability to override habitual responses under temporal stress in lab-developed measures, together with reduced automaticity in executing everyday-life habits. Hypoactive posterior putamen during habitual tasks in PD impairs the ability to effectively execute habitual behaviors.

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Poster

PSTR395. Human Cognition

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National Science Foundation (Award 1734887 to SM-C and SLM; Award 1523614 to SLM)
National Institute of Health (Award R01EY031971 to SM-C and SLM; Award R01CA258021 to SM-C, SLM, and SW)

Title: Job title and years of experience are uncertain predictors of perceptual expertise

Authors: *R. ALEXANDER[1], S. WAITE[2], A. VENKATAKRISHNAN[3], S. L. MACKNIK[4], S. MARTINEZ-CONDE[4];
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Abstract: Professionals and trainees are often classified into broad categories of expertise that are inferred from their title, level of training (i.e., medical student, resident, attending, specialist), and/or years of experience—rather than by any objective metric of performance. These categories dramatically oversimplify reality, overlooking individual differences, exceptional skills or natural talent, and potential age-related declines in sensitivity. Rank is usually tied to years on the job, and individuals typically move up but not down the ladder, even if their skills diminish over time. A more principled measure of perceptual expertise would provide the basis to optimize training assessments by allowing education programs to directly test whether trainees are behaving like experts and—if not—what specific behaviors are not yet at a desired level of performance. We conducted a psychophysical and eye-tracking study aimed at quantifying the gaze dynamics used by professional radiologists to detect abnormalities in medical images. Naive individuals with no medical imaging experience (n=19), radiology residents (n=42), and attending radiologists (n=42) searched through chest CTs, each of which contained one abnormality (a potentially cancerous nodule). Consistent with prior work, we found that some observers performed better than expected based on rank and years of experience. In fact, some residents early in their training outperformed attending radiologists, despite an extensive experience differential. These results highlight that, at best, experience is an uncertain predictor of expertise level, and at worse, it reflects little more than seniority. We therefore propose that individuals should instead be grouped based on their objectively measured performance in specific tasks. There is a need for the study of expertise to move beyond standard rank

descriptions or years of experience, and to develop more efficient strategies and methods to quantify expertise.

Disclosures: **R. Alexander:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Expertize, Inc.. **S. Waite:** None. **A. Venkatakrishnan:** None. **S.L. Macknik:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Expertize, Inc. **S. Martinez-Conde:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Expertize, Inc..

Poster

PSTR396. Alzheimer's Disease: Neuroinflammation and Immune System

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR396.01/E30

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG068215

Title: Investigating the impact of prolonged sleep fragmentation on neuroinflammation and microglia response to amyloid-beta in the APPxPS1 KI mice

Authors: F. DICKINSON, H. ALI, L. SANDERS, L. Z. GUO, A. SUBRAMONIAM, H. WHITLOCK, C. JOHNSON, B. O'HARA, M. P. MURPHY, M. DUNCAN, *A. BACHSTETTER;
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Abstract: Poor or disrupted sleep is a hallmark of multiple aging and neurodegenerative disorders, including Alzheimer’s Disease (AD). AD has been pathologically characterized by neurofibrillary tangles and amyloid-beta (Aβ) plaque deposits. Growing evidence suggests that typical glia-mediated clearance of metabolites such as Aβ, occurs during sleep. If sleep quality worsens with age, it is possible that effective glial reactivity to Aβ aggregation may be impacted. Past literature has examined the effect of acute and chronic sleep deprivation on microglia reactivity and pathology in human and animal models. We assessed the impact of sleep fragmentation (SF), a phenomenon similar to the transient arousals observed in AD patients, on inflammatory profiles in male (N=) and female (N=) WT and APP/PS1 KI mice (average age of 9 ± 1.4 months), a mouse model that mimics the human AD condition with more gradual expression of Aβ and cognitive impairment at 9 months. They were either undisturbed during sleep (US) or exposed to SF. SF consisted of four daily sessions (1 hour each) of enforced wakefulness (induced with toys and gentle paintbrush stimulation) evenly distributed across the light phase for 5 days/per week. This US and SF procedure was carried out over three weeks (Monday - Friday). SF mice slept 20% less in the light phase, and female KI mice exhibited a 70% increase in rebound sleep during the dark phase ($P<0.001$), demonstrating our SF paradigm

modified sleep patterns. Immediately after the last SF session, mice in both groups were euthanized and cortical and hippocampal tissue was dissected and frozen. At the whole tissue level, no change was found in proinflammatory cytokine protein levels following SF. The immunohistochemical analysis found a robust genotype difference in overall microglia (IBA1+) and astrocytes (GFAP+); however, SF did not modify this effect. There was also no genotype by SF interaction. Analyses using confocal microscopy and Imaris software to explore whether SF might be associated with a more specific impact on microglia and Aβ plaques are in progress. These findings indicate that a slow progressing AD-relevant mouse model can endure three weeks of sleep fragmentation without substantial neuropathological changes, implying that the negative impact of disrupted sleep on Aβ clearance may not be mediated by proinflammatory processes but other elusive factors.

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Poster

PSTR396. Alzheimer's Disease: Neuroinflammation and Immune System

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR396.02/E31

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH RF1AG069378
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NIH R01AG048993

Title: Anti-$\alpha_4\beta_7$ integrin therapy decreased M1-like microglia and promoted A2 phenotype astrocytes in the brains of $App^{NL\text{-}G\text{-}F}$ mice

Authors: *S. CHANDRASEKARAN, A. M. MCINTEE, S. NOOKALA, B. SAHU, A. M. FLODEN, C. K. COMBS;
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Abstract: Anti-$\alpha_4\beta_7$ integrin therapy decreased M1-like microglia and promoted A2 phenotype astrocytes in the brains of $App^{NL\text{-}G\text{-}F}$ mice. Alzheimer's disease (AD) brains are often characterized by the presence of reactive microglia and astrocytes and an overall proinflammatory milieu. Increasing evidence supports the notion that peripheral immune cells may influence the overall brain environment. For example, our prior work demonstrated that intestines of the $App^{NL\text{-}G\text{-}F}$ mouse model of AD have an altered immune phenotype and a colitis-like insult alters brain changes during disease. We also observed a noticeable reduction in GFAP and Iba-1 positive reactive astrocytes and microglia, respectively, in APP/PS1 mice treated with

anti-$\alpha_4\beta_1$ integrin antibody. These results suggest an immune-based communication of intestinal and peripheral changes to the brain during disease. To further explore this mechanism, we administered an inflammatory bowel disease (IBD) therapy, anti-$\alpha_4\beta_7$ antibody, to littermate control C57BL/6J wild type and *App*$^{NL\text{-}G\text{-}F}$ AD mice at 5-7 months of age to examine consequences on glial phenotype in the brain. Mice were randomly divided into control, anti-$\alpha_4\beta_7$, or isotype negative control groups for each sex. Antibodies were injected intraperitoneally at 10 mg/kg once a week for 10 weeks. The phenotypic characteristics of brain resident microglia and astrocytes were determined by immunohistochemistry for CD16, CD45, and S100A10 proteins. Both male and female *App*$^{NL\text{-}G\text{-}F}$ mice treated with anti-$\alpha_4\beta_7$ antibody showed a decrease in CD16 and CD45 immunoreactivity in the hippocampus when compared to untreated control *App*$^{NL\text{-}G\text{-}F}$ mice, suggesting a decline in the pro-inflammatory M1-like microglia population. Antibody treatment had no effect on CD16 or CD45 immunoreactivity in wild type mice. Anti-α4β7 treatment increased S100A10 immunoreactivity in male and female *App*$^{NL\text{-}G\text{-}F}$ mice compared to their respective untreated control groups suggesting an increase in neuroprotective A2-like astrocytes. Antibody treatment did not affect wild type astrocyte S100A10 immunoreactivity. These findings suggest that neuroinflammation and glial phenotype in AD may be influenced by intestinal immune cells and interventions focused on these peripheral immune cell targets may be sufficient to provide ameliorative effects in the brain.

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Poster

PSTR396. Alzheimer's Disease: Neuroinflammation and Immune System

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR396.03/E32

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 1 R01 AG071228

Title: The Effect of Mild Repetitive Traumatic Brain Injury Early in Life on the Progression of Alzheimer's Disease in 3xTgAD Mice

Authors: *C. C. H. BARKER, L. ALMUHANNA, L. A. KOZA, D. LINSEMAN;
Univ. of Denver, Denver, CO

Abstract: Repetitive traumatic brain injuries (rTBIs) have been shown to increase neuroinflammation in both mouse models and human studies and are predicted to increase risk for neurodegenerative disorders including Alzheimer’s disease (AD). Given the number of TBIs reported just in the US each year, a large part of the population could be at an increased risk of developing AD. By using a combination of behavioral tests, histopathology, and exosomal biomarker analysis, we investigated whether brain trauma accelerates cognitive dysfunction, brain pathology and appearance of exosomal biomarkers of neurodegeneration and

neuroinflammation in 3xTg-AD mice subjected early in life to repetitive mild TBI (rmTBI). Four groups of mice including naïve 3xTg-AD mice, sham 3xTg-AD mice, rmTBI 3xTg-AD mice, and naïve wild type mice, were used for this study. Mice in the rmTBI group were given 5 mTBIs, each separated by 48 hours, at 3-months old. At 11-months old, mice were assessed for cognitive function using the Barnes maze, Y-maze, and Novel Object Recognition (NOR) behavioral tests. Blood and brain tissue were taken immediately after the conclusion of the behavioral tests. Hippocampal sections of brain were stained for amyloid-beta and phosphorylated-Tau, proteins that constitute pathological hallmarks of the disease. Total exosomes were isolated from collected plasma and cell type-specific exosome subpopulations, including those from astrocytic, neuronal, and microglial origin, were isolated from brain tissue. Preliminary results from the behavioral tests indicate that there are no significant differences in cognitive function between the 3xTgAD mouse groups. However, the wild type mice do perform better across all behavioral tests than any of the 3xTg-AD mouse groups. The histopathology data shows differences in the number of amyloid-beta plaques and p-Tau staining in the hippocampus across the 3 AD mouse groups. Future biomarker analyses will examine markers of neuroinflammation (e.g., IL-6, IL-1beta, TNF-alpha) and neurodegeneration (e.g., amyloid beta, p-Tau, and TDP-43) in total plasma-derived exosomes and cell type-specific exosome subpopulations isolated from mouse brain. Our preliminary results indicate that receiving rmTBIs early in life may accelerate disease progression, indicated by histopathology, in mice which are genetically predisposed to developing AD. Biomarker analyses will reveal if the increased brain pathology correlates with an increased appearance of exosomal biomarkers of neuroinflammation and neurodegeneration in 3xTg-AD mice subjected to rmTBIs.

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Poster

PSTR396. Alzheimer's Disease: Neuroinflammation and Immune System

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Program #/Poster #: PSTR396.04/E33

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01AG058673

Title: Inhibition of NLRP3 leads to reduced tau pathology and improved social behavior in mice.

Authors: *J. GREEN[1], D. SUN[3], C. NELSON[1], Y. XU[2], S. ZHANG[2];
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Abstract: Inhibition of NLRP3 leads to reduced tau pathology and improved social behavior in 3xTg mice.
Authors: ***Jakob Green**[^], Christopher Nelson[*], Yiming Yu[#], Shijun Zhang[#], Dong Sun[*];

[^]Department of Anatomy and Neurobiology, Virginia Commonwealth University; [#]Department of Medicinal Chemistry, Virginia Commonwealth University
Abstract: Alzheimer's disease (AD) is a devastating disease that affects millions of patients around the world with limited medication available to manage the disease. In AD pathological development, neuroinflammation plays a significant role. Recent studies have identified NLRP3 inflammasome as a critical multiprotein platform regulating inflammatory response, and have shown involvement in AD pathogenesis in AD patients and animal models. In this study, we studied the role of NLRP3 in tau pathology using a combination of a pharmacological approach using a novel NLRP3 inhibitor and a transgenic approach with NLRP3 gene knock down in 3xTg-AD mice, a mouse line carrying the APP695 gene with Swedish mutations (KM670/671NL/M596L), along with the PSEN1 (M146V) and tau (P301L) mutations. The 3xTg-AD mice were treated with our novel NLRP3 inhibitor or vehicle when animals were at 5 months as single daily (20mg/kg) via oral gavage for 3 months. The 3xTg/NLRP3 KO mice were not treated. After 3 months treatment, AD related behavior changes including anxiety and cognitive functions were assessed. After completion of behavior tests, animals were euthanized, brains were collected for Western blotting and immunostaining to assess tau pathology. In comparing several phospho-tau modifications by Western blot and a novel ultrasensitive electrochemiluminescence immunoassay, we found significant effect of NLRP3 on the protein expression level of pT175, cis-pT231, pS396, pS214, and pS610 tau in female mice. Among them, pT175, cis-pT231, pS396, and pS214 showed a reduction in the treated and KO groups while pS610 only showed a significant decrease in the KO group. We also found that AT180, a similar anti-pT231 antibody, was reduced in the treatment and KO groups in both males and females. In behavior tests, no difference was found in social affiliation (sociability), a test for social motivation. In a social novelty test, an assessment for social memory, animals with NLRP3 inhibition showed significant improvement in performance. We found that NLRP3 inhibition with drug treatment or gene knock down significantly reduce AD-associated tau pathology, together with reduced anxiety like symptoms. In conclusion, our study further confirms the role of NLRP3 in AD and tau phosphorylation.

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Poster

PSTR396. Alzheimer's Disease: Neuroinflammation and Immune System

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Topic: C.02. Alzheimer's Disease and Other Dementias

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U54GM128729
NRCT N41A640191

Title: Dietary supplementation of wild edible mushroom Phallus atrovolvatus aqueous extract did not exacerbate Alzheimer's disease in a mouse model

Authors: *R. KAEWSAEN[1], E. OLSON[2], B. SAHU[3], W. P. CHANPUT[1], L. ROJANATHAMMANEE[4], C. K. COMBS[5];
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Abstract: Alzheimer's disease (AD) is a neurodegenerative disease characterized by progressive dementia and brain accumulation of Aβ peptide-containing plaques, gliosis, neuroimmune changes, and neurofibrillary tangles. Recently intestinal changes, including dysbiosis, have been suggested to also be a component of the disease process. Our prior work demonstrated intestinal dysfunction and dysbiosis in the *App*$^{NL\text{-}G\text{-}F}$ mouse model of AD and exacerbation of brain changes by inducing a colitis-like condition. This study aimed to evaluate whether a dietary intervention of the wild edible mushroom, *Phallus atrovolvatus*, recently found in Thailand could alter intestinal dysfunction and dysbiosis in the *App*$^{NL\text{-}G\text{-}F}$ mice while also reducing brain presentation of disease. Male and female 6-7 month old littermate wild type control (C57BL/6) and *App*$^{NL\text{-}G\text{-}F}$ mice were randomly assigned to either control or a diet supplemented with mushroom extract containing 30% β-glucans and 13% protein for 8 weeks to quantify changes in body weight, memory, brain cytokines, immune changes, gliosis, intestinal microbiome, and brain Aβ levels. Body weights in either genotype or sex did not significantly change throughout the study and the mushroom extract did not alter serum lipopolysaccharide compared to control diet indicating that it had no adverse effects on gut leakiness. To examine effects on behavioral performance, a cross maze test was performed. Mushroom extract feeding did not alter spatial recognition memory in either sex or genotype compared to their respective control diet groups. Our findings demonstrate that dietary intervention with a previously characterized immunomodulatory mushroom extract from *Phallus atrovolvatus* had no adverse effects on gut health or memory and support further evaluation of protective immunomodulatory or anti-Aβ effects in the brain.

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Poster

PSTR396. Alzheimer's Disease: Neuroinflammation and Immune System

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Program #/Poster #: PSTR396.06/E35

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: In vivo examination of the TREM2-dependent human microglial responses to Alzheimer's disease pathology using proteomics in chimeric mice

Authors: *Y. JIAO;
Merck, Cambridge, MA

Abstract: *In vivo* examination of the TREM2-dependent human microglial responses to Alzheimer's disease pathology using proteomics in chimeric miceAuthors: Yun Jiao[1], Hayk Davtyan[2], Carlo Ramil[1], Sepideh Kiani Shabestari[2], Jonathan Hasselmann[2], Jean Paul Chaderevian[2], Xuemei Yang[1], Alex Tamburino[1], Matt Blurton-Jones[2], Rebecca Mathew[11] Merck & Co., Inc., Cambridge, MA, USA; [2] University of California, Irvine, Irvine, CA, USA
Background: Alzheimer's disease (AD) is the most common neurodegenerative disease. Recent genome-wide association studies identified many risk genes expressed in microglia, suggesting that microglia play a central role in AD. One of the strongest risk genes, TREM2, is exclusively expressed by microglia. Loss of function mutations of TREM2 was showed to increase AD risk dramatically. TREM2 knockout in mice was shown to impair the ability of microglia to respond to beta-amyloid plaques. However, it remains unclear whether human TREM2 knockout microglia exhibit similar or perhaps additional functional deficits.Methods:To further examine the impact of TREM2 deletion on human microglia, we used CRISPR to generate TREM-knockout (TREM2-KO) induced pluripotent stem cells (iPSCs). Isogenic wildtype and TREM2-KO iPSCs were differentiated into hematopoietic progenitors (HPCs) and transplanted into postnatal immunodeficient AD mice (hCSF1-5xFAD). Six months later, human microglia were isolated from chimeric mice and examined via bulk proteomic analysis. Results:Analysis of proteomic dataset revealed significant and novel impacts of TREM2 deletion on the response of human microglia to beta-amyloid pathology. The protein abundance of a set of AD risk genes showed TREM2-dependence in their response to beta-amyloid pathology. Immune-related pathways in human microglia were altered between TREM2 genotypes in AD mice. The engulfment and phagocytosis of mouse synapses by human microglia were also altered by the loss of TREM2. These data reveal important new information about changes occurring within human microglia in response to amyloid pathology and loss of TREM2 expression.

Disclosures: Y. Jiao: A. Employment/Salary (full or part-time):; Merck & Co.

Poster

PSTR396. Alzheimer's Disease: Neuroinflammation and Immune System

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR396.07/E36

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 5R01AA026756-03
NIH Grant 5R21AA029263-02

Title: Prenatal ethanol exposure impairs fear acquisition in male transgenic F344-AD rats

Authors: *N. SAMIYA[1], S. BAKE[2], D. A. HURST[3], R. C. MIRANDA[4], F. SOHRABJI[5];
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Abstract: Alzheimer's Disease (AD) is a progressive neurodegenerative disease characterized by cognitive deficits, behavioral changes, and memory loss. Prenatal alcohol exposure (PAE) is known to affect neurodevelopment, leading to behavioral and cognitive impairment throughout the lifespan. Our previous studies related to PAE and stroke, a risk factor for dementia, have shown sex-dependent differences in behavioral outcomes; PAE male rats showed impairment during the learning phase of the Barnes maze, a spatial hippocampal task, while females did not. In this project, we examined the effect of PAE on a cued fear conditioning task in an animal model of Alzheimer's disease. Wild-type (WT) Fischer F344 females were time mated with Fischer transgenic (Tg) F344-AD males harboring the human Swedish mutation amyloid precursor protein and the presenilin-1 exon 9 deletion mutant. Pregnant dams were exposed to vapor ethanol, or room air (controls), for 1 hour daily (GD 11-16). Blood ethanol concentrations averaged 132.337 mg/DL. Male and female PAE offspring at 6 months were tested on a 3-day fear conditioning paradigm to assess cognitive function. On day 1, animals were placed in the chamber and exposed to context A for baseline recording of movement with visible light and fan switched on, then subjected to four tone-shock pairings (10 second tone co-terminated with a 1 second foot shock,0.8 mA). Fear extinction took place on day 2, in which rats were exposed to a new context (B), with 30 tone trials (1 kHz, 80 dB, 10 s) without shock. Fear extinction recall took place on day 3, also using context B, paired with 15 tone trials (1 kHz, 80 dB, 10 s). Context A had visible light and fan noise whereas context B included plastic flooring covering the metal grating, ambient white noise, near infrared lighting, a black cover over the chamber, and the scent of home cage bedding. Fear learning analysis revealed that WT male PAE rats had significantly higher freezing response (Two-way ANOVA, main effect of tone-shock, $p<0.0001$) compared to Tg-PAE males, suggesting that PAE decreased fear acquisition in AD transgenic animals. Female WT and Tg PAE animals were not significantly different in their freezing responses, however, the overall freezing response in females was lower than the males, indicating a potential sex-difference in fear learning. These findings suggest that PAE exerts sex-specific effects in associative learning in AD transgenic animals.

Disclosures: **N. samiya:** None. **S. Bake:** None. **D.A. Hurst:** None. **R.C. Miranda:** None. **F. Sohrabji:** None.

Poster

PSTR396. Alzheimer's Disease: Neuroinflammation and Immune System

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR396.08/E37

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NINDS grant 1R01NS114656

Title: Rostrace reveals APOE4 associated-neuroinflammation in a humanized mouse model of sporadic Alzheimer's disease

Authors: *Y. ZHU[1,2], A. YOUNG[2], J. CONI[1], M. SHELDON[1], M. RAJASEKARAN[1], B. GENOVESE[1], A. C. RAIKES[3], J.-P. WIEGAND[3], R. H. MACH[2], R. D. BRINTON[3], M. J. MCMANUS[1];
[1]Children's Hosp. of Philadelphia, Philadelphia, PA; [2]Dept. of Radiology, Univ. of Pennsylvania, Philadelphia, PA; [3]Ctr. for Innovation in Brain Sci., Univ. of Arizona, Tucson, AZ

Abstract: Alzheimer's disease (AD) is a devastating neurodegenerative disease affecting 6.5 million in the U.S. alone and has no cure. Mutations in APP and tau are associated with familial AD, but over 98% of AD patients have the late-onset, sporadic form. The greatest risk factors for late-onset AD (LOAD) are age, female sex, and apolipoprotein 4 (APOE4). These 3 risk factors converge at the mitochondrial-immune axis and transform the brain into a toxic, pro-inflammatory state that initiates AD pathogenesis. One or two copies of APOE4 alleles can increase the risk for AD by approximately 4- and 15-fold, respectively, compared to the healthy population. Mounting evidence suggests that APOE4 can cause unregulated production of reactive oxygen species (ROS) by inducing mitochondrial dysfunction in the neurons, modulating microglial immunometabolism, and accelerating blood-brain barrier (BBB) breakdown via the cyclophilin A pathway in pericytes. We hypothesize that these APOE4-associated effects join to create an environment of high oxidative stress in the brain that may emerge prior to clinical diagnostic AD symptoms. In the current study, we utilize a superoxide ($O_2^{\bullet-}$) -sensitive radioactive tracer, [^{18}F]ROStrace, to track oxidative stress in the living brain in humanized mice with targeted replacement of murine APOE4 and APP genes with the human genes. This study aimed to determine whether [^{18}F]ROStrace retention is higher in the early-stage pathology of LOAD mice. Age and gender-matched hAPOE4 and hAPOE4+hAPP mice were imaged with [^{18}F]ROStrace at mid-life, alongside control animals. Our microPET/CT data demonstrated increased [^{18}F]ROStrace retention in the hAPOE4+hAPP mouse group compared to hAPOE4 and control groups, which correlated with metabolic and behavioral changes. However, there was no difference in tracer retention between the hAPOE4 and control group. Moreover, the histological analysis also confirmed an abnormally elevated neuroinflammation in the hippocampus region of the hAPOE4+hAPP mouse brain, which was absent in the other two animal groups. Therefore, [^{18}F]ROStrace is sensitive to detecting ongoing oxidative stress and neuroinflammation in humanized LOAD mice. Thus, [^{18}F]ROStrace may provide a noninvasive method to identify LOAD patients before irreparable neurodegeneration occurs and evaluate anti-inflammatory AD therapeutics. This research is supported by NINDS grant 1R01NS114656.

Disclosures: Y. Zhu: None. **A. Young:** None. **J. Coni:** None. **M. Sheldon:** None. **M. Rajasekaran:** None. **B. Genovese:** None. **A.C. Raikes:** None. **J. Wiegand:** None. **R.H. Mach:** None. **R.D. Brinton:** None. **M.J. McManus:** None.

Poster

PSTR396. Alzheimer's Disease: Neuroinflammation and Immune System

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR396.09/E38

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA R01 AG068286
5T32GM132055-03

Title: Early-life Exposure to Heavy Metals Promotes Neuroinflammation and Cognitive Deficits in a Mouse Model of Alzheimer's Disease

Authors: *M. COOK[1], S. DIXON[1], S. CARROLL[2], G. WANG[2];
[1]Pathology and Lab. Med., [2]Med. Univ. of South Carolina, Charleston, SC

Abstract: Environmental or occupational exposure to heavy metals is a significant public health concern. Cadmium (Cd) and lead (Pb) pose a major public health challenge due to their ubiquitous presence in the environment and their established toxicological profiles even at low concentrations. Alzheimer's disease (AD) is a debilitating and progressive neurodegenerative disorder that causes dementia and death. A growing body of evidence demonstrates that genetic, environmental, and epigenetic factors all contribute to AD pathogenesis and progression. In line with this, epidemiological studies have identified an association between the exposure to neurotoxic heavy metals and an increased risk for neurodegenerative disorders. However, the molecular mechanisms by which heavy metal exposure affects the pathogenesis and progression of AD have not been completely understood. Using the 5xFAD transgenic mouse model of AD, we have demonstrated that early-life exposure to Pb or Cd markedly accelerates the decline of cognitive functions as determined by Y-maze tests in juvenile mice that typically will not display any AD symptoms at such as a young age. Open field tests have revealed that Pb or Cd exposure induces anxiety-like behavioral changes. Although both Cd and Pb can cause anxiety-like behavioral changes and cognitive deficits, Cd induces a greater level of cognitive deficits and anxiety-like behaviors at a 100-fold lower concentration, suggesting that Cd is much more potent than Pb in promoting AD development and progression. Mechanistic studies unveil that Cd is more effective than Pb at inducing the formation of amyloid beta (Aβ) plaques in the hippocampus and cortex. Our data also show that the levels of pro-inflammatory cytokines, including IL-1β, TNF-α and IL-6, were significantly higher in the hippocampus and cortex of mice treated with Cd or Pb than that in control mice. Furthermore, we found that Cd exposure stimulated TNF-α and IL-6 expression preferentially in the hippocampus over the cortex, indicating a spatial difference in Cd-induced upregulation of IL-6 and TNF-α expression in the brain. Given the important implications of Aβ deposition and neuroinflammation in AD, these results imply that exposure to heavy metals, such as Cd in particular, may accelerate AD pathogenesis via promoting Aβ accumulation and inflammatory cytokine-mediated neuroinflammation.

Disclosures: **M. Cook:** None. **S. Dixon:** None. **S. Carroll:** None. **G. Wang:** None.

Poster

PSTR396. Alzheimer's Disease: Neuroinflammation and Immune System

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR396.10/E39

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R03 AG067061
R21 AG071133
OSU Psychiatry seed grant

Title: High-fat diet impairs memory, alters phagocytic function, and activates the complement cascade in the 3xTg-Alzheimer's Disease mouse model

Authors: *S. MACKEY-ALFONSO[1], M. BUTLER[2], N. DEEMS[2], S. MUSCAT[2], B. GONZALEZ OLMO[2], R. BARRIENTOS[2];
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Abstract: Alzheimer's Disease (AD) is a neurodegenerative disease characterized by profound memory impairments, synaptic loss, neuroinflammation, and the hallmark pathology of amyloid beta plaques and neurofibrillary tau tangles. High fat diet (HFD) consumption has been shown to increase the risk of developing AD in humans and exacerbate neuroinflammation and memory impairment in AD mouse models. However, the underlying mechanisms linking diet to AD risk is not well characterized. Thus, this project investigated the effect of HFD consumption on 1) memory, 2) neuroinflammation, and 3) phagocytosis of synapses in the 3xTg-AD mouse model. Following the consumption of either standard chow or short-term HFD, young adult AD mice were behaviorally assessed using the novel object recognition/location memory task. In a separate cohort, AD and WT mice fed chow or HFD were transcardially saline-perfused and the hippocampus was dissected for transcript and protein analysis of pro- and anti-inflammatory markers. Protein levels of complement markers were also assessed. In another cohort, synapses were isolated from the hippocampus of AD chow and HFD-fed mice and conjugated to pH-rhodamine, which turns red when phagocytosed by cells. The amount of phagocytosis of synapses by BV2 microglial cells was tracked over 4 hours to determine any differences between groups. Only in AD mice, HFD significantly impaired hippocampal-dependent memory and increased gene and protein levels of pro- and anti-inflammatory cytokines and a marker of microglial reactivity. Complement protein expression was higher in HFD-fed AD mice. Synapses from HFD-fed AD mice were phagocytosed at a higher rate than those from chow-fed mice. These data demonstrate HFD consumption increases neuroinflammation and complement activation in 3xTg-AD mice resulting in increased phagocytosis of synapses and memory impairment. Future studies will investigate the role of complement driving these diet-induced memory and synaptic changes.

Disclosures: S. Mackey-Alfonso: None. **M. Butler:** None. **N. Deems:** None. **S. Muscat:** None. **B. Gonzalez Olmo:** None. **R. Barrientos:** None.

Poster

PSTR396. Alzheimer's Disease: Neuroinflammation and Immune System

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR396.11/E40

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Prevention of Alzheimer pathology by blocking neuregulin signaling on microglia

Authors: J. LIU, J. GERAGHTY, S. SCHRAM, J. LEI, J. A. LOEB, ***F. SONG**;
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Abstract: Plaque formation, microglial activation, and synaptic loss are pathological hallmarks of Alzheimer's disease, however, removing plaques have had little clinical benefit. Here, we show that neuregulin-1, a glial growth factor, induces inflammatory cytokines and promotes phagocytic activity in vitro and augments microglial activation and plaque formation in 5XFAD Alzheimer's mice. Brain-specific targeting of neuregulin-1 by intraventricular delivery of a novel neuregulin-1 fusion protein antagonist GlyB4 significantly alters microglial morphology and function to a non-pathogenic phenotype in early-stage 5XFAD mice and prevents plaques from forming. At later stages, once plaques have already formed, GlyB4 reduces new plaque formation and prevents synaptic loss. Selective, targeted disruption of neuregulin-1 signaling on brain microglia with GlyB4 could be a novel 'upstream' approach to slow or stop disease progression in Alzheimer's disease.

Disclosures: J. Liu: None. **J. Geraghty:** None. **S. Schram:** None. **J. Lei:** None. **J.A. Loeb:** None. **F. Song:** None.

Poster

PSTR396. Alzheimer's Disease: Neuroinflammation and Immune System

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR396.12/E41

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R44AG069622

Title: Effects of a novel anti-inflammatory on behavioral deficits and microglial morphology in a transgenic mouse model of Alzheimer's disease

Authors: *K. L. IVANICH[1], L. D. PEETERS[1], W. D. GILL[2], L. J. WILLS[1], A. M. CUOZZO[1], A. G. CORNETT[1], P. GABBITA[3], R. W. BROWN[1];
[1]Biomed. Sci., East Tennessee State Univ. Quillen Col. of Med., Johnson City, TN; [2]FDA, NCTR, ORISE/NCTR/FDA, North Little Rock, AR; [3]P2D Biosci., P2D Biosci., Cincinnati, OH

Abstract: Alzheimer's Disease (AD) is the most common form of dementia. It is a fatal neurodegenerative disease that leads to both cognitive decline and altered psychological states. There is currently no cure for AD. The pathology of AD includes the clustering of insoluble amyloid-β (Aβ) plaques, tau tangles, and increased neuroinflammation. The heightened neuroinflammatory state in AD is an essential point of focus in AD research. In this study, the novel oral anti-inflammatory tumor necrosis factor-α (TNF-α) inhibitor compound PD2244 was tested to observe its effects on sensorimotor gating using prepulse inhibition (PPI), spatial memory using Barnes Maze, and anxiety using an elevated-T maze in both female and male 3xTg mice. The 3xTg mice are the only triple-transgenic model of AD that has both Aβ plaques and tau tangles and is also an aging mouse model of AD pathology onset. A specialized diet containing variable doses of PD2244 was given to 3xTg mice beginning at 6 months of age. The doses given were 0, 1, 3, 10, and 30 mg/kg of PD2244. Testing was then performed at 9, 12, and 15 months, where 15 months equated to thorough AD pathology. Regarding behavioral improvement, it was observed that all doses of PD2244 were effective in alleviating deficits in PPI at 9, 12, and 15 months of age. On the Barnes Maze, at 9 months of age, the 10 mg/kg dose of PD2244 was effective at alleviating spatial memory deficits, whereas at 12 months the 3 and 30 mg/kg dose of PD2244 were effective, and finally, at 15 months the 3, 10, and 30 mg/kg doses of PD2244 demonstrated efficacy at alleviating deficits in spatial memory performance. On the elevated T-maze, there were no effects at 9 months of age, but the 3 mg/kg dose of PD2244 resulted in anxiolytic effects at 12 and 15 months of age. Nest building behavior is also being observed in 15-month-old mice to determine effects of PD2244 on apathy since it is a common neuropsychiatric symptom of AD. In addition, there is currently a project analyzing immunohistological staining of microglial cells in the hippocampus (HPC) and prefrontal cortex (PFC) in 15-month-old animals, which will be presented. These behavioral and neurobiological analyses are designed to discover a novel, effective, anti-inflammatory treatment for cognitive deficits and increases in anxiety associated with AD.

Disclosures: K.L. Ivanich: None. **L.D. Peeters:** None. **W.D. Gill:** None. **L.J. Wills:** None. **A.M. Cuozzo:** None. **A.G. Cornett:** None. **P. Gabbita:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); P2D Bioscience. **R.W. Brown:** None.

Poster

PSTR396. Alzheimer's Disease: Neuroinflammation and Immune System

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR396.13/F1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG057565
NIH Grant AG066198
NIH Grant AG077636
NIH Grant AG075992

Title: Brain cellular responses to peripheral inflammation in mouse model of Alzheimer's Disease

Authors: *Y. LU, C. SAIBRO-GIRARDI, N. F. FITZ, M. A. OSTACH, I. LEFTEROV, R. KOLDAMOVA;
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Abstract: Background: Peripheral infection has been implicated in the development of various neurodegenerative disorders, such as Alzheimer's Disease (AD). We aimed to test the effect of peripheral inflammation on amyloid pathology and brain transcriptome.**Methods**: We used APP/PS1 mice and had the mice intranasally exposed to *Staphylococcus aureus* (Staph) or PBS as control for different time period (chronic: 16-week-treatment, Staph n=16, 8 females and 8 males, and PBS n=20, 10 females and 10 males; acute: 1-week treatment, Staph n=4 and PBS n=4, 2 males and 2 females each group). We conducted bulk-, single-cell, and spatial-transcriptomics analysis on the mouse brain tissue, as well as cytokine assays and histology for confirmation.**Results**: Chronic exposure resulted in increased accumulation of amyloid plaques and microglia associated with plaques, leading to significant alterations in the transcriptional profile of brain barrier-associated cells and subsequent barrier dysfunction. We identified cell type- and spatial-specific transcriptional changes associated with brain barrier (BBB) functions and neuroinflammation during the acute infection. We found upregulation of BBB-related endothelial genes (*Ddit4* and *Apold1*) and functions, such as apoptosis and hypoxia. *Cldn5*, encoding an important tight junction protein, was found downregulated with Staph treatment. Both acute and chronic exposure elicited responses in brain macrophages and had detrimental effects on neuronal transcriptomics. We found upregulation of IFN-induced macrophage *Bst2* gene and other genes from the IFN-induced transmembrane proteins family, like *Ifitm2*, by acute infection. Furthermore, we observed distinct transcriptional responses in the microenvironment around amyloid plaques following acute infection, characterized by increased expression of disease-associated microglia genes (such as *Tyrobp*, *Ctss*, *Ctsd*, etc.), and a more pronounced effect on astrocytic (such as *Gfap* and *Clu*) or macrophage-associated genes (*Lyz2* and *Ccl6*), potentially facilitating the development of amyloid and related pathologies.**Conclusion**: Our findings provide insights into the underlying mechanisms connecting peripheral inflammation and AD pathology.

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Poster

PSTR396. Alzheimer's Disease: Neuroinflammation and Immune System

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR396.14/F2

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Ubiquitinated IL1B and Defective Inflammasome Formation in Alzheimer's Disease: Implications for Chronic Brain Inflammation.

Authors: *M. G. SABBIR[1], L. M. SALGUEIRO[2];
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Abstract: Chronic brain inflammation is implicated in the development and progression of Alzheimer's disease (AD). Inflammation manifests the release of cytokines, such as interleukin-1 beta (IL1B), a pro-inflammatory cytokine involved in immune responses and inflammation. Elevated levels of IL1B have been reported in AD brains. IL1B is initially synthesized as an inactive precursor and undergoes cleavage by caspase 1 associated with the inflammasome, releasing the active form. To downregulate IL1B and prevent excessive inflammation, the body employs ubiquitination, marking IL1B for proteasomal degradation. We hypothesize that ubiquitinated IL1B is present in inflammatory brains, including AD, and any defect in it may lead to unchecked inflammation, accelerating neurodegeneration. We conducted western blotting to assess the abundance of cleaved and ubiquitinated IL1B in postmortem brain tissues and examined their correlation with inflammasome formation. We used lipopolysaccharide and phorbol ester-activated human monocytic cells (THP-1) to characterize cleaved IL1B secretion and the expression of reactive inflammasome-associated proteins, including NLRP1/3, AIM2, NLRC4, ASC/TMS1, and Caspase-1. Next, we analyzed postmortem frontal/temporal cortex and hippocampus tissues from non-demented individuals of different age groups (young: 0-16 years, older: 67-80 years) and AD patients (N=74) to investigate age/dementia-related IL1B production in the brain. Our findings reveal the presence of 25 kDa cleaved IL1B in the inflammatory brains of some of the non-demented individuals across different age groups. Conversely, we did not detect cleaved IL1B in the temporal cortices of AD patients, but we observed variable amounts of 37-50 kDa ubiquitinated IL1B in 59% of AD patients' temporal cortices. The presence of NLRP1-associated inflammasome was inconsistent in the ubiquitinated IL1B-positive temporal cortices and only noted in 15% of AD brains. Isoelectric focusing demonstrated abnormal phosphorylation (negatively charged fraction) of ubiquitinated IL1B, which correlated with an increased accumulation of 37-50 kDa IL1B in AD brains compared to non-demented inflammatory brains. Overall, these findings suggest that abnormal post-translational modification of IL1B in AD brains may contribute to the accumulation of ubiquitinated IL1B and hinder the formation of the inflammasome, potentially impeding the resolution of inflammation in AD brains. Further characterization of this defective IL1B signaling pathway will provide novel insights into the neuroinflammatory origin of AD.

Disclosures: **M.G. Sabbir:** None. **L.M. Salgueiro:** None.

Poster

PSTR396. Alzheimer's Disease: Neuroinflammation and Immune System

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR396.15/F3

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Resistance exercise attenuates cognitive impairment and neuroinflammation induced by surgery performed under sevofluorane anesthesia

Authors: *A. WONG[1], R. CHANG[1,2];
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Abstract: Resistance exercise (RE) improves cognition in human and animal studies associated with Alzheimer's Disease (AD). However, the mechanisms remain poorly investigated. Previous studies have reported that pre-existing cognitive impairment imparts susceptibility to accelerated cognitive decline following an acute systemic inflammatory episode. The aim of this study was to compare cognitive impairment between sedentary and resistance-exercise-trained mice in the Alzheimer transgenic mouse model following sevoflurane anesthesia and abdominal surgery. To evaluate the role of RE in modulating pre-existing cognitive impairment, 5-month-old female *3xTg* and age-matched (C57BL/6J) controls underwent a five-week RE-training protocol. Novel-object recognition (NOR), y-maze, and fear-conditioning (FC) tests were conducted to probe for cognition and learning. The discrimination index, spontaneous alternation percentage, and freezing behavior were the major outcomes. In a second experiment, laparotomy was performed after training, and 3 days later, the same behavior tests were performed. Brain homogenates were harvested for western blot (WB) analysis, qPCR, or post-fixed in neutral-buffered formalin solution for immuno-histochemical exploration. In *3xTg* mice, RE mice exhibited improved alternation, discrimination, and freezing in NOR, y-maze, and FC test (all $P < 0.05$). qPCR revealed decreased inflammatory cytokine transcripts in the hippocampus compared to sedentary controls. No differences were observed in control mice. In post-operative *3xTg* mice, surgery induced cognitive deficits in NOR but not in FC test. RE-trained mice showed a significant increase in discrimination and freezing percentages compared to sedentary counterparts (both $P < 0.05$) and a significant reduction in inflammatory cytokines and apoptotic signaling pathways post-surgery. RE attenuated exaggerated cellular responses and improved cognitive functions in AD mice. Sevoflurane anesthesia followed by surgery induced changes in cognition and pathological changes in the hippocampus, and pre-habilitative RE prevented the acceleration of cognitive impairment triggered by the acute inflammation insult.

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Poster

PSTR396. Alzheimer's Disease: Neuroinflammation and Immune System

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Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR396.16/F4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 AG076610-01

Title: Niacin receptor HCAR2 affects amyloid pathology at an early stage of disease

Authors: *S. GEIS[1,2], I. CORONEL[2], M. MOUTINHO[2], G. LANDRETH[2];
[1]Indiana Univ. Sch. of Med., Indianapolis, IN; [2]Stark Neurosciences Res. Inst., Indianapolis, IN

Abstract: NIACIN RECEPTOR HCAR2 AFFECTS AMYLOID PATHOLOGY AT AN EARLY STAGE OF DISEASE
Samantha Geis[1], Israel Coronel[1], Miguel Moutinho[1] and Gary Landreth[1]
1) *Stark Neurosciences Research Institute, Indiana University School of Medicine, Indianapolis, IN, USA*
Background: Alzheimer's disease (AD) is a progressive neurodegenerative disease and a leading cause of dementia worldwide. Mounting evidence suggests that the accumulation and aggregation of amyloid-β (Aβ) is a key initiating factor in a cascade of events that lead to AD. The AD brain is typified by a robust microglial immune response triggered by Aβ accumulation. Although microglia have emerged as an important player in AD pathogenesis and progression, the role of these cells in disease is complex and still not fully understood. We have recently described that the niacin (nicotinic acid) receptor HCAR2 is required for an efficient microglial neuroprotective response in the 5xFAD amyloid mouse model. This analysis was performed at a stage of active and widespread plaque deposition and neuropathology. However, whether HCAR2 plays a role at an early stage of the disease remains unknown. **Methods:** We analyzed the phenotype of 2-month-old 5xFAD male and female mice lacking the HCAR2 receptor which corresponds to an early stage of disease. To examine if activation of HCAR2 at an early stage of disease exerts long-lasting beneficial effects, 2-month-old 5xFAD mice were provided with high doses of nicotinic acid through food pellets for 4 months. **Results:** Our preliminary results show that the lack of HCAR2 exacerbates amyloid burden in males at the early stages of pathology. Additionally, microglia mobilization to plaque-rich areas was reduced in both males and females at this disease stage in the absence of HCAR2. Furthermore, treatment of 2-month-old 5xFAD animals with a nicotinic acid-enriched diet for 4 months attenuated amyloid pathology. **Conclusions:** These preliminary data demonstrate that HCAR2 impacts amyloid pathology at earlier stages of the disease, highlighting an important role of this receptor in disease pathogenesis and its potential as a therapeutic target. Treatment of 2-month-old 5xFAD mice for 4 months with a nicotinic acid-enriched diet reduced amyloid pathology, highlighting the therapeutic potential of nicotinic acid in AD.

Disclosures: S. Geis: None. **I. Coronel:** None. **M. Moutinho:** None. **G. Landreth:** None.

Poster

PSTR396. Alzheimer's Disease: Neuroinflammation and Immune System

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Program #/Poster #: PSTR396.17/F5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Carlson College of Veterinary Medicine (OSU)
OSU CHAR Life Scholar's Fellowship
Biomedical Sciences Summer Research Fellowships

Title: Investigation of infectious theory of Alzheimer's Disease using HSV-1 in the 5xFAD mouse model

Authors: *I. ABOU-SEADA, K. R. MAGNUSSON, L. JIN, M. JANSEN, T. BREHON; Oregon State Univ., Corvallis, OR

Abstract: Alzheimer's Disease (AD) is a neurodegenerative disease that causes memory loss, cognitive decline, and is the cause of about 70% of all recorded dementia cases. AD is characterized by two main pathologies: amyloid beta (A-beta) plaques, and hyperphosphorylated tau proteins. Recent research has found the presence of some pathogens (herpes simplex virus-1 (HSV-1), and human herpesvirus-6 (HHV-6) being commonly found in the brains of post-mortem AD patients. From this data the infectious hypothesis of AD was proposed and states that a pathogen (virus, bacteria, prion, etc.) is the root cause of AD. In humans HSV-1 can commonly be reactivated from latency due to stress. In this study we used a mouse latency/reactivation model to investigate the infectious hypothesis of AD using HSV-1. 5XFAD heterozygous and wildtype mice (C57BL/6 background) were infected with neurotropic green fluorescent protein (GFP)-HSV-1 Mckrae virus at 8-10 weeks of age via application to the eye. HSV-1 was allowed to enter latency and then was reactivated via heat stress at 30 and 60 days post infection (dpi). Behavioral impairments were monitored using the Morris water maze, followed by euthanization and brain dissection to observe changes in cytokine and A-beta plaque levels. Behavioral results showed that reactivation of the virus accelerated memory problems at 30 dpi and cognitive flexibility deficits at 60 dpi. At 30 dpi infected and stressed mice performed significantly worse in the memory test than infected and unstressed mice ($p = 0.0425$). At 60 dpi infected and stressed mice performed significantly worse than infected, unstressed mice ($p = 0.016$) and uninfected, unstressed mice ($p = 0.014$). The hippocampus is important for spatial memory and the retrosplenial cortex is a region of the brain associated with cognitive flexibility. Although several heat-stressed groups appeared to have increased A-beta plaques in these regions, it did not reach significance. Cytokines (TNF-alpha and IL-1-beta) did show increased transcription levels following heat stress and following acute infection indicating the virus was reactivated and inflammatory processes were triggered. This study suggests that reactivation of the virus can lead to acceleration of the behavioral impairments seen in the heterozygous mice, which provides support for the infectious hypothesis of AD.

Disclosures: I. Abou-Seada: None. **K.R. Magnusson:** None. **L. Jin:** None. **M. Jansen:** None. **T. Brehon:** None.

Poster

PSTR396. Alzheimer's Disease: Neuroinflammation and Immune System

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR396.18/F6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NCATS Grant TL1-TR-002386
NIH R01 Grant 1R01AG057555
The City University of New York (Graduate Center, CNC program)

Title: Repurposing BT-11, an immunomodulatory drug, for treatment of Alzheimer's disease

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Abstract: Neuroinflammation represents a critical target for drug development in Alzheimer's disease (AD). BT-11, an orally active compound that binds to lanthionine synthetase C-like 2 (LANCL2), shows promise in AD treatment. Clinical trials conducted in Irritable Bowel Disease (IBD) have demonstrated the immunomodulatory properties of BT-11, including its ability to increase T regulatory cells in the gut. Given the characteristic imbalance in pro-inflammatory and anti-inflammatory signaling observed in AD, BT-11's capacity to modulate inflammation through its target, LANCL2, makes it a promising candidate for repurposing in AD treatment, as supported by in silico approaches.
In our research, we employed a unique transgenic rat model (TgF344-AD) exhibiting progressive age- and hippocampal-dependent spatial learning and memory deficits, as well as AD pathology resembling that observed in AD patients. For a duration of 6 months, starting at 5 months of age (pre-pathology) and continuing until 11 months of age (full AD-pathology), we administered BT-11 orally (8.5 mg/kg) to a cohort of both wild-type (WT) and Tg-AD rats. We then assessed spatial learning outcomes. Male Tg-AD rats not receiving treatment displayed significant deficits in spatial learning compared to male WT rats. Importantly, BT-11 treatment mitigated these deficits and improved learning in Tg-AD rats compared to untreated Tg-AD rats. Immunohistochemical analyses revealed a reduction in Aβ plaques in the hippocampus of treated male rats compared to untreated male controls. Intriguingly, the analysis demonstrated a decrease in microglial cells with various morphologies in female rats treated with the drug, whereas no such effect was observed in male rats.
These findings strongly support BT-11 as a potential drug candidate for AD treatment. We are currently conducting further analyses to evaluate the impact of BT-11 on tau pathology and neuronal loss. Our objective is to elucidate the mechanism(s) by which BT-11 enhances cognition and ameliorates AD pathology, exploring whether this occurs through a direct effect in the central nervous system (CNS) or a potential peripheral impact on immune cells in the gut. Recent RNAseq data indicates that BT-11's activation of LANCL2 initiates cAMP signaling, resulting in reduced expression of pro-inflammatory cytokines and increased expression of anti-inflammatory cytokines. Ongoing investigations aim to explore this effect, as well as assess the potential influence of the drug on Treg cells in the brain, similar to observations in the gut.

Disclosures: E. Birnbaum: None. **P. Rockwell:** None. **P. Serrano:** None. **M. Figueiredo-Pereira:** None.

Poster

PSTR396. Alzheimer's Disease: Neuroinflammation and Immune System

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Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR396.19/F7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA AG074331

Title: Characterization of structurally engineered lipooligosaccharide and its potential immunomodulatory role in Alzheimer's Disease.

Authors: *M. SHAKYA, M. SHERMAN, H. YANG, R. ERNST, A. SCOTT;
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Abstract: Structurally engineered variants of lipopolysaccharide (LPS) are an effective tool for the manipulation of the innate immune response, allowing for the rational design of vaccine adjuvants and inflammatory modulators. Key challenges remain, though, including cumbersome extraction methods, the effective activity of the ectopically expressed lipid A modifying enzymes, and the possibility to use these live bacterial strains to therapeutically colonize the gut. Here, we developed a first-generation strain of E. coli addressing several of these key challenges. Using a lipid-A structural engineering tool called bacterial enzymatic combinatorial chemistry (BECC), we engineered an E. coli variant, D31m4 bearing the dual lipid A modifications: 3-O-deacylation and 1-dephosphorylation (Ec DUAL). The resulting product is monophosphoryl lipid A (MPLA)-like LOS with a subset of odd chain variants and 3-O-deacylation (EcDUAL LOS). Structural characterization of EcDUAL LOS was carried out using tandem mass spectrometry, gas chromatography, and KDO assays. TLR4 structure-activity property of EcDUAL LOS was tested in human TLR4-expressing HEK293-Blue cells compared to WT LOS and a synthetic, detoxified MPLA control which showed a weak, competitive agonist-like activity of EcDUAL LOS. The parenterally administered product disseminated to the brain by 2 hours, persisted for at least 12 hours, and did not induce overt tissue pathology at the projected therapeutic dose. In a mouse gut repopulation study, the Ec DUAL live strain was found to temporarily colonize the gut and could be positively selected to improve the duration of colonization. Dysregulation of the TLR4 is repeatedly implicated in propagating and sustaining inflammation in Alzheimer’s Disease (AD) and a recent study has shown that chronic stimulation of the TLR4 pathway by a partial agonist (MPLA) significantly improved AD-related pathology in APP(swe)/PS1 mice. Similarly, our preliminary data demonstrated that APP/PS1 mouse treated once per week (IP) for twelve weeks at 30 mg/kg with EcDUAL LOS showed better spatial learning capacity (100% spontaneous alternation) in T-maze, performing better than the baseline control group suggesting further behavioral validation. Further analysis of how EcDUAL LOS affects AD-related neuropathology including Aβ accumulation, neuronal and synapse-associated proteins loss, and neuroinflammation with its mechanistic study will facilitate the understanding of the therapeutic benefits that EcDUAL LOS can produce in AD and other neuroinflammation-related diseases.

Disclosures: **M. Shakya:** None. **M. Sherman:** None. **H. Yang:** None. **R. Ernst:** None. **A. Scott:** None.

Poster

PSTR396. Alzheimer's Disease: Neuroinflammation and Immune System

Location: WCC Halls A-C

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Program #/Poster #: PSTR396.20/F8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: BrightFocus Foundation (A2021025S)
Cure Alzheimer's Fund
NIA/Mayo Clinic Alzheimer's Disease Research Center (P30 AG062677)
Glaucoma Research Foundation

Title: Deleterious immune activation is linked to impaired memory in Abca7 deficient mice

Authors: *S. REGO[1], S. P. NEVES[2], N. DELIVANOGLOU[2], Y. REN[3], M. J. BARBER[2], G. SANCHEZ[2], T. KANEKIYO[4], S. DA MESQUITA[4];
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Abstract: ATP Binding Cassette Subfamily A Member 7 (ABCA7) is a lipid transporter that has been linked to increased risk for late onset Alzheimer's disease. Deletion of *Abca7* (*Abca7*$^{-/-}$) has been shown to disrupt lipid metabolism and affect microglial activation in response to proinflammatory stimuli. In murine amyloidosis models, the lack of *Abca7* was found to exacerbate amyloid beta accumulation. Herein, we aimed at investigating the effects of *Abca7* loss-of-function on meningeal immunity and its role in modulating neuroinflammation and cognitive function in aging. Experiments involving immunofluorescence imaging and flow cytometry revealed alterations in the frequencies of brain and meningeal immune cells. Transcriptomic analysis of immune cells isolated from the brain-draining cervical lymph nodes uncovered specific gene expression signatures denoting an altered adaptive immune activation in middle-aged *Abca7*$^{-/-}$ mice. The cognitive performance of middle-aged *Abca7*$^{-/-}$ mice revealed a sex-specific impairment, which could be a cumulative result of the different changes in the immune activation profiles at the meninges and cervical LNs. Interestingly, systemic expansion of regulatory T cells (Tregs) in middle-aged *Abca7*$^{-/-}$ mice alleviated the cognitive deficits. Altogether, our data suggest that ABCA7 loss-of-function skews the adaptive immune profile towards a deleterious proinflammatory state that contributes to the appearance of cognitive deficits in aging.

Disclosures: S. Rego: None. **S.P. Neves:** None. **N. Delivanoglou:** None. **Y. Ren:** None. **M.J. Barber:** None. **G. Sanchez:** None. **T. Kanekiyo:** None. **S. Da Mesquita:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of Virginia Licensing and Ventures Group.

Poster

PSTR396. Alzheimer's Disease: Neuroinflammation and Immune System

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR396.21/G1

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Lc3-associated endocytosis plays a critical role in necroptotic neurodegeneration in AD

Authors: *S. AKTER;
Mol. Med., Univ. of South Florida, Tampa, FL

Abstract: Abstract Beta-amyloid (Aß) accumulation in the brain resulting from an imbalance between Aß production and clearance is the hallmarks of AD pathology. Our lab recently discovered a novel pathway 'LC3-associated endocytosis (LANDO)' that plays a protective role against abnormal Aß deposition by mediating the recycling of Aß receptors, a mechanism crucial for the clearance of Aß plaques, hence attenuates Aß-induced inflammatory signals, and safeguards against neurodegeneration and memory loss in 5xFAD AD mouse model. Conversely, a dearth of LANDO leads to impairment in recycling of Aß receptors, developed Aß deposition, neuroinflammation, tau hyperphosphorylation, active neurodegeneration, and severe memory loss by the age of 2yrs, leading to development of age-associated AD-like pathology in mice. While it is clear that LANDO has a protective role against neurodegeneration, the mechanism involved remains unknown. Recently through transcriptomic analysis of AD patients' brain mRNA, we identified significantly higher expression of necroptotic death markers like MLKL (mixed lineage kinase domain-like protein), RIPK1 (Receptor Interacting Protein Kinase 1), and RIPK3 (Receptor Interacting Protein Kinase 3) compared to that of a healthy individual. To investigate the potential regulatory role of LANDO in necroptotic cell death in AD brains we conducted a proteomic study where we observed a remarkably enhanced expression of activated MLKL in primary macrophages from LANDO-deficient mice when the cells were treated with Amyloid-Beta and Zvad (a Pan Caspase inhibitor). Furthermore, a protein-protein interaction study revealed that kinase domain of MLKL has a strong tendency to bind Rubicon protein, a critical regulator of LANDO pathway. Through an immunostaining study, a higher expression of activated microglia was observed colocalized with accumulated Aß in MLKL-sufficient mice brain compared to that of MLKL-deficient LANDO-deficient 5xFAD. These preliminary findings firmly suggest a role for LANDO in regulating MLKL signaling and overall neuroimmune function of microglia and neuron in AD and that pharmacological intervention of MLKL signaling may hold a great therapeutic promise for AD. Hence my investigation will focus on the role of LANDO-mediated MLKL signaling in causing necroptotic neurodegeneration in AD pathology.

Disclosures: S. akter: None.

Poster

PSTR396. Alzheimer's Disease: Neuroinflammation and Immune System

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR396.22/G2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: RISE 5R25GM059994-22
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SC1 CA182843-07S1
U54CA163069
SCI CA182843

Title: Loss of mitochondrial protein Fus1/Tusc2 causes early cognitive and molecular changes associated with Alzheimer's disease (AD)-like dementia

Authors: *T. FARRIS[1], M. MOHAMMED[1], J. TONELLO[1], T. KANAGASABAI[1], A. SHIMAMOTO[2], F. HARRISON[3], A. IVANOVA[1], A. SHANKER[1];
[1]Meharry Med. Col., Nashville, TN; [2]Meharry Med. Col., Meharry Med. Col. Neurosci. and Pharmacol., Nashville, TN; [3]Vanderbilt Univ., Nashville, TN

Abstract: Dementia is an umbrella term representing several diseases that affect cognitive health and function of the brain. The underlying mechanisms driving the development of the different forms of dementia remains elusive and yet to be precisely identified. Calcium (Ca^{2+}) regulates neuronal plasticity underlying learning, memory and neuronal survival. Chronic dysregulation of Ca^{2+} could lead to brain cell death and degeneration. Our group has found that mitochondrial protein TUSC2 (Tumor Suppressor Candidate 2), initially named Fus1, is involved in regulation of mitochondrial/cytoplasmic Ca^{2+} fluxes in cells. Fus1 protein resides on the inner membrane of the mitochondria and assists in Ca^{2+} uptake and extrusion. The deficiency of Fus1 leads to increased oxidative stress, altered mitochondrial membrane potential and energy production in immune and cancer cells. The Fus1 function in brain cells has not been addressed in detail. Here we examined the role of Fus1 in memory and neuroimmune and molecular phenotypes by using the Fus1 knockout (Fus1 KO) mouse model. We used 4-5 month old Fus1 KO and wild-type (WT) male mice. Mice underwent behavioral testing including Y-maze and Morris Water Maze. Independently, immunophenotyping (via flow cytometry) and molecular pathways analyses (via immunoblotting) were performed. Fus1 KO mice showed impaired short-term spatial memory as assessed with Y- maze test ($p<0.05$), and long-term memory as assessed with MWM ($p<0.05$). Immune analysis of Fus1 KO brain immune subsets showed changes associated with neurodegeneration, such as increased number of activated microglia expressing IFN and TNF, increased number of activated astrocytes and T-regulatory cells. Western Blot analysis of hippocampal tissue revealed increased tauopathy in Fus1 KO brain. In addition, prominent activation of mTOR pathway (higher S6 phosphorylation), as well as increased levels of Calbindin (Ca^{2+}-binding protein), an indicator of disrupted calcium homeostasis potentially leading to synapse loss, suggested active neurodegenerative processes in Fus1 KO brain. Decreased GFAP, an early predictor of Mild Cognitive Impairment (MCI), was in line with other molecular pathologies observed in Fus1 KO mice. The studies are underway to further characterize early pathologies linked to AD in Fus1 KO mice. Overall, Fus1 deficiency plays a pivotal role in the development of pathological processes leading to cognitive impairment and AD.

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Poster

PSTR396. Alzheimer's Disease: Neuroinflammation and Immune System

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR396.23/G3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant AG079141
NIH grant AG077610

Title: The effect of ubiquitous BIN1 overexpression in microglia on neuroinflammation and Alzheimer's disease pathophysiology

Authors: ***M. YUKSEL**[1,2], D. MOREIRA-SILVA[1,2], M. HANSEN[1,2], M. DELOZIER[1,2], S. NIDAMANUR[1,2], V. SKOROBOVENKO[1,2], L. COLLIER[1,2], G. THINAKARAN[1,2];
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Abstract: Bridging Integrator 1 (BIN1) is the second most prevalent risk factor locus for late-onset Alzheimer's Disease (LOAD). It is expressed as more than 10 isoforms with diverse tissue and cellular distribution. A reduction in neuronal BIN1 and an increase in the ubiquitous isoform (BIN1Ubi) expression have been observed in the brains of individuals with LOAD. Bin1Ubi is one of the major BIN1 isoforms expressed in oligodendrocytes and microglia. Recently, our lab observed that microglial BIN1 plays a critical role in the neuroinflammatory response. However, how BIN1Ubi expression contributes to AD pathology remains enigmatic. This study aims to investigate the role of elevated microglial BIN1Ubi expression in neuroinflammation in health and AD pathogenesis. To this end, we generated a tamoxifen-inducible BIN1Ubi microglial overexpression model (referred to as *BIN1*^{OE}) by crossing *Rosa26*-targeted human *BIN1Ubi* transgenic mice with *TMEM119-CreERT2* (referred to as Cre). To assess how elevated BIN1 expression modifies LPS-induced neuroinflammation, we administered LPS or saline to 8-month-old *BIN1*^{OE} and Cre mice. qRT-PCR analysis showed that 8-month-old male and female Cre vs. *BIN1*^{OE} showed similar expression levels of disease-associated microglial (DAM) genes under saline conditions. However, LPS stimulation caused a significant upregulation of DAM genes *Itgax, Trem2,* and *Ifitm3* in female *BIN1*^{OE} compared to controls, showing the potential involvement of BIN1Ubi in the regulation of LPS-induced inflammatory response in a sex-dependent manner. To test how elevated BIN1Ubi in microglia alters AD pathology and innate immune response, we further crossed *BIN1*^{OE} mice to 5XFAD or PS19 mice (which develop amyloidosis or tau pathology, respectively). The qRT-PCR of DAM inflammatory gene *Cst7* showed up to a 50% reduction in both male and female 5XFAD as well as male PS19 overexpression mice compared to their respective controls. *Cst7,* coding for the protein cystatin F, is an endogenous inhibitor of cysteine proteases. This decrease in *Cst7* expression suggests a

possible role of BIN1Ubi in microglial endolysosomal homeostasis during AD pathology. Thus, these results indicate a potential involvement of microglial BIN1Ubi in regulating pro-inflammatory responses and AD neuropathology.

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Poster

PSTR396. Alzheimer's Disease: Neuroinflammation and Immune System

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR396.24/G4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG069436

Title: Androgen deprivation therapy exacerbates Alzheimer's disease-associated cognitive decline through increasing brain infiltration of immune cells in a clinically relevant mouse model

Authors: C. ZHANG[1], ***M. AIDA**[5], S. SAGGU[6], H. YU[1], L. ZHOU[2], K. JIAO[7], R. LIU[3], L. WANG[4], Q. WANG[8];
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Abstract: Androgen deprivation therapy exacerbates Alzheimer's disease-associated cognitive decline through increasing brain infiltration of immune cells in a clinically relevant mouse model Chao Zhang[1*], Mae Aida[2, 5*], Shalini Saggu[2, 5], Haiyan Yu[1], Lianna Zhou[2], Kai Jiao[1, 6], Runhua Liu [1,3], Lizhong Wang[1,3,4], and Qin Wang[2, 4, 5]**Authors' Affiliations:** Departments of [1]Genetics, [2]Cell, Molecular, & Developmental Biology, [3]Center for Clinical and Translational Science, [4]Comprehensive Neuroscience Center, University of Alabama at Birmingham, Birmingham, AL 35294; [5]Department of Neuroscience & Regenerative Medicine, Medical College of Georgia at Augusta University, Augusta, GA 30912; [6]Center for Biotechnology and Genomic Medicine, Medical College of Georgia at Augusta University, Augusta, GA 30912
Androgen deprivation therapy (ADT) is widely applied to treat prostate cancer, which is predominantly a disease of the elderly. While effective in improving life span, ADT is associated with an increased risk of dementia. Alzheimer’s disease (AD) is the most common cause of dementia in older adults. The role of ADT for prostate cancer in AD development and

progression has not been clearly defined. The high heterogeneity of prostate cancer and AD, together with the potentially complex interaction between ADT and AD, pose major barriers for clinical mechanistic studies. Here we report establishment of a clinically relevant tumor-bearing AD mouse model by engrafting PTEN-CaP8 prostate cancer cells that recapitulate prostate cancer features and responses to ADT treatment in human patients into a well-established AD model, *App*$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ knock-in (*AppKI*) mice. Using this unique model, we revealed cognitive deficits accompanied by complex changes in immune and inflammatory responses in peripheral blood and in the central nervous system following ADT. In particular, ADT induced proinflammatory cytokines (e.g., IL-6) but decreased anti-inflammatory cytokines (e.g., IL-12), leading to increased proinflammatory responses in the brain of tumor-bearing *AppKI* mice. Pathological analyses further demonstrated enhanced gliosis without significant changes in amyloid accumulations in the brain of tumor-bearing *AppKI* mice treated with ADT. Together, these data suggest that increased neuroinflammation underlies ADT-exacerbated cognitive deficits. Our study provides critical information regarding the complex interaction among prostate cancer, ADT, and AD, and paves the way for future mechanistic and translational studies to test whether ADT can be improved to alleviate its detrimental effects on AD-related deficits.

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Poster

PSTR397. Astrocytic Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR397.01/G5

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Examining the impact of progressive tauopathy on hippocampal astrocyte morphology and function

Authors: *R. C. MCREYNOLDS, III, Y. KOMURO, A. GLEICHMAN, S. T. CARMICHAEL, J. HINMAN;
Neurol., UCLA, Los Angeles, CA

Abstract: The prodromal stage of Alzheimer’s disease (AD) is characterized by age-associated, region-specific changes in glial-cell gene transcription and activation. Alterations to cytoskeletal glial fibrillary acidic protein (GFAP) staining suggest that hippocampal astrocytes display disease-associated cellular atrophy. These atrophy-associated changes are well-recognized in amyloid-based models of AD, however, whether a similar phenotype occurs among astrocytes in tau-mediated neurodegeneration remains unknown. **The aim of this study is to investigate cytoskeletal, morphological, and transcriptional changes occurring in hippocampal astrocytes during progressive tauopathy as modeled by the P301S mouse model.** We hypothesize that distinct morphological changes in hippocampal astrocytes precede the

development of tauopathy and are driven by defined molecular pathways active at the transcription level. We sparsely labeled the full morphology of astrocytes using an adeno-associated virus that encodes a membrane associated and cytosolic modified GFP protein containing V5 epitope tags (smV5). Visualizing non-GFAP-filled processes enables greater insight into tau-mediated morphological changes in astrocytes. Differences in surface area and volume of cytoskeletal and morphological astrocytic features were measured by immunofluorescent co-staining of GFAP and viral smV5. We find variation in GFAP surface area and volume as well as primary process number within the hippocampus in wild-type and P301S mice. Cellular morphology is dictated by transcriptional states, so we profiled hippocampal astrocyte transcriptomes using vTRAP, which enables the identification of genes undergoing active translation that are associated with astrocyte function and morphology during tauopathy. In wild-type mice, astrocyte vTRAP labeling results in the enrichment of astrocytic marker genes (GFAP, ALDH1L1) compared to input hippocampus samples. Multiple candidate genes associated with morphologic differences in P301S mice were identified and verified using RNAscope. A selection of differentially expressed RNA transcripts were spatially analyzed in the hippocampus across disease progression. The application of comprehensive morphologic labeling of astrocytes, vTRAP, and RNAscope in P301S mice, will identify molecular pathways driving structural changes in astrocytes. Early and progression-dependent astrocytic morphological and cytoskeletal atrophy could drive AD pathology by astrocytes retracting their peri-synaptic astrocytic processes or disrupting intercellular channels implicating a new mechanism of vulnerability to AD.

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Poster

PSTR397. Astrocytic Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR397.02/G7

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Exploring Astrocyte States and Altered Pathways in Alzheimer's Disease: A Transcriptomics Approach

Authors: *K. GHAFARI[1], A. K. KUNISKY[1], S. BABU[1], H. ZHAO[1,2], H. MATHYS[1]; [1]Neurobio., Univ. of Pittsburgh, Pittsburgh, PA; [2]Tsinghua Univ., Beijing, China

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder associated with aging, marked by cognitive deterioration and a complex set of molecular mechanisms that are not yet fully understood. Astrocytes play crucial roles in maintaining the normal functioning of the nervous system and are actively involved in the development of neurodegenerative disorders like Alzheimer's disease. New findings strongly support the notion that different astrocyte states are linked to particular stages of Alzheimer's disease. The emergence of transcriptomics technologies

has facilitated rapid advancements in understanding and characterizing these abnormal astrocyte states associated with the disease. In this study, we analyzed over 145,000 astrocytes from more than 400 aged post-mortem brain samples exhibiting varying levels of pathologies related to AD. Integration of this dataset identified eight transcriptomically distinct astrocyte cell states (including non-reactive, reactive, inflammatory, stressed, and ribosomal translation), and the clusters were validated across the publicly available datasets. Comparison with the datasets revealed that the inflammatory cell type shows a similar profile to that observed in Multiple Sclerosis and indicates a common response to neurodegeneration. Using a pseudobulk-based differential gene expression analysis approach, we identified genes that were differentially expressed in high-resolution cell states across various measures of AD pathology including global AD pathology, neurofibrillary tangle burden, tangle density, overall amyloid level, neuritic plaque burden, diffuse plaque burden. We also explored the associated biological and functional ontologies, revealing that genes and gene modules related to the modulation of chemical synaptic transmission, DNA damage response, RNA metabolism, Cholesterol, and Lipid metabolism are altered in AD. Furthermore, a systematic analysis of protein complexes revealed that the TRBP-containing complex and several other complexes are altered in AD. We also analyzed the sex differences across the high-resolution cell types and their association with multiple AD pathologies. In summary, our examination of a transcriptomic atlas involving over 400 postmortem human brain samples has yielded valuable insights into astrocyte cell states, pathology-associated types, gene and pathway alterations, modified protein complexes, and the impact of sexual dimorphism on brain aging.

Disclosures: K. Ghafari: None. **A.K. Kunisky:** None. **S. Babu:** None. **H. Zhao:** None. **H. Mathys:** None.

Poster

PSTR397. Astrocytic Roles in Alzheimer's Disease

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Program #/Poster #: PSTR397.03/G8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA (R01AG059848)
Chan Zuckerberg Initiative (Ben Barres Early Career Acceleration Award; grant ID 199150)
Stanford Alzheimer Disease Research Center (NIH/NIA P30 AG066515)

Title: Transcriptomic profiling of human astrocytes in Alzheimer’s disease

Authors: *J. PAN[1], A. SANKARAESWARAN[1], F. JIANG[2], J. FORES MARTOS[1], J. E. OBERHAUSER[1], K. VALLEJO[1], M. OTERO-GARCIA[1], V. DOAN[1], J. J. PALOP[2], I. COBOS[1];
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Abstract: Astrocytes play crucial roles in β-amyloid metabolism, neurotransmitter trafficking, neuroinflammation, and synapse remodeling. However, the precise functions of different astrocyte populations during aging and neurodegeneration remain largely unknown. The characterization of reactive astrocyte states under disease conditions is challenging due to the continuous spectrum of transcriptomic states exhibited by these cells. To address this complexity, we conducted single-nucleus RNA sequencing (snRNA-seq) on human Alzheimer's disease (AD) brain tissue from 21 donors at various stages of AD progression as well age-matched healthy controls. To enrich for astrocytes, we utilized fluorescence-activated nuclear sorting (FANS) of $Pax6^{+}NeuN^{-}$ populations from neocortical gray and white matter. Our resulting dataset consists of approximately 140,000 high-quality nuclei, encompassing astrocytes (28%), oligodendrocytes (44%), excitatory neurons (14.7%), inhibitory neurons (6.9%), microglia (2.8%), endothelial cells (2.0%), and oligodendrocyte precursor cells (1.5%). Within the astrocyte population, we identified five distinct transcriptomic states. Two states are non-reactive and expressed either *WIF1* and *RERG* (cluster 1) or *SMTN* and *MMD2* (cluster 2). Additionally, we found three reactive populations that exhibited downregulated expression of the glutamate transporters *SLC1A2* and *SLC1A3,* and expressed *GRIA1* and *SEMA5A* (cluster 3), *CHI3L1* and *CA1* (cluster 4), or *WDR49, VCAN,* and *AQP1* (cluster 5). Furthermore, we integrated this dataset with previously generated snRNA-seq data from the human AD brain to examine cell-cell interactions. This analysis revealed reactive astrocyte populations with increased interactions with distinct neuronal and microglial populations at different stages of disease progression. In early AD, reactive astrocytes exhibited heightened interactions with $CUX2^{+}/RORB^{+}$ middle cortical layer excitatory neurons, while in late AD, they displayed increased interactions -with $LAMP5^{+}/KIT^{+}$ inhibitory neurons and reactive microglia. Our dataset provides a resource for understanding the dynamics and interactions of different molecular subtypes of astrocytes in AD progression.

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Poster

PSTR397. Astrocytic Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR397.04/G9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant NS085171
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Neurodegeneration Consortium at MDAnderson

Title: Reduced Morphological Complexity of Astrocytes in the Thalmic Reticular Nucleus of APP Mice

Authors: *J. CAMPBELL[1], R. JAGIRDAR[2], N. RIVERA-RAMIREZ[3], M. BEIERLEIN[4], J. R. CHIN[1];
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Abstract: Effective strategies to treat or prevent Alzheimer's disease (AD) are still lacking, in part because significant pathological changes in the brain have already occurred by the time individuals are diagnosed. Therefore, earlier intervention strategies are needed. Recent insights into the initial stages of AD suggest that deficits in sleep maintenance and slow wave sleep (SWS) can impact the production and clearance of amyloid beta (Aβ), contributing to disease progression. We previously demonstrated that reduced activity of the thalamic reticular nucleus (TRN) contributes to deficits in sleep maintenance and SWS in mice that express mutant human amyloid precursor protein (APP). However, the mechanisms underlying hypofunction of the TRN are unclear. Alterations in astrocyte function may play a role in TRN hypofunction, as they undergo morphological and functional changes in AD that can disrupt neuronal function. To assess whether astrocytes in the TRN are altered early in disease progression, we characterized astrocytes in the TRN of APP mice and nontransgenic (NTG) littermate controls at 4 months of age, when sleep deficits are robust but plaque deposition has not yet begun. We found that although the overall numbers of astrocytes remained largely stable in the TRN of APP mice, the number of GFAP-expressing astrocytes increased. The increase in GFAP-expressing astrocytes was particularly robust in the somatosensory segment of the TRN, which is responsible for sleep related rhythms. We also found a number of differentially expressed genes in the TRN of APP mice that impact astrocyte morphology. To test whether the morphology of TRN astrocytes is altered, we used an AAV that drives TdTomato expression under a truncated GFAP promotor to fully reveal their highly ramified structure. We found that both astrocyte volume and surface area are markedly decreased in TRN astrocytes in APP mice compared to those in NTG mice. Our findings suggest that TRN astrocytes undergo state dependent changes early in disease progression that could impact their influence on neuronal and synaptic function. Reciprocal interactions between neurons and astrocytes may play key roles in shaping neuronal activity in the TRN, with consequences for sleep stability in AD. Therefore, astrocytes may represent promising targets for innovative therapies to improve sleep, and slow or stop the progression of AD.

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Poster

PSTR397. Astrocytic Roles in Alzheimer's Disease

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Program #/Poster #: PSTR397.05/G10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: FWO fellowship 11658ON
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Scientific Fund Willy Gepts
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Strategic research Program VUB SRP49

Title: Targeting xCT does not improve hippocampal function in the 5XFAD mouse model

Authors: *A. MASSIE[1], J. O. ADEYEMI[2], A. VILLERS[3], C. DELEUZE[3], H. SATO[4], G. ATES[2], L. RIS[3], L. DE PAUW[1];
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Abstract: Genetic deletion of the specific xCT subunit of the cystine/glutamate antiporter system x_c^- protects against age-related hippocampal dysfunction and memory decline as well as toxin-induced neurodegeneration. Oxidative stress, (neuro)inflammation and glutamate toxicity are common pathogenic mechanisms that are active in several neurodegenerative diseases, including Alzheimer's disease (AD). Given the implication of system x_c^- in these pathways and given our previous findings in aging and other disease models, we hypothesized that targeting system x_c^- would be beneficial in a model of AD. To study the effects of genetic xCT deletion in AD, we cross-bred 5XFAD mice (C57BL/6J background) with $xCT^{-/-}$ mice. Both control ($5XFAD^{0/0}$), hemizygous ($5XFAD^{+/0}$) and homozygous ($5XFAD^{+/+}$) 5XFAD mice, with and without xCT, were obtained. Female mice were behaviourally characterized at a pre- (4 months) and symptomatic age (6 months). Next, brain tissue was collected at 5 and 7 months, and evaluated for amyloid plaques load (Aβ), neuroinflammation (Iba1, GFAP) and -degeneration (NeuN) using immunohistochemistry/-fluorescence. Hippocampal neurotransmission and long-term potentiation (LTP) were analysed at 8-10 months, using slice electrophysiology. Deficient spatial memory as well as impairment of LTP were observed in symptomatic female 5XFAD mice, mostly independent of xCT. Analysis of neuroinflammation and -degeneration is ongoing. Our first (preliminary) findings do not seem to support protective effects of xCT deletion on the development of an AD phenotype in the 5XFAD model.

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Poster

PSTR397. Astrocytic Roles in Alzheimer's Disease

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Program #/Poster #: PSTR397.06/H1

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: SAO-FRA 2021/0023
FWO fellowship 12B3223N

Title: System x_c^- inhibition to preserve metabolic health in Alzheimer's disease

Authors: *G. ATES[1], J. O. ADEYEMI[1], L. MACKENS[1], J. WALCKIERS[1], O. VANONCKELEN[1], H. SATO[2], P. JANSSEN[1], O. LARA[1], B. GUILLAUME[3], L. DE PAUW[1], A. MASSIE[1];
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Abstract: The cystine/glutamate antiporter system x_c^- is mainly expressed on astrocytes in the central nervous system and on peripheral immune cells. Deletion of its functional subunit xCT, results in lifespan extension, memory preservation in aged mice, as well as alterations in the aging hippocampal metabolome. Since aging is the main risk factor for developing Alzheimer's disease (AD), and both aging and AD are characterized by metabolic dysfunction, we investigated the metabolic health of the 5XFAD mouse model for AD, in the presence and absence of xCT.Hereto, body weight and glucose tolerance were monitored in male and female $5XFAD^{0/+}xCT^{+/+}$ and $5XFAD^{0/+}xCT^{-/-}$ mice at the pre-symptomatic and symptomatic stage. Visceral fat was weighed at the time of sacrifice, blood was collected for general blood chemistry, and metabolic health and flexibility of adult astrocytes were measured using the Seahorse metabolic flux analyzer. Results show significant peripheral metabolic changes in the $5XFAD^{0/+}$ mice, compared to age-matched $5XFAD^{0/0}$ controls, with lipid/cholesterol metabolism differently affected in the absence of xCT. Moreover, the mitochondrial health of AD astrocytes is compromised, with significant decreases in basal and maximal respiration, spare respiratory capacity, and ATP-coupled respiration, especially in the astrocytes from symptomatic $5XFAD^{0/+}xCT^{+/+}$ mice. The mitochondria of $xCT^{-/-}$ astrocytes, however, maintain their flexibility and health. Targeting xCT thus seems beneficial for the metabolic health in $5XFAD^{0/+}$ mice, putting system x_c^- forward as an interesting target to prevent AD-related metabolic decline.

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Poster

PSTR397. Astrocytic Roles in Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG027297
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Title: Development of a monoclonal antibody specific for a calpain-cleaved 48 kDa calcineurin fragment, a marker of distressed astrocytes

Authors: S. PRATEEPTRANG[1], S. HONGTHONG[1], N. THONGSOPHA[1], *S. D. KRANER[2], P. SOMPOL[2], P. T. NELSON[2], C. M. NORRIS[2];
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Abstract: Calcineurin (CN) is a Ca2+/calmodulin-dependent protein phosphatase expressed at high levels in brain. In healthy tissue, CN exists mainly as a full-length (~60 kDa) highly-regulated protein involved in essential cellular functions. However, in diseased or injured tissue, CN is proteolyticaly converted to a constitutively active fragment that has been causatively-linked to numerous pathophysiologic processes. These calpain-cleaved CN fragments (ΔCN) appear at high levels in human brain at early stages of cognitive decline associated with Alzheimer's disease (AD). ΔCN tends to show-up in regions of frank amyloid and cerebrovascular pathology, especially in select subsets of astrocytes, both in humans and in animal models. Our goal was to develop a monoclonal antibody to ΔCN for use in neuropathology research. Monoclonal antibodies were produced at GenScript using the immunizing peptide corresponding to the C-terminal end of the 48 kDa calpain-cleaved fragment (Wu et al., 2004, Pleiss et al., 2016). Notably, Δ48 kDa CN lacks a key autoinhibitory domain (AID) and is constitutively active. Antibodies were initially screened in ELISAs against the immunizing peptide, but ***decision-making screens*** were carried out as a Western analysis of calpain-cleaved calcineurin, to show specificity towards the Δ48 kDa CN fragment. Using the above criteria, we obtain a mouse monoclonal antibody, designated 26A6, that selectively detects the 48 kDa ΔCN in Western analysis of calpain-cleaved recombinant human CN. Using this antibody, we screened both pathological (9 cases) and normal brain (2 cases) sections provided by the human bank at the University of Kentucky's Alzheimer's Disease Research Center. 26A6 showed low reactivity towards normal brain tissue, but detected astrocytes both surrounding AD amyloid plaques and throughout AD brain tissue. In brain tissue with infarcts, there was considerable concentration of 26A6-positive astrocytes within/around infarcts, suggesting a link with anoxic/ischemia pathways. Additionally there was staining of some non-astrocytic cells. The new monoclonal 26A6 is highly selective for the 48 kDa ΔCN proteolytic fragment and labels a subset of astrocytes, and possibly other cell types, under pathological conditions apparently identifying distressed astrocyte phenotypes. The production of a constitutively active form of CN by Ca^{2+}-driven processes is consistent with the overall Ca^{2+} dysregulation theory of brain aging and cognitive decline proposed by PW Landfield and colleagues. **References: 1)** Wu *et al*., 2004 JBC 279: 4929-4940. **2)** Pleiss *et al*., 2016, Biochimica et Biophysica Acta 1862a; 1521-1532

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Poster

PSTR397. Astrocytic Roles in Alzheimer's Disease

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Program #/Poster #: PSTR397.08/H2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: IBS (Korea) IBS-R001-D2

Title: Molecular identification of ALDH1A1 and SIRT2 in the astrocytic putrescine-to-GABA metabolic pathway

Authors: *M. BHALLA[1,3], J. SHIN[2], J. JOO[1,3], Y. JU[4], Y. M. PARK[1,3], S. HYEON[5], S. YOO[6], H. LEE[6], H. RYU[7], C. J. LEE[1];
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Abstract: GABA (γ-aminobutyric acid) is the primary inhibitory neurotransmitter in the CNS. In astrocytes, GABA is synthesized by degradation of putrescine by monoamine oxidase B (MAO-B), a process which is known to mediate tonic inhibition of neuronal excitability. This astrocytic tonic GABA and related enzymes are also reported to be involved in memory impairment in Alzheimer's Disease (AD), and therefore are potential therapeutic targets to rescue memory in AD patients. However, the enzymes downstream of MAO-B in this pathway have not been elucidated yet. To fill this gap in knowledge, we performed transcriptomic and literature database analysis and identified Aldehyde dehydrogenase 1 family, member A1 (ALDH1A1) and a histone deacetylase enzyme Sirtuin2 (SIRT2) as plausible candidate enzymes in primary cultured astrocytes. Immunostaining, metabolite analyses, and 2-cell sniffer patch recordings performed in the presence or absence of suitable inhibitors, or with genetic ablation of ALDH1A1 and SIRT2 *in vitro* and *ex vivo* mouse brain slices recapitulated their participation in GABA production from putrescine. Immunohistochemistry from an animal model of AD and human AD patients reveals elevated levels of SIRT2 in hippocampal astrocytes. Genetic ablation of SIRT2 and ALDH1A1 in hippocampal astrocytes of AD mouse models rescues memory deficits and neuronal firing, further highlighting their role in pathological astrocytic GABA production. Altogether, we uncover this previously unexplored role of SIRT2 in astrocytic GABA production and propose it as a therapeutic target to ameliorate Alzheimer's Disease pathology.

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Poster

PSTR397. Astrocytic Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR397.09/H3

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Cholesterol hydroxylation in astrocytes under an inflammatory context as a predisposing factor for Alzheimer's disease

Authors: *G. CATALDI, M. MARTIN;
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Abstract: Cholesterol 24-hydroxylase (or CYP46) carries out the hydroxylation of cholesterol to 24(S)HOC, which is the main mechanism of cholesterol elimination from the brain. CYP46 has been mainly reported in neuronal populations, however, in cases of brain damage such as traumatic brain injury or Alzheimer's disease CYP46 increases its expression in astrocytes. At the moment, the role that CYP46 would play in astrocytes in pathological conditions is unknown. We found that CYP46 levels are greatly increased in reactive astrocytes challenged with lipopolysaccharide (LPS) or the proinflammatory cytokine IL-6. In addition, our data show that IL-6 was is able to increase APP synthesis in rat primary astrocytes by a mechanism mediated by CYP46. Indeed, the IL-6 ability to trigger APP synthesis in astrocytes is impaired by CYP46 inhibition. Further providing a link between CYP46 and APP, our results show a marked increase in APP levels in 24(S)HOC-treated primary cortical astrocytes compared to control cells. Preliminary Our preliminary data indicate that 24(S)HOC would exert its function through epigenetic mechanisms associated with histone 3 remodeling and acetylation.We propose that under a proinflammatrory context, as for example a microbial infection in the brain, 24(S)HOC would mediate the production and processing of APP and Aβ in astrocytes to face the aggression but on the other side it would predispose to Alzheimer's disease.

Disclosures: G. cataldi: None. **M. Martin:** None.

Poster

PSTR397. Astrocytic Roles in Alzheimer's Disease

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Program #/Poster #: PSTR397.10/H4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The Swedish Research Council 2020-02956

Title: Modifications of Brain Creatine Kinase associated with dementia

Authors: *T. ZHENG[1], J. MULDER[1], N. MITSIOS[1], D. KOTOL[2], M. UHLÉN[2], F. EDFORS[2], W. ZHONG[1];
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Abstract: Alzheimer's disease (AD) and dementia with Lewy body (DLB) are the most common forms of dementia. Oxidative stress is a major component of dementia pathology leading to

neuronal damage and cell death. Identification of proteins that are sensitive to oxidation is acrucial step to decipher how oxidative modification alter cellular physiology in dementia. Brain Creatine Kinase (CKB) is an enzyme that is involved in storage of energy in cells and regulates available ATP. Dysfunction of CKB enzyme activity in AD is reported. However, CKB distribution in the human AD and DLB brains is not explored in detail. To identify cellular expression, changes in protein levels and distribution associated with disease, both antibody-based and targeted proteomics approaches were used. Multiplex fluorescence analysis revealed co-existance of CKB immunoreactivity with astrocyte markers in the grey matter of the human cortex. No co-existance of CKB with neuronal markers, oligodendrocyte and microglia markers could be identified. To identify CKB distribution patterns in dementia, we investigated CKB-ir in samples from the temporal cortex (TCX) of donors diagnosed with Alzheimer's disease (n=10), dementia with Lewy bodies (n=10), and non-demented age-matched controls (n=9). Reduced CKB immunoreactivity was found in dementia patients, which was confirmed by Western Blot. Especifically, cases with a high level of amyloidosis revealed decreased CKB-ir intensity. Targeted proteomics analysis of revealed no overall change in the CKB levels in dementia, in line with CKB mRNA levels reported in the Allen Aging study. However, our quantitative mass spectrometry approach revealed disease specific pattern in CKB peptide ratios that suggest variation in efficacy or proteolytic cleavage or detection of CKB peptides disease affected brain tissue. All together these finding demonstrate a clear link between CKB and pathological processes associated with dementia. The loss of immunoreactivity and altered pepetide ratios suggest oxidation or other post-translational modification of CKB that lead to loss of CKB function in dementia.

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Poster

PSTR397. Astrocytic Roles in Alzheimer's Disease

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Program #/Poster #: PSTR397.11/H5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: JST JPMJFS2128

Title: Analysis for the regulation of ApoE expression and Aβ metabolismby astrocytic SphK2/S1P signaling

Authors: *M. KOMAI, T. UEHARA, N. TAKASUGI;
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Abstract: [Background] Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive cognitive decline. Although the current therapeutic strategy is based on the

amyloid hypothesis, which places neuronal toxicity induced by amyloid β (Aβ) at the center of pathogenesis, the therapeutic effect is not sufficient. AD is also considered a complication with several pathologies present at the time of its diagnosis, suggesting that pathologies other than neuronal-derived Aβ need to be addressed. Recently, attention has focused on the importance of chronic inflammation by activated astrocytes. Therefore, a target that could simultaneously control neuronal and astrocytic pathology would be a major advantage as a therapeutic strategy. Our laboratory has reported that sphingosine-1-phosphate (S1P), a lipid messenger, is increased in the brains of AD patients, which may promote Aβ production in neuronal cells. Interestingly, S1P is known to have epigenetic regulatory functions in the nucleus and is involved in immunoinflammatory effects. Therefore, we hypothesized that this signal is involved in astrocyte pathology and performed the following experiments. [Methods]To investigate S1P function, we established Sphingosine kinase 2 (SphK2), S1P-producing enzyme stable expression in U87 human astrocytoma cells. We evaluated the effect of S1P on astrocytic function by analyzing Apolipoprotein E (ApoE) as an astrocyte-related factor. The effect of SphK2 inhibitor was evaluated using biochemical approach.[Results] We found that ApoE induction by LXR/RXR agonists is suppressed by SphK2 overexpression, while SphK2 inhibitor enhances ApoE expression. Since SphK2 localizes predominantly to the nucleus, we next performed ChIP analysis and found that RXR binding to ApoE promoter is lowered by SphK2 activation. In addition, ApoE induced by SphK2 inhibitor binds Aβ and promotes Aβ uptake.[Conclusion] We uncovered a novel role of nuclear SphK2/S1P in ApoE expression, and suggested SphK2 inhibitor regulates astrocytic function and Aβ metabolism and will be a promising multi-target for AD therapy.

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Poster

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AEI-PID2019-109059RB-I00
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AEI-SAF2016-80419-R
H2020-JTI-IMI2-115975-2

Title: Apoe polymorphism affects functional and morphological features during the inflammatory process of ipsc-derived astrocytes from alzheimer´s disease patients

Authors: R. VECINO[1,2], E. DÍAZ-GUERRA[2,1], E. ARRIBAS-GONZÁLEZ[2,1], D. SANZ GIL[2], A. RODERO ROMERO[2], M. J. ROMÁN[2], I. SERRA-HUETO[2], M. GONZÁLEZ MARTÍN[2], E.

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Abstract: Alzheimer´s disease (AD) is the main cause of dementia in the aging population, with the presence of the ε4 allele of Apolipoprotein E (*APOE*), the major brain lipid transporter, being the strongest genetic risk factor. Astrocytes, the main producers of APOE in the brain, mediate key processes during the progression of AD, such as the clearance of amyloid-beta (Aβ) aggregates and the inflammatory response, among others. However, the impact of the different *APOE* alleles not only on astrocyte function but also on astrocyte development and maturation remains to be elucidated. To clarify these questions, we obtained induced pluripotent stem cells (iPSCs) from fibroblasts of AD patients carrying ε3 and ε4 alleles (in homozygosis) and from healthy patients. We also used gene-edited iPSC lines homozygous for the main *APOE* variants and an *APOE* knock-out line. iPSC-derived human astrocytes were generated by establishing a differentiation protocol through the consecutive addition of small molecules and growth factors. Then, the expression of typical markers (GFAP, GLT1, AQP4, and S100beta) and APOE was analyzed by RT-PCR and immunofluorescence to confirm its astrocytic phenotype. In addition, astrocytes exhibited functional features like calcium waves production and glutamate uptake capacity, confirmed by detecting the calcium indicator Fluo-4 and by ELISA, respectively. They also responded to an inflammatory stimulus (IL-1beta and TNF-alpha) or to the presence of amyloid-beta 1-42 peptide by increasing the expression levels and release of pro-inflammatory factors and cytokines (such as IL-6) and changing their morphology. Our results show that *APOE* polymorphism not only affects the basal state of astrocytes, but also their capacity to react to both stimuli by acquiring different morphologies, which could be relevant during the disease's inflammatory process. Furthermore, the presence of the ε4 allele of *APOE* could alter the uptake/degradation capacity of Aβ by astrocytes given the differences in the percentage of astrocytes capturing a fluorescence-labeled Aβ and its distinct distribution within the cell. Our findings shed light on the relevance of *APOE* polymorphism in the morphological and functional profile of astrocytes and their potential correlation with the different risks of developing AD.

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Poster

PSTR397. Astrocytic Roles in Alzheimer's Disease

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National Institute on Aging R01 AG061796 to N.E.T
Alzheimer's Association Zenith Awards to N.E.T.

Title: Reactive to neurogenic astrocytic conversion through induced Ngfr signaling reduces Alzheimer's pathology via Lcn2/Slc22a17 axis in mouse and human

Authors: T. SIDDIQUI[1], P. BHATTARAI[2], M. COSACAK[1], S. POPOVA[1], E. YILMAZ[2], A. J. LEE[3], Y. MIN[6], X. WANG[6], M. A. YOUNKIN[6], Ö. IS[7], N. RODRIGUEZ-MUELA[1], B. N. VARDARAJAN[3], D. A. FLAHERTY[2], A. F. TEICH[4], I. SANTA-MARIA[9], U. FREUDENBERG[10], C. WERNER[10], G. TOSTO[3], R. MAYEUX[5], N. ERTEKIN TANER[8], ***C. KIZIL**[2];
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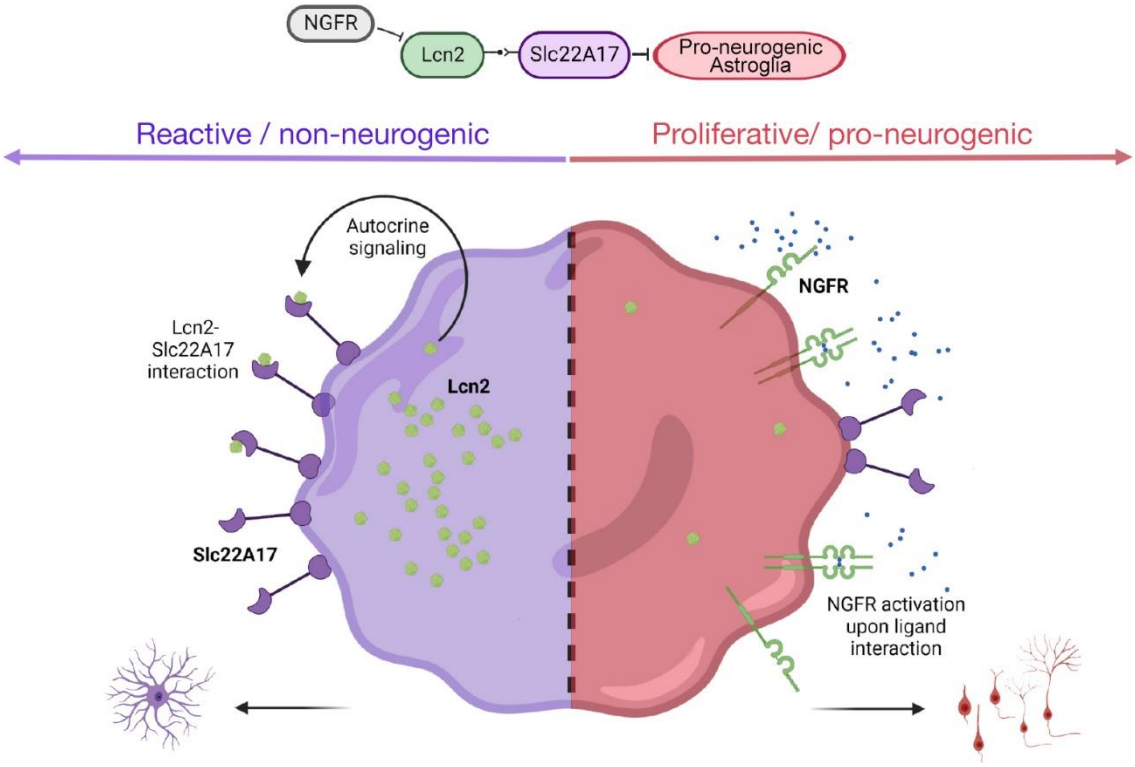
Abstract: Neurogenesis relates to the brain resilience and is reduced in Alzheimer's disease (AD). Restoring healthy levels of neurogenesis could have beneficial effects for coping with neurodegenerative pathology. Yet, the molecular mechanisms with which astroglial pro-neurogenic fate can be promoted under AD pathology are unknown. Here, we used APP/PS1dE9 mouse AD model, where astroglia adopt reactive states. We induced the expression of Nerve growth factor receptor (Ngfr) through lentiviral transduction in the hippocampus. Ngfr, a neuro-regenerative inducer in zebrafish brain model of amyloidosis, stimulated proliferative and neurogenic outcome in mice. Histological analyses of the changes in proliferation and neurogenesis, single-cell transcriptomics, spatial proteomics, and functional knockdowns showed that induced expression of Ngfr reduced reactive astrocyte marker Lipocalin-2 (Lcn2), which we found was sufficient to reduce neurogenic outcome in astroglia. We determined that anti-neurogenic effect of Lcn2 is mediated by Slc22a17, blockage of which recapitulated the pro-neurogenic effects of Ngfr. Long-term Ngfr expression reduced amyloid plaques and Tau hyperphosphorylation. Analyses on postmortem human AD hippocampi and 3D human neurogenesis cultures showed elevated LCN2 levels correlate with reactive gliosis and reduced neurogenesis. By comparing transcriptional changes in mouse, zebrafish and human cohort brains with AD for cell intrinsic differential gene expression and weighted gene co-expression networks, we observed common downstream effectors of NGFR signaling, such as PFKP, which is altered in AD patients. When we blocked PFKP, proliferation and neurogenesis was enhanced. Overall, we show the reactive and non-neurogenic fate of astroglia in AD can be switched to a pro-neurogenic fate with Ngfr, which also modulates pathological hallmarks of AD. We propose that enhancing pro-neurogenic astroglial fate may have therapeutic ramifications in AD.



Disclosures: **T. Siddiqui:** None. **P. Bhattarai:** None. **M. Cosacak:** None. **S. Popova:** A. Employment/Salary (full or part-time):; Neuron D GmbH. **E. Yilmaz:** None. **A.J. Lee:** None. **Y. Min:** None. **X. Wang:** None. **M.A. Younkin:** None. **Ö. Is:** None. **N. Rodriguez-Muela:** None. **B.N. Vardarajan:** None. **D.A. Flaherty:** None. **A.F. Teich:** None. **I. Santa-Maria:** None. **U. Freudenberg:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuron D GmbH. **C. Werner:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuron D GmbH. **G. Tosto:** None. **R. Mayeux:** None. **N. Ertekin Taner:** None. **C. Kizil:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Abcam. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuron D GmbH. F. Consulting Fees (e.g., advisory boards); Neuron D GmbH.

Poster

PSTR397. Astrocytic Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR397.14/H7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AC-G was funded by the Michael J fox Foundation (grant #000858)
Spanish Ministry of Science and Innovation (grant (#PI2020-119236RB-100)
María de Maeztu Unit of Excellence(Institute of Neurosciences, University of Barcelona) MDM-2017-0729

Title: Astrocytic RTP801 is involved in neurodegeneration and neuroinflammation in Alzheimer's disease

Authors: *A. CHICOTE[1,2], J. SOLANA[1,2], G. CAMPOY[1,2], P. GARCÍA[1,2], E. PEREZ[1,2,3], J. ALBERCH[1,2,3], G. SORIA[1,4], A. GIRALT[1,2,3,4], C. MALAGELADA[1,2];
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Abstract: Neuroinflammation is a key player in many neurodegenerative diseases, including Parkinson's, Huntington's and Alzheimer's disease (AD). In this process, astrocytes and microglia are the main cells involved, releasing cytokines, chemokines, prostaglandins, NO, and ROS. The release of these pro-inflammatory molecules has devastating consequences, as it leads to neuronal death, synaptic dysfunction, and inhibition of neurogenesis. The protein RTP801, also known as REDD1, has been recently involved in neuroinflammation. RTP801 levels are higher in the hippocampus of AD patients. Such increased levels of hippocampal RTP801 correlated with the severity of neurofibrillary tangles distribution, progressive depositions of Aβ, and astrogliosis. Moreover, silencing RTP801 in hippocampal neurons prevented cognitive impairment and neuroinflammation in the 5xFAD mouse model of AD. This study aims to assess whether astrocytic RTP801 affects memory and neuroinflammation in the 5xFAD mouse model of AD (7-month-old). RTP801 was knocked-down specifically in astrocytes injecting bilaterally adeno-associated viral particles containing AAV2/5-GFAP-miRNA-CONTROL-GFP or AAV2/5-GFAP-miRNA-RTP801-GFP. Four weeks later, we performed a battery of behavior tests including the light/dark box test and the Plus maze test to assess anxiety like behavior and Novel object location task, T-maze and Morris water maze to test spatial learning and memory. Silencing astrocytic RTP801 (miRTP801) in 5xFAD mice recovers the anxiety-like phenotype at the Plus Maze behavioral test and improved significantly the spatial learning and memory evaluated by the T-maze and Morris water maze. Mice were subjected to 1H-MRS (Magnetic resonance spectroscopy). MRI experiments were conducted on a 7.0 T BioSpec 70/30 horizontal animal scanner equipped with an actively shielded gradient system (400 mT/m, 12 cm inner diameter). Thus, brain metabolites were detected and quantified by linearly fitting each spectrum with a simulated basis set including 19 metabolites, as well as macromolecules. Silencing RTP801 in hippocampal astrocytes in the 5xFAD mice prevented the decrease of GABA/Creatin ratio observed in 5xFAD control mice. Silencing RTP801 in 5xFAD hippocampal astrocytes also reduced the levels of the inflammasome effectors NLRP3, ASC, and pro-caspase-1 compared to miCT injected 5xFAD mice, by western blot. Hence, we conclude that astrocytic RTP801 is contributing to cognitive impairment by affecting GABA and the inflammatory response in the pathogenic context of AD.

Disclosures: **A. Chicote:** None. **J. Solana:** None. **G. Campoy:** None. **P. García:** None. **E. Perez:** None. **J. Alberch:** None. **G. Soria:** None. **A. Giralt:** None. **C. Malagelada:** None.

Poster

PSTR397. Astrocytic Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR397.15/H8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Research UK Grant

Title: Validation of astrocytic cAMP signalling to study therapeutic targets for Alzheimer's disease

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Abstract: Astrocytes play a fundamental role in pathological processes associated with neurodegenerative diseases, including neuroinflammation, impaired glutamate uptake, reduced neurotrophic support and defective metabolism. Activation of cAMP signalling in astrocytes elevates glycolytic rate, increases glutamate transporter and neurotrophic factor expression, and suppresses the immune response. Molecules that regulate astrocytic cAMP signalling are therefore potential therapeutic targets for neurodegenerative diseases such as Alzheimer's disease (AD).

To aid astrocyte-targeted drug discovery, we developed an astrocyte-focused *in vitro* platform to be used for target validation and drug screening. Using primary rat and human iPSC-derived healthy and familial AD astrocytes in monoculture and in co-culture with neurons, we have optimised pharmacological assays including cAMP, Ca^{2+}, RNA-seq, multi-electrode array, and seahorse assays which can quantitatively and reliably measure changes to astrocyte function and neuronal activity.

We have used the astrocyte platform to validate the potential of astrocytic cAMP signalling as a therapeutic target for AD. Activation of adenylyl cyclase using forskolin or stimulation of a Gs-coupled GPCR using a tool compound induced cAMP signalling in rat and hiPSC-derived astrocytes. Alongside increased cAMP levels, forskolin and the tool compound led to elevated glycolytic rate, increased glutamate transporter expression, and the downregulation of pro-inflammatory pathway genes in astrocytes. We observed that addition of the tool compound in rat primary astrocyte and neuron co-culture induced an acute increase in neuronal excitability. The positive effects of cAMP activation on metabolism, glutamate transporter levels and inflammatory gene expression provide evidence that activating astrocytic cAMP signalling may translate to therapeutic benefit in AD. Our astrocyte platform has validated the pleiotropic effect of astrocytic cAMP signalling and can be applied to future novel drug discovery programs targeting astrocytes.

Disclosures: **F.E. Ducotterd:** None. **L. Granat:** None. **J. Bilsland:** A. Employment/Salary (full or part-time):; Astronautx Tx. **S. Jolly:** None.

Poster

PSTR397. Astrocytic Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR397.16/H9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSF GRFP
NIH AI166772
NIH CA252162
NSF NNCI-2025233

Title: Monomeric and oligomeric amyloid-β_{42} exposure demonstrate different reactive characteristics in human primary astrocytes within glymphatics-on-chip in vitro microfluidic model

Authors: *A. R. YSLAS, R. PARK, E. LEE;
Nancy E. and Peter C. Meinig Sch. of Biomed. Engin., Cornell Univ., Ithaca, NY

Abstract: Amyloid-β_{42} (Aβ42) is one of the two proteins responsible for the chronic and fatal neurodegenerative disease Alzheimer's disease (AD), where it is a neurotoxic extracellular aggregate. AD is characterized by dysfunction in the glymphatic system, the perivascular waste removal system of the brain parenchyma that is highly reliant upon astrocytes. To better understand how Aβ42 affects astrocytes and leads to their AD pathophysiology, including glymphatic dysfunction, we developed a three-dimensional microfluidic in vitro model of the glymphatic system (glymphatics-on-chip) containing two acellular perivascular channels and an astrocyte parenchyma embedded with either monomeric Aβ42 (mAβ42), oligomeric/aggregate Aβ42 (oAβ42), or no Aβ42 (control). The glymphatics-on-chip was manufactured via soft lithography of polydimethylsiloxane and contains human primary astrocyte cells in a 3D hydrogel of collagen 1, hyaluronan, and fibronectin as an extracellular matrix with embedded mAβ42 or oAβ42 (or no Aβ42 for the control). Following culture, these devices were either treated with fluo-4 AM calcium ion (Ca^{2+}) indicator and imaged live or fixed and imaged for immunofluorescence via confocal microscopy. Using imageJ analysis tools, the size and shape parameters were measured, with the astrocytes exposed to mAβ42 exhibiting greater size and decreased circularity and solidity, indicative of hypertrophy, compared to either of the other groups. The astrocytes exposed to mAβ42 also showed a higher ratio of cytoskeletal components nestin compared to actin, which is an accepted indicator of reactive astrocyte pathology in neurodegenerative diseases including AD. However, astrocytes exposed to oAβ42 showed greater intracellular Ca^{2+} concentrations with large temporal variations via changes in mean fluo-4 signal, demonstrating the calcium dyshomeostasis seen in AD astrocytes, both the increased intracellular Ca^{2+} concentration and the increased transients. Preliminary results show that

mAβ42 and oAβ42 have different effects on interstitial fluid flow through the glymphatic system, but confirming this is future work. Altogether, this work illustrates that mAβ42 and oAβ42 have different effects on astrocytes, and this can be used to elucidate the progression of AD pathology in astrocytes via Aβ42 aggregation, with later disruption to the glymphatics system. Our current work shows that some of the accepted hallmarks of AD pathophysiology in astrocytes may not develop simultaneously but rather in separate stages depending on whether the Aβ42 is in its monomeric or oligomeric forms.

Disclosures: A.R. Yslas: None. **R. Park:** None. **E. Lee:** None.

Poster

PSTR398. Mechanisms of Vascular Changes in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR398.01/H10

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Structural and Functional Aspects of Meningeal Lymphatic Vasculature in Aged Human Brain

Authors: *O. ALBAYRAM;
Med. Univ. of South Carolina Neurosci. Grad. Program, MT PLEASANT, SC

Abstract: Structural ad Functional Aspects of Meningeal Lymphatic Vasculature in Aged Human BrainM.S. Albayram[1], K. Yagmurlu[2], E. Karakaya[3], G. Smith[1], J. Edwards[3], A. Ergul[3], **O. Albayram[3, 4]**[1]Depart. of Radiology, University of Florida, College of Medicine, Gainesville, FL; [2]Depart. of Neurosurgery, University of Tennessee, Memphis, Tennessee; [3]Depart. of Path. & Lab. Med. Medical University of South Carolina (MUSC), Charleston, SC; [4]Depart. of Neuroscience, MUSC, Charleston, SC, None of the authors has disclosure.
The brain was historically considered an immune-privileged organ separated from the peripheral lymphatic system by the blood-brain barrier. Currently, meningeal lymphatics in the brain have garnered increasing attention in the scientific literature, which has left us to investigate how the lymphatic vasculature network may be positioned within the dural matters in the brain, and the novel potential role of the meningeal lymphatic system in brain aging and age-related neurodegenerative disorders. Indeed, using the term “brain lymphatics” is still unclear because a direct connection between lymphatic channels and brain parenchyma is not recognized yet. Using a novel 3D T2-Fluid Attenuated Inversion Recovery (FLAIR) Magnetic Resonance Imaging technique, we have demonstrated a progressive age-related cervical lymph node atrophy and thickening of lymphatics channels in both dorsal and ventral regions of the human brain. This non-invasive imaging technique relies on internal signals of protein-rich lymphatic fluid rather than contrast media and visualizes the major human dural lymphatic structures. Moreover, we detect direct connections between lymphatic fluid channels along the cranial nerves, vascular systems, and cervical lymph nodes. Furthermore, by using selective antibodies against lymphatic endothelium, including Podoplanin, VEGF-R3, Prox-1, and Lyve-1, we identified the lymphatic

vasculatures in the parasagittal dura of the brain along with subarachnoid space and the possible intramural lymphatic drainage pathway in the arterial wall of the brain by using high-definition microscopy in post-mortem human brain-meningeal biospecimens. Taking advantage of emerging imaging and microscopy techniques in the live human brain and human brain tissues, we investigate to develop novel strategies to detect meningeal lymphatic vasculature in humans and translational therapeutic interventions to prevent age-associated neurodegeneration by improving brain lymphatic drainage.

Disclosures: O. Albayram: None.

Poster

PSTR398. Mechanisms of Vascular Changes in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR398.02/I1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AHA 19PABH134580006
NIH 1P30AG072959-01
NIH RM1 GM142002
Brockman Foundation

Title: 15-pgdh mediates blood-brain barrier deterioration in alzheimer's disease

Authors: *Y. KOH[1], E. VÁZQUEZ-ROSA[2], F. GAO[3], H. LI[4], S. BARKER[1], Z. BUD[5], P. SRIDHARAN[6], B. CORDOVA[1], Y. YU[2], J. HYUNG[2], R. KATABATHULA[4], J. LUTTERBAUGH[4], L. BEARD[4], U. KANDJOZE[2], S. CORELLA[1], C. CINTRÓN-PÉREZ[2], K. FRANKE[2], X. ZHU[1], B. WILSON[8], M. FLANAGAN[9], H. FUJIOKA[7], S. FINK[4], S. FINK[4], A. DESAI[1], D. DAWSON[1], J. READY[10], M.-K. SHIN[11], S. MARKOWITZ[4], A. PIEPER[2,12,8]; [1]Dept. of Pathology, [2]Dept. of Psychiatry, [3]Dept. of Genet. and Genome Sci., [4]Case Comprehensive Cancer Ctr., [5]Frances Payne Bolton Sch. of Nursing, [6]Dept. of Neurosci., [7]Cryo-Electron Microscopy Core, Case Western Reserve Univ., Cleveland, OH; [8]Geriatric Psychiatry, Louis Stokes VA Med. Ctr., Cleveland, OH; [9]Mesulam Ctr. for Cognitive Neurol. and Alzheimer’s Dis., Northwestern Univ., Chicago, IL; [10]Dept. of Biochem., UT Southwestern Med. Ctr., Dallas, TX; [11]Col. of Pharm. and Res. Inst. of Pharmaceut. Sci., Seoul Natl. Univ., Seoul, Korea, Republic of; [12]Harrington Discovery Inst., Univ. Hosp. Cleveland Med. Ctr., Cleveland, OH

Abstract: Despite its massive worldwide prevalence and staggering socioeconomic burden, there are still no medicines that halt, or even slow, Alzheimer’s disease (AD). We have identified a novel therapeutic target within the blood-brain barrier (BBB) in AD, 15-hydroxyprostaglandin dehydrogenase (15-PGDH) enzyme, that degrades various eicosanoids, including prostaglandin D2 and E2 (PGD2 and PGE2). AD pathology is characterized by early and progressive BBB deterioration, and in the 5xFAD mouse model of AD we identified an abnormal and pathological

increase of 15-PGDH activity at 6 months of age. 15-PGDH activity increases further with disease progression, and 15-PGDH expression is enriched in myeloid cells intimately associated with the BBB. We also observe similar increase in 15-PGDH in human AD brain. Notably, 15-PGDH-deficient 5xFAD mice are protected from BBB deterioration, as well as neurodegeneration and cognitive impairment. This protective efficacy is independent of any changes in amyloid pathology. Thus, 15-PGDH may represent a novel and non-amyloid based therapeutic target for AD or other forms of neurodegenerative disease.

Disclosures: **Y. Koh:** None. **E. Vázquez-Rosa:** None. **F. Gao:** None. **H. Li:** None. **S. Barker:** None. **Z. Bud:** None. **P. Sridharan:** None. **B. Cordova:** None. **Y. Yu:** None. **J. Hyung:** None. **R. Katabathula:** None. **J. Lutterbaugh:** None. **L. Beard:** None. **U. Kandjoze:** None. **S. Corella:** None. **C. Cintrón-Pérez:** None. **K. Franke:** None. **X. Zhu:** None. **B. Wilson:** None. **M. Flanagan:** None. **H. Fujioka:** None. **S. Fink:** None. **S. Fink:** None. **A. Desai:** None. **D. Dawson:** None. **J. Ready:** None. **M. Shin:** None. **S. Markowitz:** None. **A. Pieper:** None.

Poster

PSTR398. Mechanisms of Vascular Changes in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR398.03/I2

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Effects of voluntary physical exercise and its correlation on the neurovascular unit in the 3xTg-AD model for Alzheimer's disease

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Abstract: Alzheimer's disease (AD) is the most common neurodegenerative disease in theworld. It is characterized by the deterioration of memory, thinking, orientation andlearning, as well as the quality of life of the people who suffer from it. The twopathognomonic hallmarks are neuritic plaques and neurofibrillary tangles. However,it has been proposed that the cerebral vascular system is also affected and evenaggravates the symptoms of the disease. One of the most widely used animalmodels to study disease mechanisms is the 3xTg-AD triple transgenic mouse thatcontains three associated with familial AD (PS1M146V, APPSWE and tauP301L). Itshould be emphasized that up to now there is no effective treatment for AD. Anadjuvant non-pharmacological therapeutic approach for AD is physical exercise,which is a variety of planned, repetitive, and dosed physical activity, however itseffect on the cerebral vascular system is unknown. The objective of this work was toanalyze the effect of physical exercise on the neurovascular unit in the 3xTg-ADmodel. A total of 40 ten-month-old female mice, 3xTg-AD (n=20) and non-Tg (n=20),divided equally into exercise and sedentary groups, were used. A

voluntary physicalexercise intervention was carried out for 3 months with a frequency of 5 times aweek. Subsequently, memory and learning were assessed using the Barnes mazetest. Vascular morpho structure was analyzed by histological assays using vascularbasement membrane markers (anti-Collagen IV), pericytes (anti-PDGFR-β) andastrocytic feet (Aquaporin 4), other amyloid beta deposits (BAM-10) hematoxylinstaining and eosin for structural evaluation. Voluntary physical exercise did notcause improvement in cognitive ability; however, it promoted the recovery of thevascular system, anatomical changes, as well as the reduction of amyloid betadeposits, both in the form of plaques and vascular. The intervention with voluntaryphysical exercise favors the stability of the vascular system, as well as the reductionof amyloid deposits in the symptomatic stages of the disease.

Disclosures: J. Andrade: None. **E. Orta:** None. **O. Soto:** None. **S. Diaz-Cintra:** None.

Poster

PSTR398. Mechanisms of Vascular Changes in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR398.04/I3

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Coexistence of Alzheimer’s disease, cerebrovascular disease, and arthritis in humans: is there a common risk factor(s)?

Authors: A. FROLOV[1], N. MAGLASANG[1], M. A. GUZMAN[1], S. ECHOLS[2], N. LAPRESTA[1], ***D. DALY**[1];
[1]St. Louis Univ. Sch. of Med., Saint Louis, MO; [2]Scarlett Imaging, Murray, UT

Abstract: Cerebrovascular dysfunction leading to impairment of the blood-brain barrier (BBB) has been known to play an important role in dementia development and progression, including that associated with Alzheimer’s disease (AD). Recently, an increased risk for AD was found in a large population of patients with rheumatoid arthritis (RA) (PMID: 27470609). It has been also shown that RA is a significant risk factor for cognitive impairment (PMID: 34656753; PMID: 22647255) and dementia (PMID: 26754993). Together, these findings raise a question about the existence of common risk factor linking AD, cerebrovascular disease (cVD), and RA. To address this question, we performed a postmortem neuropathological examination and genetic screen of two individuals. The first individual (donor 1, D1) was a 74-year-old male who was diagnosed with both AD and RA, and also underwent hip replacement surgery bilaterally. The cause of death (COD) in D1 was listed as AD type dementia. The second individual (donor 2, D2) was a 90-year-old male with a reported diagnosis of RA as well as two left hip replacements. The COD for D2 was community acquired pneumonia. A thorough histochemical (H&E) and immunohistochemical (β-amyloid, tau-protein, TDP43) examination of D1 and D2 brains revealed the presence of AD related pathology in both individuals, with AD stages being mild in D1 and intermediate in D2. The cVD related pathology was also microscopically evident in both cases and was characterized by several microbleeds indicative of a compromised BBB integrity.

Blood vessel wall thickening was significant in D1 but minor in D2. The whole exome sequencing of DNA procured from D1 and D2 employing the Next Generation Sequencing (Illumina platform) was followed by a very stringent bioinformatics analysis that yielded seven rare (minor allele frequency, MAF ≤ 0.01) genetic pathologic variants: *AQP7, ARSD, FAM160A1, HYDIN, IGSF3, OTOP1,* and *PRSS1*, with all but the variant of *FAM160A1* being identical in both donors. Intriguingly, subsequent analysis of the respective literature indicated that *AQP7, IGSF3,* and *PRSS1* could be linked to either AD, cVD, or RA. Analysis of less rare (MAF < 0.05) genetic pathologic variants revealed an additional common variant, *CSPG4*, which was identical in D1 and D2. *CSPG4* is a known pericyte (BBB) marker and could be also linked to either AD, cVD, or RA. Altogether, the data presented herein are consistent with the notion that AD, cVD, and RA, when they coexist in humans, could be linked to a common genetic risk factor(s).

Disclosures: A. Frolov: None. **N. Maglasang:** None. **M.A. Guzman:** None. **S. Echols:** None. **N. LaPresta:** None. **D. Daly:** None.

Poster

PSTR398. Mechanisms of Vascular Changes in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR398.05/I4

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: High fat diet-induced atherosclerosis in ApoE knockout mice causes cerebrovascular remodeling and cognitive behavioral deficits linked with Alzheimer’s disease

Authors: *J. A. MINTEER[1], H. C. BENNETT[2], Y. DU[3], Y. JIANG[3], D. J. VANSELOW[4], P. HE[3], Y. KIM[2];
[1]Dept. of Neural and Behavioral Sci., [2]Dept. of Neural & Behavioral Sci., [3]Dept. of Cell. and Mol. Physiol., [4]Dept. of Pathology, Penn State Col. of Med., Hershey, PA

Abstract: High fat diet-induced atherosclerosis in ApoE knockout mice causes cerebrovascular remodeling and cognitive behavioral deficits linked with Alzheimer’s disease
Author list: Jennifer A. Minteer[1], Hannah C. Bennett[1], Yong Du[2], Yanyan Jiang[2], Daniel J. Vanselow[3], Ping He[2], Yongsoo Kim[1]
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Atherosclerosis is a major risk factor in the development of Alzheimer's disease (AD). Disruptions to the apolipoprotein E (ApoE) gene and high-fat diet (HFD) cause hyperlipidemia and increased formation of atherosclerotic plaques. The causal relationship between atherosclerosis and AD-associated cognitive deficits remains unclear. Here, we used a murine atherosclerosis model, ApoE knockout (KO) mice with HFD feeding, to investigate the

contribution of atherosclerosis on the cerebrovascular structure and its impact on memory performance. We examined cerebrovascular alterations in ApoE KO mice fed HFD for 24 weeks (32 weeks old) using tissue clearing, 3D immunolabeling, and light sheet fluorescence microscopy. We observed increased numbers of penetrating cortical arterioles and heightened vascular tortuosity in HFD ApoE KO brains compared to age-matched ApoE KO mice with control diet. We also conducted longitudinal behavioral tests with novel object recognition (NOR) task and Y-Maze to determine whether increased atherosclerotic lesions in HFD-fed ApoE KO mice induce impairment of cognitive performance. Indeed, ApoE KO males exhibited significant impairment in the NOR and Y-maze tasks by 12 weeks (20 weeks old) of HFD feeding and ApoE KO females showed delayed onset of behavioral deficit at 16 weeks (24 weeks old) of HFD. In contrast, control diet-fed ApoE KO mice had no impairment in either test up to 30 weeks old. In summary, our findings demonstrate atherosclerosis-induced cerebrovascular remodeling and the associated memory deficit, providing insights into atherosclerosis-initiated AD-related behavioral deficit.

Disclosures: **J.A. Minteer:** None. **H.C. Bennett:** None. **Y. Du:** None. **Y. Jiang:** None. **D.J. Vanselow:** None. **P. He:** None. **Y. Kim:** None.

Poster

PSTR398. Mechanisms of Vascular Changes in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR398.06/I5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA AG060238
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NS114413
U54 TR002003
AHA 21PRE836269

Title: Reduced endothelial Caveolin-1 underlies deficits in brain insulin signaling in type 2 diabetes: Implications for Alzheimer's disease

Authors: *A. SHETTI[1], A. RAMAKRISHNAN[2], L. ROMANOVA[3], W. LI[4], K. VO[1], I. RATNAYAKE[1], T. STEPHEN[4], R. MINSHALL[1], S. COLOGNA[1], O. LAZAROV[5]; [1]Univ. of Illinois Chicago, Chicago, IL; [2]Northwestern Univ., Chicago, IL; [3]Rush Univ. Med. Ctr., Chicago, IL; [4]Univ. of Illinois at Chicago, Chicago, IL; [5]The Univ. of Illinois at Chicago, Chicago, IL

Abstract: Patients with type-2 diabetes exhibit severe impairments in insulin signaling in the brain and are more likely to develop Alzheimer's disease. However, what leads to these impairments is not fully understood. Here, we show reduced expression of endothelial cell caveolin-1 (Cav-1) in the *db/db* (*Lepr^{db})* mouse model of type-2 diabetes. This reduction correlated with alterations in insulin receptor expression and signaling in brain microvessels as well as brain parenchyma. These findings were recapitulated in the brains of endothelial cell-specific Cav-1 knock-out (Tie2Cre;Cav-1$^{fl/fl}$) mice. Lack of Cav-1 in endothelial cells led to reduced response to insulin as well as reduced insulin uptake. Furthermore, we observed that Cav-1 was necessary for the stabilization of insulin receptors in lipid rafts. Interactome analysis revealed that insulin receptor interacts with Cav-1 and caveolae-associated proteins, insulin degrading enzyme, and the tight junction protein Zonula Occludence-1 in brain endothelial cells. Restoration of Cav-1 in Cav-1 knockout brain endothelial cells rescued insulin receptor expression and localization. Overall, these results suggest that Cav-1 regulates insulin signaling and uptake by brain endothelial cells by regulating IR-α and IR-β localization and function in lipid rafts. Furthermore, depletion of endothelial cell specific Cav-1 and the resulting impairment in insulin transport leads to alteration in insulin signaling in the brain of type-2 diabetes.

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Poster

PSTR398. Mechanisms of Vascular Changes in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR398.07/I6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH AG071746

Title: Interaction of estrogen loss and cardiovascular disease on neurovascular coupling

Authors: *C. RICHARD[1], B. VISNIAUSKAS[2], I. FERNANDEZ-UGIDOS[2], Z. DIAZ[2], A. MCNALLY[2], R. MOSTANY[2], S. H. LINDSEY[2];
[2]Tulane Brain Inst., [1]Tulane Univ., New Orleans, LA

Abstract: While animal studies show that estrogen provides protection against cognitive decline, some clinical studies fail to find cognitive benefits in response to menopausal hormone therapy. Since human studies enrolled older patients that were many years past the menopausal transition, the results may have been influenced by subclinical disease progression. Therefore, the current study was designed to test the hypothesis that cardiovascular disease inhibits the protective effects of estrogen in the brain through detrimental effects on neurovascular coupling. Female C57BL/6J mice were ovariectomized at 10.5 months of age to match menopausal age in humans and randomized to receive either vehicle or estradiol (E2) at the time of surgery. Some mice

were infused with angiotensin II infusion (Ang; 700 ng/kg/min) four weeks before ovariectomy to promote hypertension and associated cardiovascular damage. Data from this 2x2 design (±E2/±Ang, N=3-5 per group) was analyzed by two-way ANOVA with Sidak's multiple comparisons test. Blood pressure was measured via tail cuff plethysmography, arterial stiffness was measured via ultrasound, and neurovascular coupling was measured through cranial window two-photon microscopy. As expected, E2 significantly increased uterine weight (P=0.03), and this effect was maintained in the presence of Ang (P=0.003). E2 also significantly decreased body weight (P=0.02), but this effect was lost in the presence of Ang (P=0.89). Ang significantly increased blood pressure in ovariectomized mice (P=0.005), but E2 was protective against the hypertensive response (P=0.99). Arterial stiffness, measured as pulse wave velocity, followed the same pattern as blood pressure, with Ang increasing stiffness in the -E2 group (P=0.04) but not the +E2 group (P=0.98). We expect our ongoing analysis to provide evidence that neurovascular coupling is protected by E2, but this protection is lost in the presence of Ang. These studies have significance implications for understanding the interactions between estrogen and cardiovascular disease in the aging female brain.

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Poster

PSTR398. Mechanisms of Vascular Changes in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR398.08/I7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Open Philanthropy

Title: Dysregulation of the gut microbiome contributes to compromised blood-brain barrier integrity inApoE4 transgenicmice

Authors: *M. ZHANG[1], M. T. HUUSKONEN[1], A. P. SAGARE[1], A. CHAKHOYAN[1], K. KISLER[1], A. R. NELSON[2], Y. WANG[1], B. V. ZLOKOVIC[1];
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Abstract: Recent studies indicate that cognitive impairment and Alzheimer's disease (AD) can be influenced by dysfunction in brain blood vessels. *APOE4*, a prominent genetic risk factor for AD, worsens the breakdown of the blood-brain barrier (BBB) and the degeneration of brain vascular pericytes. This leads to the disruption of cerebral blood flow regulation and an increase in the deposition of amyloid, which ultimately affects neuronal function. Alongside genetic risk factors, emerging evidence suggests a strong connection between the gut microbiome and age-related neurodegenerative diseases, such as AD. Here, we investigated the difference in gut bacterial taxa in *ApoE3* and *ApoE4* transgenic mice and its contribution to the BBB permeability by treating animals with a cocktail of antibiotics amoxicillin-clavulanic acid (ABX) through

drinking water for two weeks. Our findings indicate that the gut microbiome undergoes changes caused by ABX treatment, leading to the restoration of blood-brain barrier (BBB) integrity in *ApoE4* mice. We assessed this restoration through dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) and the observation of reduced accumulation of pericapillary fibrinogen deposits. By conducting shotgun metagenomics analysis on fecal pellets collected before and after ABX treatment, we identified disruptions in the balance of three major bacterial families from the dominant phyla Bacteroidetes and Firmicutes. These disruptions may contribute to the compromised integrity of the BBB in *ApoE4* transgenic mice. These preliminary findings suggest that differences in gut microbiome between *ApoE3* and *ApoE4* transgenic mice may influence BBB permeability and that ABX treatment suppressed the proinflammatory CypA-MMP9 BBB-degrading pathway in pericytes which may help restore *APOE4*-mediated BBB disruption in mice after ABX treatment.

Disclosures: M. Zhang: None. **M.T. Huuskonen:** None. **A.P. Sagare:** None. **A. Chakhoyan:** None. **K. Kisler:** None. **A.R. Nelson:** None. **Y. Wang:** None. **B.V. Zlokovic:** None.

Poster

PSTR398. Mechanisms of Vascular Changes in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR398.09/I8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH, NINDS BINP R01 NS117754
T32AG052375

Title: Chronic stress accelerates cerebral hypoperfusion and cognitive decline in Alzheimer’s triple transgenic mice

Authors: *S. PRABHU[1], N. EMINHIZER[2], A. HANSHEW[2], K. KARELINA[3], E. KELLEY[4], J. W. SIMPKINS[4], P. CHANTLER[2];
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Abstract: Chronic psychosocial stress is a risk factor for cognitive decline in Alzheimer’s disease (AD). However, there is a critical need to identify the contributing molecular factors linking stress and AD. Our previous work has shown that chronic mild stress (UCMS) in C57BL/6 mice had reduced endothelial dependent dilation of the middle cerebral artery vs non-UCMS control mice. In this study, we examined the effects of early exposure to UCMS in AD mice. Male/female 3xTg-AD (APPSWE, PS1M146V, and tauP301L) and WT-AD (C57BL6/129S) mice underwent 8 weeks of UCMS starting at 4 months of age for 5 days/week and 7 Hrs/day, while control mice did not undergo stress. To determine the role of age as a confounding variable in accelerating AD progression, all mice were taken off UCMS at 6 months of age and euthanized at 12 months of age. Prior to euthanasia, cerebral blood flow (CBF) was

measured using Laser Doppler contrast imaging and mean global flux was calculated across all treatment groups. After euthansia, tissues were harvested and homogenized for protein quantification using Western blot analysis. Cognitive outcomes such as open field, Y-maze, and Novel Object recognition were examined. Our data shows that the WT UCMS mice had a 19% reduction in CBF ($p<0.05$) compared to the WT controls. The 3xTg UCMS group showed a 10% decrease in global flux levels as compared to the 3xTg controls. Male controls showed a significant reduction in global flux ($p<0.05$) compared to females in the 3xTg Controls. Female 3xTg UCMS mice showed significantly reduced CBF ($p<0.05$) compared to female 3xTg control mice indicating the effects of stress to be more prominent in females. The recognition index for the WT control mice (time investigating the novel object) was significantly higher than the 3xTg controls ($p<0.05$). The WT UCMS group showed a significant reduction in the recognition index as compared to the WT controls. Thus, the study establishes the premise that chronic stress accelerates AD progression and cognitive decline in our early-onset model with potential sex differences. The study also lays the foundation for future studies to develop effective treatment strategies to prevent early cognitive decline in AD.

Disclosures: **S. Prabhu:** None. **N. Eminhizer:** None. **A. Hanshew:** None. **K. Karelina:** None. **E. Kelley:** None. **J.W. Simpkins:** None. **P. Chantler:** None.

Poster

PSTR398. Mechanisms of Vascular Changes in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR398.10/J1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: UAMS Neurodegeneration Creativity Hub.

Title: Investigating the role of p-glycoprotein in amyloid β-peptide clearance: implications for alzheimer's disease.

Authors: *J. ASANTE, C. L. NAGEL, M. BALASUBRAMANIAM, S. W. BARGER; Univ. of Arkansas for Med. Sci., LITTLE ROCK, AR

Abstract: Alzheimer's disease (AD) is characterized by the accumulation of amyloid β-peptide (Aβ) in the brain, with impaired clearance playing a significant role in its pathogenesis. One key player in the clearance of Aβ is the multidrug resistance protein (MDR1), also known as P-glycoprotein (P-gp), which functions as an active transporter in the plasma membrane of cerebrovascular endothelial cells. P-gp facilitates the removal of Aβ from the brain into the bloodstream. Various substrates of P-gp can act competitively or cooperatively on one another's transport rate, depending on their binding site on the enzyme. In this cohort study, we analyzed data from the PharMetrics Plus database (IQVIA) including 471,539 participants, 65 and older who were receiving various medications known to be P-gp substrates and were free of AD at the baseline. A nested case-control analysis was performed, matching each AD case with four

controls (1:4) based on age and time of study entry using density-based sampling. AD cases (1359) and matched controls (5436) were then analyzed using time-dependent Cox regression to assess the association between the use of P-gp substrate medications and the risk of AD while adjusting for confounding factors such as age, sex, and comorbidities including stroke, hypertension, diabetes, coronary artery disease, depression, and chronic kidney disease. This provided estimated odds ratios for a number of P-gp substrates regarding risk for AD-related dementia.To supplement the epidemiological approach, we employed computational modeling and molecular dynamic simulations to identify the binding sites of Aβ and other substrates on P-gp, allowing assignment into complementation groups. Our modeling results revealed that Aβ binds at the so-called “R-site” of P-gp. Furthermore, results predict that P-gp substrates, including berotralstat, remdesivir and actinomycin D, exhibited higher binding affinity to a P-gp-Aβ complex versus P-gp alone. Our long-term goal is to gain a comprehensive understanding of the impact of P-gp substrates on the clearance of Aβ from the brain. This knowledge will contribute to the development of health policies and therapeutic strategies aimed at preventing Aβ accumulation and, ultimately, Alzheimer's disease.

Disclosures: J. Asante: None. **C.L. Nagel:** None. **M. Balasubramaniam:** None. **S.W. Barger:** None.

Poster

PSTR398. Mechanisms of Vascular Changes in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR398.11/J2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01AG044404

Title: Oligomeric Tau Alters Tight Junction Protein Expressions and Cerebral Endothelial Permeability: Role of RhoA/ROCK Signaling Pathway

Authors: *F. HOSSEN, J. C. LEE;
Univ. of Illinios, Chicago, Chicago, IL

Abstract: Oligomeric Tau Alters Tight Junction Protein Expressions and Cerebral Endothelial Permeability: Role of RhoA/ROCK Signaling Pathway Faruk Hossen[1], James C. Lee[1]
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Introduction: Tight junction (TJ) proteins in Cerebral Endothelial Cells (CECs) play an important role in maintaining the integrity of the blood-brain barrier (BBB). In Alzheimer’s disease (AD), BBB dysfunction has been associated with amyloid beta (Aβ) pathology, but how oligomeric Tau (oTau) impacts the BBB function has yet to be fully elucidated. **Materials and Methods:** Primary mouse CECs cultured in a transwell chamber was employed as our BBB

model. Cells in the chamber were treated with oTau. To examine the involvement of the RhoA/ROCK pathway, cells were pre-treated with Fasudil, a RhoA/ROCK signaling inhibitor. Permeability of the CEC layer was measured by trans-endothelial transport of FITC-dextran and electrical resistances (TEER) assays. The expressions of TJ proteins (occludin, claudin-5, ZO-1) and p-p47phox in CECs were assayed by western blot analysis. Oxidatively modified proteins levels and proteasome activity were quantified by Protein Carbonyl Assay and Proteasome Activity Assay, respectively.**Results:** Treating CECs with oTau significantly decreased TJ protein expressions and increased the permeability of the CEC layer. It also upregulated p-p47phox (subunit of NADPH oxidase) and increased oxidative damage of proteins but decreased proteasome activity in CECs. These functional alterations of our BBB model induced by oTau were significantly suppressed by the pre-treatment with RhoA/ROCK signaling inhibitor, Fasudil. **Conclusion:** Our findings suggest that the RhoA/ROCK pathway involved in oTau-induced disruption of BBB. Regulation of the RhoA/ROCK pathway can be a potential therapeutic strategy to maintain the BBB integrity in AD.**Funding:** This work was supported by National Institutes of Health R01AG044404 (JCL).

Disclosures: F. Hossen: None. **J.C. Lee:** None.

Poster

PSTR398. Mechanisms of Vascular Changes in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR398.12/J3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant P01AG052350
Cure Alzheimer’s Fund
NIH grant R01HL142975

Title: Multi-omic signature of therapeutic activated protein C analog on APOE4 neurovascular dysregulation

Authors: *K. KISLER[1], G. BARISANO[1], A. P. SAGARE[1], V. CLEMENTEL[1], A. CHAKHOYAN[1], M. T. HUUSKONEN[1], J. A. FERNÁNDEZ[2], J. H. GRIFFIN[2], M. P. COBA[1], B. V. ZLOKOVIC[1];
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Abstract: Apolipoprotein E4 (*APOE4*), the main susceptibility gene for Alzheimer's disease (AD), leads to blood-brain barrier (BBB) breakdown in humans and mouse models. Vascular and BBB dysfunction has also been linked to AD evolution in experimental, imaging, pathological, and epidemiological studies. Importantly, BBB dysfunction can predict cognitive decline and precedes synaptic deficits in *APOE4* carriers and *APOE4* transgenic mice. We have previously shown that *APOE4* compared with *APOE3* non-risk allele leads to an early disruption of the

BBB transcriptome in 2-3-mo-old *APOE4* knock-in (KI) mice, followed by dysregulation in protein signaling networks controlling cell junctions, cytoskeleton, clathrin-mediated transport, and translation in brain endothelium. Currently, no treatment exists to restore BBB integrity and function in *APOE4* carriers.
Here, we treated *APOE4* KI mice with 3K3A-activated protein C (APC), a cell-signaling analogue of endogenous blood serine protease APC that exerts vasculoprotective, neuroprotective, and anti-inflammatory activities in a variety of rodent disease models. Using single-nucleus RNA-sequencing and phosphoproteome and proteome analysis, we show that *APOE4* KI mice treated with 3K3A-APC had restoration of their neurovascular transcriptomic and proteomic profiles towards levels comparable to age-matched *APOE3* KI control mice. Additionally, *APOE4* KI mice treated with 3K3A-APC showed improved BBB integrity in vivo by DCE-MRI. Together these data provide a multi-omic signature of the restorative therapeutic effects of 3K3A-APC. 3K3A-APC may be a promising therapeutic to counteract the effects of *APOE4* mediated transcriptomic, proteomic, and functional BBB disruption.

Disclosures: **K. Kisler:** None. **G. Barisano:** None. **A.P. Sagare:** None. **V. Clementel:** None. **A. Chakhoyan:** None. **M.T. Huuskonen:** None. **J.A. Fernández:** None. **J.H. Griffin:** None. **M.P. Coba:** None. **B.V. Zlokovic:** None.

Poster

PSTR398. Mechanisms of Vascular Changes in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR398.13/J4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Columbia University Schaefer Research Scholar Award to C.K.
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National Institute on Aging U01 AG046139 to N.E.T
National Institute on Aging R01 AG061796 to N.E.T
Alzheimer’s Association Zenith Awards to N.E.T.

Title: Single cell/nucleus transcriptomics of neuro-vascular cell types in zebrafish and human patients reveal evolutionary conserved pathomechanisms of vascular contribution to Alzheimer's disease

Authors: *E. YILMAZ[1,2], P. BHATTARAI[2], Ö. IS[3], X. WANG[4], A. J. LEE[5], M. COSACAK[6], B. VARDARAJAN[5], R. MAYEUX[1,7], N. ERTEKIN-TANER[8], C. KIZIL[2];
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Physicians and Surgeons, Columbia Univ., New York, NY; [6]German Ctr. for Neurodegenerative Dis. (DZNE), Dresden, Germany; [7]Neurology, The TAUB Institute, Sergievsky Center, Psychiatry, and Epidemiology, Col. of Physicians and Surgeons, Columbia Univ., New York, NY; [8]Dept. of Neuroscience, Dept. of Neurology, Mayo Clin. Florida, Jacksonville, FL

Abstract: Recent studies have discovered novel genes that are associated with the co-existence of vascular pathologies and Alzheimer's disease (AD). However, the biological functions and the mechanism of action by which they contribute to the disease pathology are still to be further elucidated. Therefore, animal models that allow streamlined high-throughput functional screens in a biologically relevant manner are essential. Towards this goal, we generated adult zebrafish models of amyloid toxicity to transcriptionally compare the candidate genes identified from genetic association studies in multi-ethnic AD and control cohorts as well as single nucleus transcriptome datasets from human brains. Integration of single cell/nucleus transcriptomics of human AD patients and zebrafish brains showed remarkable similarities in the transcriptional alterations of neurons in response to amyloid in zebrafish and AD patients. By performing immunohistological analyses, gene editing for generating genetic knockouts, pharmacological intervention of selected signaling pathways and comparing the findings to human AD brains, we determined the similarities in vascular pathological mechanisms contributing to AD pathology. In this presentation, we will present our findings on an evolutionarily conserved molecular regulatory pathway that controls blood-brain-barrier integrity. Our studies propose zebrafish as a useful model for transcriptional and functional investigation of vascular pathology and AD-related genes identified in clinical studies by providing in vivo biological knowledge with which novel drug development strategies can be designed.

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Poster

PSTR398. Mechanisms of Vascular Changes in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR398.14/J5

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Whole-brain 3D imaging of Aβ plaques following treatment with a BBB shuttle-enhanced aducanumab biosimilar in a mouse model of AD.

Authors: *M. RAMOS VEGA[1], F. WICHERN[3], A. JENSEN[3], C. S. JENSEN[4], J. LERCKE[1], C. GRAVERSEN SALINAS[1], S. VERGO[4], H. HANSEN[2], J. HECKSHER-SØRENSEN[5]; [1]Gubra, Hørsholm, Denmark; [2]Gubra, Gubra, Horsholm, Denmark; [3]Lundbeck AS, Vallby, Denmark; [4]Lundbeck AS, Lundbeck AS, Valby, Denmark; [5]Gubra ApS, Gubra ApS, Hørsholm, Denmark

Abstract: Accumulation of amyloid β (Aβ) in the brain is the neuropathological hallmark of Alzheimer's disease (AD). Recently approved Aβ-directed antibodies (aducanumab, lecanemab) have demonstrated modest efficacy in AD, which may potentially be explained by poor blood-brain barrier (BBB) penetration. Transferrin receptor (TfR) mediated enhanced brain delivery of monoclonal antibodies across the BBB is a promising concept in drug development for CNS disorders. Using an advanced light sheet fluorescence microscopy (LSFM) pipeline coupled with deep learning computational analysis, the present study aimed to visualize, map and quantify amyloid plaque load following long-term therapy with a BBB shuttle-enhanced Aβ-directed antibody in a mouse model of AD. 5-9 month-old APP/PS1 transgenic mice (n=8-10 per group) were treated with an aducanumab biosimilar (AduBS, 10 or 50 nmol/kg), aducanumab biosimilar fused with a mTfR binder as BBB-shuttle (AduBS-BBB, 10 nmol/kg), control hIgG (Contr, 50 nmol/kg), or saline for 12 weeks. Whole brains were stained with an antibody against Aβ, cleared and scanned on a LSFM. A deep-learning image analysis algorithm was developed and validated for automated whole-brain visualization, segmentation, anatomical mapping and quantification of Aβ plaques using a custom mouse brain atlas. Amyloid plaque deposition was detected in the brain at micrometre resolution. Regions with marked Aβ plaque load included the cortex, hippocampus, thalamus and lateral septum. Deep-learning image analysis was employed to map potential quantitative differences in the distribution and number of Aβ plaques following long-term therapy with a BBB shuttle-enhanced Aβ antibody compared to an AduBS. The LSFM pipeline enables high-throughput quantitative LSFM 3D imaging of Aβ plaque load in mouse models of AD for preclinically profiling brain-wide effects of anti-amyloid therapies.

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Poster

PSTR398. Mechanisms of Vascular Changes in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR398.15/J7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 MH130862

Title: An insight into embryonic-heart-expressed sema6d in Alzheimer's disease

Authors: *K. JIAO, S. MALIK, S. SAGGU, M. AIDA, Q. WANG;
Med. Col. of Georgia at Augusta Univ., Augusta, GA

Abstract: An insight into embryonic-heart-expressed SEMA6D in Alzheimer's disease
Saima Shakil Malik[1], Shalini Saggu[2], Mae Aida[2], Qin Wang[2], Kai Jiao[1]* *[1]Center for Biotechnology & Genomic Medicine (CBGM), Medical College of Georgia, Augusta University [2]Department of Neuroscience and Regenerative Medicine, Medical College of*

Georgia, Augusta University
Alzheimer's disease (AD) is a progressive degenerative condition that primarily affects cognitive abilities, memory, and other cognitive functions. The brain is a highly vascularized organ, receiving cardiac output and vulnerable to the impairment of cerebral perfusion, which is a frequent event of heart failure. SEMA6D is a member of the Semaphorin family of signaling molecules and our previous study suggests it is essential to sustain late fetal or early neonatal cardiomyocytes at a proliferative and less mature status. In this study, we generated a novel model, *cTnt-Cre;Sema6D$^{loxp/loxp}$;APP$^{Ki/Ki}$*, to investigate the cardiac function impairment in hearts and its effects on AD-related pathological changes. Via echocardiography, we found that embryonic deletion of *Sema6D* in cardiomyocytes caused decreased heart function, i.e., lower ejection fraction, fractional shortening and cardiac output with various other abnormalities in the heart. Histopathological examinations revealed significantly enlarged hearts associated with thinning of LV walls, which could possibly lead to heart failure. Furthermore, we examined whether cardiomyopathy caused by embryonic heart deletion of *Sema6D* could accelerate the onset and aggravates the severity of AD-related deficits. To reveal this, we are in the process of assessing the cognitive function and testing that it is significantly decreased in mice with myocardial deletion of *Sema6D*. We are also examining amyloid deposition and glial cell reactivation in the brain of these mice. Evaluating how congenital cardiomyopathies can impact AD is a new area to explore and we are the first ones to design this mouse model and investigating the comorbid effects. Successfully accomplishing our research will open the door for future studies improving the diagnostic criteria and therapeutic approaches.
Conflict of interest: The authors have declared that no conflict of interest exists.

Disclosures: K. Jiao: None. **S. Malik:** None. **S. Saggu:** None. **M. Aida:** None. **Q. Wang:** None.

Poster

PSTR398. Mechanisms of Vascular Changes in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR398.16/Web Only

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant NS047229

Title: Presenilin 1-FAD mutants and γ-secretase inhibitor disrupt the VEGF-induced VEGFR2 endocytosis, trafficking and in vitro and in vivo brain angiogenic functions.

Authors: *A. ZARROUK, R. PANDEY, G. TZIKAS, E. LEVENDOSKY, P. DEY, A. GEORGAKOPOULOS, N. K. ROBAKIS;
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Abstract: Brain vascular alterations observed in Alzheimer’s disease (AD) such as reduction in capillary density, may be the result of decreased angiogenesis. Angiogenesis, a process by which

new blood vessels arise from pre-existing vessels is regulated by endothelial cells (EC) and angiogenic factors such as the Vascular Endothelial Growth factor (VEGF) and its receptor VEGFR2. When VEGF binds to VEGFR2, it triggers VEGFR2 activation, dissociation from VE-cadherin, endocytosis, and activation of subsequent downstream signaling events that promote angiogenesis. Here, we explore the effects of Presenilin 1 (PS1)-Familial AD (FAD) mutants and γ-secretase inhibitor on the VEGF-induced VEGFR2 internalization, trafficking, interaction with VE-Cadherin and angiogenic functions in brain ECs and brain neovascularization. *In vitro* angiogenesis assays were performed using primary cortical EC (pCEC) from brains of wild-type and knock-in mice expressing FAD mutant PS1 I213T. Surface biotinylation assay was used to assess the VEGFR2 internalization in HEK293 cells overexpressing VEGFR2 in the presence or absence of VEGF-A and γ-secretase inhibitor (RO4929097). Immunofluorescence staining was performed in Human Umbilical Vein Endothelial cells (HUVEC) to detect VEGFR2 trafficking, using the endosomal markers Rab5 and Rab7. The role of γ-secretase in VEGF-induced formation of VEGFR2/VE-cadherin complexes was examined using RO4929097, with immunoprecipitation. Brain neovascularization was measured following VEGF injection in mice with collagen IV immunostaining and confocal microscopy. Our data show that VEGF-induced sprouting and migration are decreased in PS1 FAD mutant-expressing pCEC and that the VEGF-induced neovascularization is decreased in the brains of PS1 I213T mice. Additionally, VEGF-induced endocytosis of VEGFR2 is reduced by RO4929097 in HEK293 cells. Co-localization analysis in HUVEC showed that VEGF stimulation promotes VEGFR2 internalization to Rab5-positive early endosomes, followed by trafficking to Rab7-positive late endosomes. RO4929097 increased the VEGF-stimulated localization of VEGFR2 to Rab7-positive late endosomes. Furthermore, we found that VEGF-stimulated decrease of the VE-cadherin/VEGFR2 complexes is inhibited by RO4929097 in HUVEC. Our data suggest that PS1 FAD mutants and γ-secretase inhibitors decrease brain angiogenic functions by changing of the VEGFR2/VE-cadherin complexes, decreasing the VEGFR2 endocytosis and increasing its degradation in the lysosomes. Our findings suggest a molecular mechanism through which PS1 FAD mutants impair brain angiogenesis.

Disclosures: **A. Zarrouk:** None. **R. Pandey:** None. **G. Tzikas:** None. **E. Levendosky:** None. **P. Dey:** None. **A. Georgakopoulos:** None. **N.K. Robakis:** None.

Poster

PSTR398. Mechanisms of Vascular Changes in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR398.17/J8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA RF1AG071226

Title: Endothelial senescence mediated by $p16^{INK4a}$ leads to cerebrovascular dysfunction in mouse brains

Authors: *K. KAWATANI[1], T. AIKAWA[1], Y. INOUE[1], F. SHUE[1], T. PARSONS[1], C. YUANXIN[1], B. Y. KIM[2], T. KANEKIYO[1];
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Abstract: Vascular cognitive impairment of dementia (VCID) is observed in around 8-15 % of aged patients with cognitive dysfunctions and is the second major cause of dementia. Aging is a strong risk factor for VCID and is predicted to be caused by an accumulation of senescent cells, in which the increase of $p16^{INK4a}$ is one of the key hallmarks in cellular senescence. Thus, we investigated how the forced expression of $p16^{INK4a}$ in vascular endothelial cells impacts cerebrovascular function, using an in vivo gene delivery system through a unique recombinant adeno-associated virus 2 (rAAV2) with a modified capsid. When $p16^{INK4a}$ or EGFP was expressed in cerebrovascular endothelial cells in wild-type mice 1 month after the rAAV2 injection, results showed an increase in IgG leakage into the brain parenchyma in the $p16^{INK4a}$-expressed mice. When cerebral blood flow (CBF) was measured by in vivo 2-photon imaging 2.5 months after the injection in the mice, we found that cerebrovascular endothelial $p16^{INK4a}$ overexpression substantially reduced CBF in arterioles and capillaries in the cortex. Furthermore, we assessed neurovascular coupling (NVC) with whisker stimulation by measuring CBF using laser speckle contrast analysis (LASCA) to analyze the function of the cerebrovascular system. NVC was disrupted in the $p16^{INK4a}$-expressed mice 6 months after the injection. RNA-sequencing in the cortex found that most top-ranked master regulator genes targeted *Cdkn1a* and *Hspa5*. In weighted gene co-expression network analysis, the pathways associated with endoplasmic reticulum (ER) stress/ unfolded protein response (UPR) activation and lipid metabolism were altered. These findings implied that forced expression of $p16^{INK4a}$ in cerebrovascular endothelial cells specifically influences senescence in the brain. In the Morris water maze test, $p16^{INK4a}$-overexpression impaired spatial memory and learning. Our results show that overexpression of 16^{INK4a} in cerebrovascular endothelial cells disturbs BBB integrity and neurovascular coupling, resulting in impaired cognitive function, which may contribute to the pathogenesis of VCID.

Disclosures: K. Kawatani: A. Employment/Salary (full or part-time):; Mayo Clinic. **T. Aikawa:** A. Employment/Salary (full or part-time):; Mayo Clinic. **Y. Inoue:** A. Employment/Salary (full or part-time):; Mayo Clinic. **F. Shue:** None. **T. Parsons:** A. Employment/Salary (full or part-time):; Mayo Clinic. **C. Yuanxin:** A. Employment/Salary (full or part-time):; Mayo Clinic. **B.Y. Kim:** A. Employment/Salary (full or part-time):; University of Texas MD Anderson Cancer Center. **T. Kanekiyo:** A. Employment/Salary (full or part-time):; Mayo Clinic.

Poster

PSTR398. Mechanisms of Vascular Changes in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR398.18/J9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH AG060731

Title: Effects of locus coeruleus (LC) deafferentation and noradrenergic rescue on cognitive function and cerebrovascular markers in a transgenic rat model of Alzheimer's disease

Authors: *M. GIFANI[1,2], J. S. BECK[2], Y. CHEN[3], Z. FERNANDEZ[3], C. QIAN[3], L. CHAMBERS[4], D. C. ZHU[3], R. WISEMAN[3], A. M. DORRANCE[4], S. E. COUNTS[2]; [1]Michigan State Univ., Grand rapids, MI; [2]Translational Neurosci., Michigan State Univ., Grand Rapids, MI; [3]Dept. of Radiology, [4]Pharmacol. toxicology, Michigan State Univ., East Lansing, MI

Abstract: Degeneration of noradrenergic locus coeruleus (LC) neurons, which modulate attentional and memory function in cognitive forebrain regions such as prefrontal cortex (PFC), is an early feature of Alzheimer's disease (AD). Recently, we demonstrated that experimental LC degeneration results in elevated amyloid plaque pathology, cerebral amyloid angiopathy (CAA), astrogliosis, and cerebrovascular leakage in the Tg344-19-AD rat model of AD. To explore additional pathology associated with LC loss in this model and to test whether these perturbations could be ameliorated by norepinephrine (NE)-based therapies, 6-month-old Tg344-19 AD rats were stereotactically lesioned by administering the noradrenergic immunotoxin, dopamine-β-hydroxylase IgG-saporin (DBH-sap), or control IgG-sap (n = 16/group) into PFC. The rats were then randomized to receive the NE pro-drug L-DOPS + the NE reuptake inhibitor atomoxetine (ATM) or solvent placebo weekly. Rats were tested behaviorally 8 weeks later on Open Field, Novel Object, Elevated Plus Maze and Barnes Maze. After 4 weeks, cerebral perfusion was assessed in the PFC of a subset of rats by MRI using a pulsed Arterial Spin Labeling (ASL) technique called FAIR (Flow Sensitive Alternating Inversion Recovery) and echo planar imaging, followed by pressure myography studies of parenchymal arterioles and processing for pathological analysis. Based on our published data, we expect DBH-sap-lesioned rats to exhibit significant deficits in spatial and working memory compared to IgG-sap rats in addition to increased plaque, CAA, vascular leakage, and astroglial pathology. We also expect that LC lesions will reduce perfusion, alter arteriole vasoreactivity, and exacerbate additional markers of forebrain pathology including microglial activation and vascular cell senescence. Finally, we anticipate that L-DOPS/ATM treatments will rescue these behavioral and pathological deficits since L-DOPS will provide NE replacement and there are noradrenergic terminals remaining for ATM to be efficacious. If so, this would provide critical preclinical evidence that cerebrovascular pathology induced by LC projection system loss can be mitigated by different pathways promoting endogenous NE bioavailability. Thus, understanding the potential impact of LC degeneration on cerebrovascular dysfunction and vascular risk factors in AD may open up new therapeutic avenues.

Disclosures: M. Gifani: None. **J.S. Beck:** None. **Y. Chen:** None. **Z. Fernandez:** None. **C. Qian:** None. **L. Chambers:** None. **D.C. Zhu:** None. **R. Wiseman:** None. **A.M. Dorrance:** None. **S.E. Counts:** None.

Poster

PSTR398. Mechanisms of Vascular Changes in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR398.19/J10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Bright Focus

Title: Apolipoprotein E as a potential treatment target in cerebral amyloid angiopathy

Authors: *O. BONNAR[1], M. SANCHEZ-MICO[1], L. H. MARESCO[1], B. J. BACSKAI[1], S. M. GREENBERG[1], D. M. HOLTZMAN[2], S. J. VAN VELUW[1];
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Abstract: Deposition of amyloid beta (Aβ) within the walls of small blood vessels, known as cerebral amyloid angiopathy (CAA), is commonly observed in the brains of Alzheimer's disease (AD) patients alongside parenchymal Aβ plaques. The presence of CAA increases the risk of hemorrhage and independently contributes to cognitive impairment. Although anti-Aβ immunotherapy may seem like a logical treatment for CAA, safety concerns involving bleeding and edema, known collectively as amyloid-related imaging abnormalities (ARIA), have led to caution against its use in this patient group (PMID: 34237272). Recently, a promising alternative has been suggested, where targeting non-lipidated ApoE4 that co-deposits with Aβ resulted in a reduction in CAA without bleeding (PMID**: 33597265).** Despite this promise, several questions remain. Firstly, can CAA that has already been deposited be removed by anti-ApoE immunotherapy and secondly, what is the impact of removal on vascular function? We explore these questions in this study by using multiphoton microscopy to longitudinally image the vasculature of mice administered anti-ApoE immunotherapy. Nineteen mice (9 females and 10 males) expressing human APOE4 and five AD-linked mutations (APOE4 5xFAD) had a cranial window surgically implanted over the right visual cortex and underwent repeated unanesthetized imaging following a post-surgical recovery period. After an initial imaging session at 10 months of age, mice received weekly intraperitoneal (IP) injections of control IgG or anti-human ApoE antibody (50mg/kg). Throughout the treatment period, mice were imaged monthly for a further three months. CAA and Aβ plaque burden were visualized with methoxy-X04 and vascular function, including resting-state vasomotion and evoked vascular reactivity, were also recorded. After the final imaging session brains were extracted for post-mortem examination. Tissue sections were processed for immunohistochemistry to assess Aβ, Iron (for bleeds) and smooth muscle actin. After 3 months of treatment, CAA burden did not differ between mice that received anti-ApoE immunotherapy compared to those who received control IgG ($p = 0.54$). Evoked vascular activity also didn't differ between treatment groups ($p = 0.36$). Analyses conducted on in vivo data suggest that anti-ApoE immunotherapy does not actively remove CAA that is already deposited, but it may instead work to inhibit further accumulation of Aβ. This hypothesis will be explored in ongoing post-mortem studies. It is the hope that this study will add to the growing body of evidence that will help to determine the feasibility of anti-ApoE immunotherapy as a therapeutic agent in CAA patients.

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Poster

PSTR398. Mechanisms of Vascular Changes in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR398.20/K1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA 1RF1AG077570

Title: Effects of Aducanumab on brain pericytes in two mouse models of Alzheimer's Disease

Authors: Y. WU, N. BHAT, *M. LIU;
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Abstract: Aducanumab, an FDA-approved medication for treating Alzheimer's disease (AD), is a monoclonal antibody targeting amyloid-beta (Aβ). Besides its role in clearing brain parenchymal Aβ plaques, how Aducanumab affects the brain-blood barrier (BBB) and neurovascular unit (NVU) is still unknown. Brain capillary pericytes, a primary component of BBB and NVU, maintain BBB integrity and regulate cerebral blood flow. Damage and compromised pericytes are involved in AD pathogenesis. The present study examined the effects of Aducanumab on brain pericytes in two distinct AD/amyloidosis mice models: APP/PS1 mice (parenchymal dense-core amyloid plaque dominant) and Tg-SwDI mice (vascular diffuse amyloid deposition). All mice received Aducanumab (0.1 mg/kg) or vehicle via weekly intracerebroventricular (ICV) injections for three weeks. Then we measured the level of cerebrospinal fluid (CSF) soluble platelet-derived growth factor receptor beta (sPDGFRβ) with *ELISA* assay. Multiple studies have found that injured brain pericytes shed sPDGFRβ into CSF. Therefore, an elevated CSF sPDGFRβ level has become a sensitive marker for detecting pericyte injury. Our results demonstrated that Aducanumab treatment in APP/PS1 mice dramatically decreased CSF sPDGFRβ level, which was nearly 5-fold lower when compared to the vehicle control. In contrast, Aducanumab treatment caused a 2-fold increase in CSF sPDGFRβ level in the Tg-SwDI control mice compared to the vehicle control. Furthermore, we identified that Aducanumab triggered cerebral microhemorrhage (microbleeds) in Tg-SwDI mice rather than APP/PS1 mice. These results indicate that Aducanumab may alleviate pericyte damage in mice with dominant parenchymal Aβ while exacerbating pericyte damage in mice with dominant vascular amyloid deposition. The intensified damage to capillary pericytes may be associated with increased cerebral microhemorrhage, a common side effect observed in patients receiving anti-Aβ treatment.

Disclosures: Y. Wu: None. **N. Bhat:** None. **M. Liu:** None.

Poster

PSTR399. APP and ABeta Mechanisms of Action and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR399.01/K2

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: Veterans Affairs Merit Award I01BX005976

Title: Pharmacologic normalization of brain NAD+/NADH with P7C3-A20 prevents and reverses Alzheimer's disease

Authors: *K. CHAUBEY[1,2,3,4], E. VÁZQUEZ-ROSA[1,2,3,4], M.-K. SHIN[1,2,3,4,5], Y. YU[1,2,3,4], X. WANG[6], S. TRIPATHY[7,8,9,10], S. CHAKRABORTY[7,8,9,10], P. SRIDHARAN[1,2,3,4,11], E. MILLER[1,2,3,4,11], Z. BUD[1,2,3,4,12], K. FRANKE[1,2,3,4], C. CINTRÓN-PÉREZ[1,2,3,4], S. BARKER[1,2,3,4,6], H. FANG[1,2,4,3,13], S. ROSE[1,2,4,3,14], M. DHAR[1,2,3,4], Y. KOH[1,2,3,4,6], X. ZU[6], H. FUJIOKA[15], M. FLANAGAN[16,17,18], F. ORTIZ[19], N. WILLIAMS[19], B. WILSON[3,20], B. PAUL[7,8,9,10], J.-A. WOO[6], D. KANG[6,20], A. A. PIEPER[2,1,4,3,21];
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Abstract: Alzheimer's disease (AD) is late-onset even when patients carry lifelong genetic susceptibility, suggesting the brain defends itself for decades before succumbing to AD. The underlying basis for this loss of resilience, however, is unknown. Here, we report impaired brain metabolism as diminished NAD^+/NADH in human AD and two AD mouse models: amyloid-driven 5xFAD mice and tau-driven PS19 mice. P7C3-A20-mediated pharmacologic normalization, but not elevation above normal, of brain NAD^+/NADH in 5xFAD mice from the 2-month-old presymptomatic stage until 6 months of age prevents acquisition of blood-brain barrier deterioration, neurodegeneration, neuroinflammation, impaired hippocampal neurotransmission, aberrant neuropsychiatric behavior, and cognitive impairment. Furthermore, normalization of brain NAD^+/NADH from the 6-month-old symptomatic stage to 12 months of

age reverses these features. AD-like cognitive impairment was also reversed in 12-month-old severely symptomatic PS19 mice after one month of P7C3-A20-mediated brain NAD^+/NADH normalization. Thus, normalizing brain NAD^+/NADH confers brain resilience that prevents and reverses AD. These results illustrate the potential for not only preventing or slowing AD, but also for disease reversal through restoring brain resilience.

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Poster

PSTR399. APP and ABeta Mechanisms of Action and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR399.02/K3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: DOD Grant W81XWH-18-1-0701

Title: Hormesis-like interactions between amyloidβ and environmental stressors in C. elegans, mediated by key stress response pathways

Authors: ***J. LICHTY**, A. SAN MIGUEL;
Chem. and Biomolecular Engin., North Carolina State Univ., Raleigh, NC

Abstract: Alzheimer’s Disease (AD) is a neurodegenerative disorder which presents as a progressive loss of mental and physical function in those afflicted with it. AD is characterized by many abnormalities in the brain, such as widespread oxidative stress, immunity activation, neurodegeneration, inflammation, and protein aggregation. Amyloid β (Aβ) accumulation and aggregation has been associated with several AD traits, implicating it in the disease. To study this peptide’s role in AD, several models using the organism *C. elegans* have been engineered to express Aβ pan-neuronally. Previous work has shown Aβ’s ability to aggregate and induce oxidative stress, neurodegeneration, and reduced lifespan in *C. elegans,* but there are several other ways in which Aβ may influence the organism’s health. To fully capture Aβ’s impact on the organism and accurately translate it into a physiologically relevant result for humans, we need understand exactly what and how Aβ is interacting with the worm’s own physiology. Environmental stressors, including heat stress, oxidative stress, starvation, and hypoxia, are known to significantly impact worm health, but their interactions with Aβ have been largely unexplored. This work focuses on elucidating how Aβ influences worm health and survival in response to several different stressors. We initially hypothesized that environmental stressors and

Aβ would act synergistically to the detriment of worm health and survival. By exposing Aβ-expressing *C. elegans* to different stressors and scoring for survival, we show Aβ induces lowered oxidative stress resistance. Contrary to expectations though, Aβ expression increases resistance to several other stressors, indicating a possible hormesis-like effect. Additionally, we show, with a temperature inducible Aβ, a correlation between Aβ levels and heat stress resistance. By expressing Aβ in different tissues, we show that this a neuron-specific effect. Using gene-expression analysis techniques, we identified the daf-16 pathway as a possible route for this interaction. Targeted gene suppression indicated the possibility of a generalized daf-16 activation in response to Aβ expression. We also explore contributions from other stress response pathways. This work aims to improve our understanding of how Aβ affects *C. elegans* to better design AD experiments and interpret the results in this model organism.

Disclosures: **J. Lichty:** None. **A. San Miguel:** None.

Poster

PSTR399. APP and ABeta Mechanisms of Action and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR399.03/K4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 AG054551

Title: Soluble amyloid-beta impairs place cell network and circuit function in the APP/PS1 mouse model of Alzheimer’s disease

Authors: *H. LI, Z. ZHAO, R. J. F. GREEN, S. N. GOMPERTS;
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Abstract: Early in the course of Alzheimer’s disease, soluble amyloid-beta (Aβ) deposits to form Aβ plaques in a cascade that causes progressive hippocampal failure. Prior work has shown that Aβ plaques are associated with impaired hippocampal activity and place cell representations, but the effects of soluble Aβ on neural systems function, before plaque deposition, are less well understood. We acquired chronic electrophysiological recordings together with simultaneous dynamic calcium imaging with GCaMP6f in hippocampal CA1 of young APP/PS1 mice expressing soluble Aβ to investigate the impact of soluble Aβ on hippocampal place cell network and circuit function linked to memory encoding and consolidation. Although hippocampal place cell spatial information was comparable in young (5.2 ± 0.2 months) APP/PS1 mice compared to age-matched (4.7 ± 0.2 months) littermate controls, young APP/PS1 mice showed delayed place field formation and diminished hippocampal theta-gamma phase-amplitude coupling. While coordination of hippocampal sharp wave ripples and cortical spindles in slow wave sleep (SWS) remained intact in young APP/PS1 mice, place cell pairwise reactivation was reduced. Compared to control mice, young APP/PS1 mice showed elevated synchronization in the theta and delta range between CA1 neuronal somatic calcium fluctuations and the local field potential in both

exploratory behavior and SWS. Together, these results suggest that soluble Aβ is sufficient to impair hippocampal function both online and offline, identifying early manifestations of Aβ-associated hippocampal dysfunction in Alzheimer's disease.

Disclosures: H. Li: None. **Z. Zhao:** None. **R.J.F. Green:** None. **S.N. Gomperts:** None.

Poster

PSTR399. APP and ABeta Mechanisms of Action and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR399.04/K5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Brain Research Foundation

Title: PrP^C expression affects APP processing and Aβ secretion

Authors: *N. MEHTA[1], L. GREGORY[2], A. SOLANKI[2], J. MASTRIANNI[1];
[1]Neurol. Dept. and Committee on Neurobio., The Univ. of Chicago, Chicago, IL; [2]Neurol. Dept., Univ. of Chicago, Chicago, IL

Abstract: The cellular isoform of prion protein (PrP^C) is a GPI-anchored membrane protein that is highly expressed in neurons. Although central to the development of prion disease, evidence suggests that PrP^C interacts with amyloid-β (Aβ), a 38 to 42 amino acid peptide released from amyloid precursor protein (APP) through sequential enzymatic cleavage, and deposited extracellularly as amyloid plaques, a hallmark of Alzheimer's Disease (AD). We previously established a direct relationship between PrP^C expression and $Aβ_{42}$ secretion in mouse neuroblastoma (N2a) cells stably expressing human APP carrying the Swedish mutation linked to familial AD (N2a-APPswe), although the mechanism by which this occurs is unclear. We questioned whether PrP^C exerts its effect on Aβ secretion by modulating APP processing. To do this, we employed MSD electrochemiluminescence, qRT-PCR, and ELISA to quantify changes in Aβ secretion and the balance between the non-amyloidogenic (α-secretase-generated) and amyloidogenic (β-secretase-generated) processing pathways of APP, before and after siRNA-induced knockdown of PrP^C in N2a-APPswe and N2a-APPwt cells. We show that a ~70% PrP^C knockdown significantly reduced all major species of Aβ ($Aβ_{38}$, $Aβ_{40}$ and $Aβ_{42}$) in both cell lines. Using qRT-PCR, APP mRNA levels were unchanged, arguing against reduced APP expression as the cause. To determine whether PrP^C affects the processing of APP by α or β-secretase, we measured the changes in soluble(s) APPα and APPβ, the secretion products of α-secretase and β-secretase cleavage, respectively. We show that PrP^C knockdown significantly reduced sAPPβ levels in the media of both N2a-APPswe (2148 $\pm$ 43 vs 1833 $\pm$ 69 ng/mL, n=12 samples, $p<0.0059$) and N2a-APPwt (28996 $\pm$ 499 vs 24370 $\pm$ 611 pg/mL, n=6 samples, $p<0.0048$) cells. Although not yet tested in N2a-APPswe cells, PrP^C knockdown produced a striking increase in sAPPα in N2a-APPwt cells (19459 $\pm$ 278 vs 29221 $\pm$ 1241 pg/mL, n=6 samples, $p<0.0001$). Overall, the unaltered APP mRNA levels, substantial increase in sAPPα,

and moderate reduction in sAPPβ following PrP^C knockdown all suggest that PrP^C may normally suppress α-secretase activity and its loss shifts APP processing to the non-amyloidogenic pathway, reducing Aβ levels. Other effects are still possible, such as enhanced β-secretase activity or changes in APP trafficking. Additional studies are planned in cells and mouse models of AD with normal or absent PrP^C expression, to further clarify this relationship. In conclusion, our results provide insight into a possible role for PrP^C in modulating APP processing by promoting cleavage in the amyloidogenic pathway, leading to increased Aβ secretion at baseline.

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Poster

PSTR399. APP and ABeta Mechanisms of Action and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR399.05/K6

Topic:

Support: NTU LKC Medicine Dean's Postdoctoral Fellowship 021207-00001
Mistletoe Research Fellowship 022522-00001

Title: Defective lysosomal acidification contributes to TNF-TNFR1 mediated neuronal cell death

Authors: *C. H. LO[1], G. W. Z. LOI[1], E. N. SAIPULJUMRI[1], L. M. O'CONNOR[2], R. REYNOLDS[3], A. M. BARRON[1], J. ZENG[1];
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Abstract: Tumor necrosis factor receptor 1 (TNFR1) signaling contributes to the pathogenesis of neurodegenerative diseases including Alzheimer's disease (AD). Recently, it has been shown that TNF induced TNFR1 activation leads to autophagic dysfunction and subsequently results in neuronal cell death. Autophagy is a homeostatic mechanism involved in the disposal of damaged organelles and toxic protein aggregates. However, the exact mechanism of how TNFR1 induced autophagic dysfunction leads to neuronal death remains unclear. In this study, we reveal that TNFR1 activation results in a downregulation of genes associated with lysosomal, autophagic, and mitochondrial functions and an upregulation of genes in cell death pathways in TNF treated SH-SY5Y neuronal cells and human iPSC derived primary neurons. Functionally, we show for the first time that lysosomal acidification is impaired only in neurons treated with TNF but not with other cytokines, contributing to inhibition of autophagic flux. Furthermore, we illustrate that there is defective mitophagy in neurons with TNFR1 activation, together with a decrease in mitochondrial membrane potential and an increase in reactive oxygen species. Importantly, we demonstrate that a novel type of lysosome-targeting acidic nanoparticles restores lysosomal acidification, autophagic activity, and mitochondrial function, as well as rescues neuronal cell

death in both cellular models and APP knock-in mouse model of AD. This opens avenues for new therapeutic directions to target lysosomal dysfunction, in addition to the existing efforts in developing receptor-specific inhibitors that target TNFR1 signaling.

Disclosures: C.H. Lo: None. **G.W.Z. Loi:** None. **E.N. Saipuljumri:** None. **L.M. O'Connor:** None. **R. Reynolds:** None. **A.M. Barron:** None. **J. Zeng:** None.

Poster

PSTR399. APP and ABeta Mechanisms of Action and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR399.06/K7

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Cell-based small molecule screening for γ-secretase inhibitors and modulators

Authors: *J. KIM, S. PARK, D.-G. JO;
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Abstract: The accumulation of amyloid-beta (Aβ) plaques in the brain is a critical factor in the development of Alzheimer's disease (AD). Aβ is generated through the processing of amyloid precursor protein (APP) by β and γ-secretase. Although numerous attempts have been made to regulate Aβ generation by inhibiting γ-secretase, most clinical trials have failed due to the side effects caused by the inhibition of notch, a substrate of γ-secretase. Accordingly, modulating γ-secreatse activity has become a potent therapeutic strategy in treating AD pathology. In this study, we developed a fluorescence-based γ-secretase reporter system to assess γ-secretase activity in neuronal cell line. Using this reporter system, we screened FDA-approved drugs and identified several compounds as potential γ-secretase inhibitors or modulators. In addition, one of these compounds has shown improvements in cognitive function in the 5XFAD mouse model. To confirm the regulation of γ-secretase activity by this compound, we measure Aβ plaque and APP C-terminal fragment protein levels in the brain. Our findings highlight the therapeutic potential of this compound as a γ-secretase modulator for treating Alzheimer's disease.

Disclosures: J. Kim: None. **S. Park:** None. **D. Jo:** None.

Poster

PSTR399. APP and ABeta Mechanisms of Action and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR399.07/K8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 1U19AG073172-01
1R01AG062547
1R01AG082093-01

Title: Aβ42 induces neuronal process degeneration and mitochondrial dsDNA release in an inducible human cortical organoid model of Alzheimer's disease

Authors: *M. HEBISCH, J. PARK, S. KWAK, T. KALATTURU, B. TAILOR, K. WASHICOSKY, R. E. TANZI, D. KIM;
Genet. and Aging Res. Unit, Inst. for Neurodegenerative Dis., Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Alzheimer's disease (AD) is the most common cause of dementia, currently affecting 55 million patients. The amyloid-β (Aβ) hypothesis posits that accumulation of pathogenic Aβ42, but not the Aβ40 isoform, triggers a pathogenic cascade leading to neurodegeneration. However, there is no human AD model clearly recapitulating Aβ42-induced neurodegeneration. Previously, our lab has developed Aβ-driven 3D AD cellular models that demonstrate the functional link between amyloid generation and phospho-tau pathology in a 3D gel matrix. Here, we created a human cortical organoid (CO) model of Aβ-driven AD pathology using a doxycycline-inducible transgene in the AAVS1 locus. This novel model generates large quantities of Aβ species with either pathogenic (Aβ42-CO) or non-pathogenic (Aβ40-CO) isoform ratios and enables the isoform-specific interrogation of Aβ effects without confounding effects from altered development or other amyloid precursor protein (APP) fragments such as sAPPα/β or C99. Both Aβ conditions, as well as non-transgenic controls, initially develop cortical plate structures, neurons, and astrocytes. However, upon transgene induction, Aβ42-COs deposit Congo red-positive Aβ aggregates and accumulate oligomeric tau protein. Importantly, we found 60% less neuronal immunoreactivity after 8 weeks in Aβ42-COs, whereas Aβ40-COs closely resembled non-transgenic controls, clearly demonstrating Aβ42-driven neurodegeneration. Single-cell RNAseq analysis confirmed dysregulation of neuronal development, synapse maintenance, mitochondrial dynamics, and axon outgrowth (including Stathmin 2 depletion) in Aβ42-COs. Furthermore, Aβ42-COs contained approx. 50-70% less mitochondrial mass as indicated by TOMM20, and mitochondria in Aβ42-COs showed reduced DNA content in immunocytochemical analyses. Interestingly, we also detected cytoplasmic double-stranded (ds) DNA-positive specks in Aβ42-COs, likely representing released dsDNA from mitochondria. Previous studies showed that cytosolic release of mitochondrial dsDNA triggers innate cellular immune responses, including the cGAS-STING pathway. Indeed, another study from our lab found that Aβ42 accumulation activates the cGAS-STING pathway and accelerates the AD pathogenic cascade in human neurons cultured in a 3D gel matrix. Thus, Aβ42-mediated mitochondrial impairment and innate immune responses may explain the robust neurodegeneration in our human cortical organoid model of Alzheimer's disease. We are currently exploring underlying molecular mechanisms of Aβ42-induced mitochondrial damage and neuronal process degeneration.

Disclosures: M. Hebisch: None. **J. Park:** None. **S. Kwak:** None. **T. Kalatturu:** None. **B. Tailor:** None. **K. Washicosky:** None. **R.E. Tanzi:** None. **D. Kim:** None.

Poster

PSTR399. APP and ABeta Mechanisms of Action and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR399.08/K9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH 1R01AG054598 - 01A1
BrightFocus A2023021F

Title: Understanding the effect of Abeta pathology on astroglial oxidative stress by using in vivo multiphoton microscopy

Authors: *M. SANCHEZ-MICO, E. KIRONDE, S. HOU, M. ALGAMAL, M. CALVO-RODRIGUEZ, M. ARBEL-ORNATH, B. BACSKAI;
Dept. of Neurol., Massachusetts Gen. Hosp., Boston, MA

Abstract: Amyloid beta (Aβ) plaques are one of the key hallmarks of Alzheimer's disease (AD) that can lead to astroglial dysfunction resulting in reduced metabolic support for neurons. This pathological cascade has been linked to disease progression. Using a genetically encoded calcium sensor targeted to astrocytes and multiphoton microscopy, we recently reported evidence of calcium dysregulation in astrocytes *in vivo* from an Aβ plaque-depositing AD transgenic mouse model (APPswe/PS1dE9; PMID: 19251629), and after direct application of naturally secreted soluble Aβ oligomers (Aβo) to the healthy brain (PMID: 37236808). Calcium dyshomeostasis is closely associated with the increase of reactive oxygen species (ROS) in AD and other neurodegenerative diseases. Increased ROS is responsible for promoting oxidative damage to DNA, RNA, proteins and lipids, triggering cell death, a key hallmark of AD pathology. While most studies in AD have focused on neurodegeneration or neuroinflammation, the extent and the mechanism of astroglial dysfunction in AD remains poorly understood. For that reason, we aimed to specifically investigate how Aβ plaques and soluble Aβo contribute to the ROS insult in astrocytes *in vivo*. We leveraged intravital microscopy to characterize *in vivo* the intracellular oxidative stress of astrocytes, by using a genetically encoded indicator (*roGFP*) that selectively reports the reduced/oxidized glutathione (GSH/GSSG) ratio specifically targeted to astrocytes (GFA2.roGFP). We evaluated astroglial oxidative stress after the deposition of Aβ plaques in APPswe/PS1dE9 mice compared to aged-matched non-transgenic mice, as well as after acute application of naturally secreted soluble Aβo (conditioned medium from cultured transgenic primary neurons) onto the living brain of 4-6-mo-old non-transgenic mice. Our preliminary data show an increase in astroglial oxidative stress in APPswe/PS1dE9 mice compared to non-transgenic mice, as well as upon topical soluble Aβo application, in the astroglial cytosol. Aβ-immunodepleted transgenic conditioned media and wild-type conditioned media did not alter astrocyte cytosolic oxidative stress, supporting the specificity of the observed effects. These results together support a detrimental role of Aβ which leads to astrocyte oxidative stress *in vivo*, implying that Aβ accumulation is involved in the astrocytic dysfunction observed in AD. Future studies will address whether this oxidative stress is cause or consequence of the pathology, the specific pathways affected, and the implications for neuronal activity, survival and synaptic functionality.

Disclosures: **M. Sanchez-Mico:** None. **E. Kironde:** None. **S. Hou:** None. **M. Algamal:** None. **M. Calvo-Rodriguez:** None. **M. Arbel-Ornath:** None. **B. Bacskai:** None.

Poster

PSTR399. APP and ABeta Mechanisms of Action and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR399.09/K10

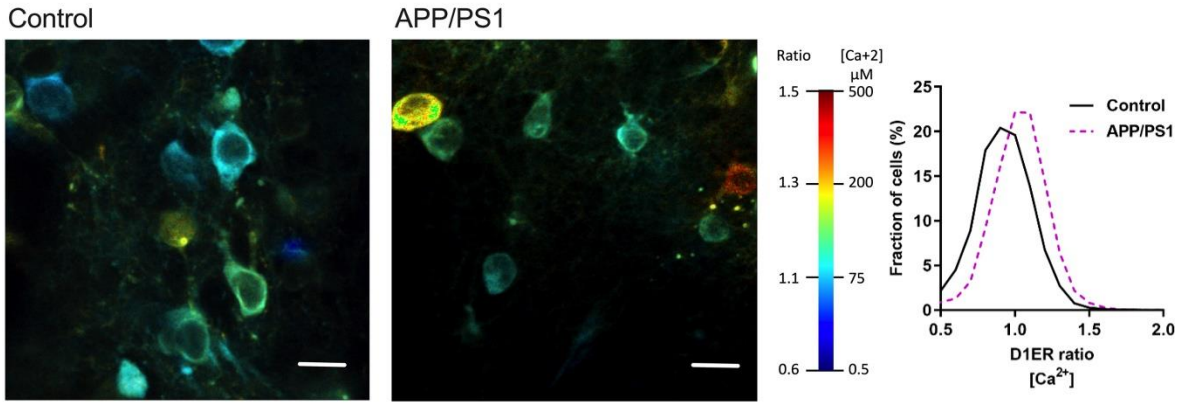
Topic: C.02. Alzheimer's Disease and Other Dementias

Support: BrightFocus Grant A2021001F

Title: Role of soluble amyloid β in increased ER calcium levels in a mouse model of Alzheimer's disease in vivo

Authors: ***M. ALGAMAL**[1], M. CALVO RODRIGUEZ[2], S. S. HOU[2], B. J. BACSKAI[2]; [1]Massachusetts Gen. Hosp., Boston, MA; [2]Neurol., Massachusetts Gen. Hospital-Harvard Med. Sch., Charlestown, MA

Abstract: The hallmarks of Alzheimer's disease (AD) include the deposition of Aβ plaques and neurofibrillary tangles, synapse loss, and neuronal cell death. Calcium (Ca^{2+}) dyshomeostasis is a neurodegenerative mechanism that impacts neuronal function and survival in AD. The endoplasmic reticulum (ER), mitochondria, and their contacts are crucial for regulating Ca^{2+} signaling. We have recently shown that mitochondrial Ca^{2+} overload plays a pivotal role in neuronal death in AD mouse models. However, the reason behind increased Ca^{2+} levels in neuronal mitochondria and the contribution of ER to mitochondrial Ca^{2+} dyshomeostasis remain unexplored in vivo. To address this, we employed viral delivery of a Ca^{2+} reporter targeted to ER (D1ER) and longitudinal in vivo multiphoton microscopy to assess ER Ca^{2+} levels in the cortex of an APP/PS1 mouse model of cerebral β-amyloidosis. We also test the effect of acute exposure to soluble Aβ on neuronal ER calcium using canula-mediated intracortical injections. Our results show increased ER Ca^{2+} levels in 4-10 months-old APP/PS1 mice compared to non-transgenic controls. Longitudinal imaging of ER calcium levels in APP/PS1 mice over several months indicated that the onset of plaque deposition does not impact ER Ca^{2+} levels, suggesting a role for soluble Aβ. In support of this hypothesis, acute exposure to a transgenic culture media containing soluble Aβ increased ER calcium levels in non-transgenic mice replicating the phenotype observed in APP/PS1 mice. In contrast, the exposure to non-transgenic control media did not result in significant ER calcium elevations. These findings highlight the pivotal role of soluble Aβ in mediating calcium dyshomeostasis and cellular toxicity in AD.



Disclosures: **M. Algamal:** None. **M. Calvo Rodriguez:** None. **S.S. Hou:** None. **B.J. Bacskai:** None.

Poster

PSTR399. APP and ABeta Mechanisms of Action and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR399.10/Web Only

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 1123564
1132524
1143848
1136241
2000660
20001572)
DP170100781

Title: Amyloid-β Precursor Protein facilitates tau spreading in Alzheimers Disease models

Authors: ***M. PRZYBYLA**[1], J. VAN EERSEL[1], M. CHU[1], G. CHAN[1], M. SABALE[1], A. VAN HUMMEL[1], L. HOU[1], J. VAN DER HOVEN[1], A. F. FEITEN[1], T. FATH[1], A. ITTNER[1], J. KRIL[2], G. SUTHERLAND[2], Y. D. KE[1], L. ITTNER[1];
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Abstract: Tau spreading within the brain correlates with disease progression and leads to distinct neuropathological changes in Alzheimer's disease (AD). In AD, abnormal cleavage of the amyloid-β (Aβ) precursor protein (APP) results in Aβ formation, which is a disease initiating step upstream of tau pathology. Here, we show that APP facilitates neuronal release and spreading of tau, highlighting a novel role of APP in disease progression towards dementia. We further demonstrate that this process is amplified by pathogenic APP mutations and increased

APP levels. In summary, our data shows that APP is an integral part of tau pathology spreading and further delineates a novel role for pathogenic APP mutations in AD.

Disclosures: **M. Przybyla:** None. **J. van Eersel:** None. **M. Chu:** None. **G. Chan:** None. **M. Sabale:** None. **A. Van Hummel:** None. **L. Hou:** None. **J. van der Hoven:** None. **A.F. Feiten:** None. **T. Fath:** None. **A. Ittner:** None. **J. Kril:** None. **G. Sutherland:** None. **Y.D. Ke:** None. **L. Ittner:** None.

Poster

PSTR400. Tau: Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR400.01/L1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA R01-AG060718

Title: Calcineurin inhibition counteracts hippocampal synaptic plasticity impairment induced by brain-derived tau oligomer

Authors: *P. SCADUTO, R. KAYED, G. TAGLIALATELA;
Neurol., UTMB, Galveston, TX

Abstract: Background: Cognitive decline in Alzheimer’s disease (AD) is closely linked with tau protein build-up, with the most toxic species being in form of oligomers. Given the polymorphic nature of tau oligomers, isolating and testing brain-derived tau oligomers (BDTO) directly from AD patients could provide new insights into tau toxicity. BDTO are potent inhibitors of long-term potentiation (LTP) in hippocampal brain slices, but the implicated mechanism is yet to be fully elucidated. We previously reported significantly reduced incidence of AD in aging humans chronically treated with an FDA-approved calcineurin inhibitor, FK506, used as immunosuppressant after solid organ transplant. Here, we found that synaptic function deterioration provoked by BDTO is rectified by calcineurin inhibition using FK506, via selective transcriptomic changes. **Methods:** Combination of electrophysiological and RNA-seq techniques were used. *Ex-vivo* slices from 8-12 weeks old C67BL/6J mice were treated with aCSF (Ctrl), BDTO (100nM) or BDTO+FK506. Field excitatory post-synaptic potentials (fEPSPs) recordings were performed by stimulating the Schaffer collateral pathway, and recording electrode was located at the junction of the alveus and cornu ammonius 1 (CA1). After electrophysiological recordings, the hippocampus was isolated from the brain slice, snap frozen and processed for RNA-seq. **Results:** FK506 treatment significantly ameliorated the BDTO-induced attenuation of fEPSP. One-way ANOVA followed by Tukey’s test, showed that BDTO caused reduction of fEPSP compared to Ctrl ($p=0.068$). fEPSP was re-established at physiological levels in the group BDTO+FK506 (vs Ctrl $p=0.603$; vs BDTO $p=0.0163$). A similar trend was observed when we analyzed the paired-pulse ratio. We performed a Gene Ontology enrichment analysis on the differentially expressed genes to hierarchically cluster the experimental groups. This revealed

two distinct clusters: 1) BDTO cluster, characterized by enhanced mRNAs associated with the regulation of protein catabolic processes and transcriptional regulation, and 2) CTRL and BDTO+FK506 cluster, characterized by upregulation of genes related with energy and metabolic processes. **Conclusion:** Our study provides evidence that FK506 has the potential to block the BDTO toxic effect on synaptic plasticity. These results suggest that FK506 may represent a promising therapeutic strategy for the treatment of AD. Further research is needed to elucidate the precise molecular mechanisms underlying FK506-mediated neuroprotection and to explore its potential in *in-vivo* models of AD.

Disclosures: **P. Scaduto:** None. **R. Kayed:** None. **G. Taglialatela:** None.

Poster

PSTR400. Tau: Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR400.02/L2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Busch Biomedical Grant, Rutgers University

Title: The role of WAVE1 in the regulation of tau phosphorylation

Authors: *C. BERDASCO[1,3], B. CARABELLI[3], Y. KIM[3,2];
[2]Brain Hlth. Inst., [1]Rutgers Univ., Piscataway, NJ; [3]Dept. of Neurosurg., Robert Wood Johnson Med. School, Rutgers Univ., Piscataway, NJ

Abstract: Alzheimer’s disease (AD) is the most common cause of dementia and a progressive neurodegenerative disorder. Wiskott-Aldrich syndrome protein (WASP) and WASP-family verprolin-homologous protein 1 (WAVE1) is a major regulator of Arp2/3 complex-mediated actin polymerization in the brain. Previously, we found a significant downregulation of WAVE1 gene (*WASF1*) expression in AD brains. We also observed the role of WAVE1 in the trafficking of amyloid precursor protein (APP)-containing vesicles and thereby Aβ production. Because experimentally reducing WAVE1 gene expression dramatically reduced Aβ levels and restored memory deficits in APP/PS1 mice harboring APPs and PS1ΔE9, WAVE1 reduction found in AD brains might be a cellular compensatory mechanism to control Aβ production. However, the role of WAVE1 in the regulation of tau hyperphosphorylation has not been studied. To examine the potential effect of WAVE1 reduction on tau hyperphosphorylation, we crossed P301S tau PS19 mice with WAVE1 KO mice. P301S tau mice carrying WAVE1 wild type (WT), heterozygous or homozygous alleles were sacrificed, and the hippocampi were lysed to perform Western blotting. We found that the protein level of WAVE1, but not Cdk5 or p35, is significantly reduced in the hippocampus of P301S tau mice harboring WAVE1 WT alleles compared to non-transgenic control mice. We also found that tau hyperphosphorylation at S202/T205 (detected by AT-8 antibody) and T231 (AT-180) sites were significantly reduced in P301S mice harboring either heterozygous or homozygous WAVE1 deletion compared to the levels in the P301S mice

expressing WT WAVE1. However, phosphorylation at S199 was not significantly altered by WAVE1 deletion, indicating that the effect of WAVE1 KO on tau hyperphosphorylation is site-specific. These results suggest that WAVE1-mediated actin polymerization is a potential therapeutic target for amyloid as well as tau pathology in AD.

Disclosures: C. Berdasco: None. **B. Carabelli:** None. **Y. Kim:** None.

Poster

PSTR400. Tau: Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR400.03/L3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Association AARF-21-720991

Title: Pathological Tau Mediates Dysfunction of the Nuclear Lamina in Alzheimer's Diseased Brains

Authors: *Z. WALTON[1], M. MONTALBANO[2], R. KAYED[3];
[1]John Sealy Sch. of Med., [2]Neurol., [3]Univ. of Texas Med. Br., Galveston, TX

Abstract: Background: Tau is a microtubule stabilizing protein belonging to the microtubule-associated protein (MAP) family. Tau aggregation is associated with neurodegenerative disorders, such as Alzheimer's Disease (AD). Recent findings from our group suggest a connection between oligomeric forms of tau and nuclear dysfunction, including alterations in the nuclear lamina (NL). The NL is a meshwork formed from various combinations of four intermediate filament lamin proteins (lamins A, B1, B2, and C) located along the nuclear perimeter. The NL provides a structure for attachment and organization of heterochromatin and is a vital connection between the nucleoskeleton and cytoskeleton. We hypothesized that Tau aggregates modify the NL structure, thus altering nuclear structure and morphology. **Methods:** We performed immunofluorescence (IF) imaging of lamin proteins in cortical human brain tissue samples from age-matched patients with AD and non-demented controls. Alterations in lamin expression and structure between control and AD nuclei were quantified and analyzed using Imaris Explore 10.0 software. To corroborate our observations, we performed western blot (WB) of subcellular fractionations from human brain tissue samples. To determine tau's impact on the NL, we treated inducible cells with tetracycline (Tet) to induce tau expression and further examined NL dysfunction via immunocytochemistry.**Results:** IF imaging revealed changes in nuclear morphology, structure, and lamin expression in AD brains. Large amounts of lamin proteins were mislocalized throughout the nucleoplasm and cytoplasm from the nuclear margin. WB confirmed abnormal localization of lamin proteins in the cytoplasmatic and cytoskeletal fractions of AD brains. Confocal Imaging revealed that AD brains contain less nuclei positive to lamin A but more nuclei positive to lamin C. We also observed invaginations and ruptures of the nuclear envelope. Additionally, lamin A and B1 components of the NL are significantly thicker

in AD nuclei compared to control nuclei. In our in vitro model, cells treated with Tet disclosed a thicker NL with a higher number of cells containing invaginations comparable to our observations in human samples.**Conclusions:** In AD brains, the NL is morphologically and structurally altered as characterized by defective lamin expression and cellular localization. Furthermore, our in vitro experiments highlight tau's role in NL dysfunction. Our data contributes to additional molecular insights of tau-induced toxicity in cell nuclei.

Disclosures: Z. Walton: None. **M. Montalbano:** None. **R. Kayed:** None.

Poster

PSTR400. Tau: Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR400.04/L4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Office of the Assistant Secretary of Defense for Health Affairs through the Peer Reviewed Alzheimer's Research Program Award W81XWH-20-1-0174
NIH NIA Michigan Alzheimer's Disease Research Center P30AG072031
Alzheimer's Association AARG 20-682085
NIH NINDS R01 NS082730
NIH NIA R01 AG067762

Title: Pathogenic tau mutants activate p38 MAPK and disrupt fast axonal transport in primary neurons

Authors: *B. COMBS[1], N. M. KANAAN[1,2,3];
[1]Translational Neurosci., Michigan State Univ., Grand Rapids, MI; [2]Neurosci. Program, Michigan State Univ., East Lansing, MI; [3]Hauenstein Neurosci. Ctr., Mercy Hlth. St. Mary's, Grand Rapids, MI

Abstract: Synaptic and axonal degeneration are early hallmarks of Alzheimer's disease and related disorders (ADRD) that are also characterized by pathological aggregation of the tau protein. Normal microtubule-based fast axonal transport (FAT) is disrupted in ADRD and many other neurodegenerative diseases and represents a potential mechanism of tau-induced degeneration. FAT involves the bidirectional movement of cargo carried out in the anterograde direction by kinesin motor proteins and in the retrograde direction by cytoplasmic dynein motor protein complex. Pathological forms of tau, including tau aggregates and tau monomers with disease-associated pseudophosphorylation or frontotemporal lobar degeneration (FTLD) tau mutations, disrupt FAT in squid axoplasm and/or mammalian primary neuron models. We previously found that pathological tau aberrantly activates a phosphorylation-based signaling pathway involving protein phosphatase 1 and glycogen synthase kinase 3β resulting in changes to kinesin function. However, a subset of these pathological tau forms also disrupt dynein-based

retrograde FAT but the mechanisms mediating tau's effects on transport in this direction are not yet known. Here, we sought to determine how expression of pathogenic FTLD-tau mutants (R5L, A152T, P301L, and R406W) affect FAT compared to wild-type (WT) tau expression. We co-transfected mixed-sex primary hippocampal neurons from tau knockout (TKO) mice with WT or one of the FTLD-tau mutants and a fluorescent-tagged cargo protein (synaptophysin or Rab6) then generated live cell movies of cargo transport on a confocal microscope at ~28 frames/second. Expressing pathogenic tau increases pause frequency of both cargo proteins compared to WT tau or GFP-expressing cells, indicating a disruption of normal FAT. To identify potential mechanisms of the effect on retrograde FAT we expressed WT or mutant tau in primary TKO hippocampal neurons using lentiviruses. We measured active and total levels of several kinases known to modulate retrograde FAT to determine how tau expression affected their activity. Multiple pathogenic tau mutants induced a significant increase in levels of active p38 (MAPK14) compared to WT tau or GFP-expressing neurons while the activities of other kinases were unaffected. The p38 kinase is linked to disease-associated FAT disruptions in amyotrophic lateral sclerosis (ALS) but a role in ADRD is not yet identified. Using p38 inhibitors we are determining how its activation is linked to pathogenic tau-induced disruption of retrograde FAT in our primary neuron model. This represents a potential mechanism for tau-induced disruptions to retrograde FAT.

Disclosures: B. Combs: None. **N.M. Kanaan:** None.

Poster

PSTR400. Tau: Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR400.05/L5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 AG076610-01

Title: Nicotinic acid modulates microglia phenotypes and tau phosphorylation in the PS19 tauopathy model

Authors: *I. CORONEL[1,2], R. DELVECCHIO[3,2], D. SONI[2], H. PATEL[2], S. PUNTAMBEKAR[2,4], P. MARTINEZ[2], A. OBLAK[2], C. LASAGNA-REEVES[2], G. LANDRETH[2], M. MOUTINHO[2];
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Abstract: Alzheimer's disease (AD) is the most common type of dementia, for which there is no effective treatment. AD is characterized by a robust immune response, the presence of extracellular amyloid-β (Aβ) plaques, and intraneuronal neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau. Dietary intake of nicotinic acid has been correlated with

decreased risk of AD and age-related cognitive decline. Our recent evidence showed that nicotinic acid activates the microglial receptor HCAR2 to induce a protective phenotype in an amyloid mouse model of AD, attenuating disease severity. We hypothesized that the beneficial effects of nicotinic acid extend to tau pathology as well. To investigate the role of HCAR2 in tau pathology we used the tauopathy mouse model PS19. We treated a cohort of 6-month-old female PS19 mice with the FDA-approved formulation of nicotinic acid (Niaspan®) or vehicle by daily oral *gavage* for 30 days, and evaluated microglia phenotype, synaptic markers, and tau species expression. Although 6-month-old PS19 mice exhibit a mild disease phenotype, we can already observe a significant increase in microgliosis within the hippocampus of PS19 compared to *wild-type* control mice (B6). Interestingly, oral Niaspan® treatment decreased hippocampal microglial Iba-1 staining and mRNA expression with respect to vehicle control mice. Niaspan® treatment also reduced expression of high-molecular-weight phosphorylated tau determined in soluble and insoluble fractions, but not the expression of synaptic proteins such as PSD95 and synaptophysin. Our results indicate that oral Niaspan® treatment reduces microglial activation and ameliorates the expression of phosphorylated tau aggregates in PS19 mice. Recent studies have shown that microglia contribute to the progression of tau pathology through the spreading of tau seeds. Nonetheless, the activation of HCAR2 did not elicit changes in tau seeding, thus, it is possible that activation of microglial HCAR2 with niacin, and the subsequent mitigation of microgliosis, lead to increased clearance of tau aggregation and phosphorylated tau. Pharmacological evaluation of the effect of Niaspan® on the PS19 tauopathy model at multiple ages will further our understanding of the potential use of this FDA-approved drug to be repurposed for AD at different stages of the disease.

Disclosures: I. Coronel: None. **R. DelVecchio:** None. **D. Soni:** None. **H. Patel:** None. **S. Puntambekar:** None. **P. Martinez:** None. **A. Oblak:** None. **C. Lasagna-Reeves:** None. **G. Landreth:** None. **M. Moutinho:** None.

Poster

PSTR400. Tau: Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR400.06/L6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: JP21dm0207073

Title: Screening for the regulators of tau secretion

Authors: *H. SATO[1], K. KASUGA[2], N. ISOO[3], T. HAYASHI[4], T. IKEUCHI[5], H. YUKIKO[6], T. TOMITA[7];
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Abstract: Alzheimer disease (AD) is the most common neurodegenerative disorder, which is characterized by two types of pathological amyloid deposition, amyloid β peptide (Aβ) and tau. Extracellular Aβ deposition has already appeared 15-20 years before the onset of cognitive decline in the brains of patients with AD and is known as an initiator for intracellular tau deposition and spreading throughout the brain. However, the detailed mechanism by which Aβ induces and spreads the tau pathology is still unknown. Several studies have reported that the intracellular tau is physiologically and pathologically released into the extracellular milieu, and that tau secretion mediates the spread of tau pathology. We, therefore, examined the relationship between tau secretion and Aβ deposition. As candidates, we selected several molecules whose expression is increased along with Aβ deposition in the human AD brain and investigated the effect of them on tau secretion from tau overexpressing mouse neuroblastoma Neuro2a (N2a) cells. In the result, we identified amyloid precursor protein, APP, and its soluble N-terminal fragment, sAPPβ, as a positive regulator. APP is accumulated in neuronal cells around Aβ plaques, and the production of sAPPβ is also increased by APP cleavage via BACE1. We revealed that overexpression of APP increased tau secretion from the tau stable overexpressing N2a cell line. On the other hand, knockout of APP dramatically decreased tau secretion, indicating APP-dependent tau secretion. In addition, tau secretion was also enhanced by overexpression of sAPPβ, but not by other cleavage fragments from APP. These results suggest a new tau secretion pathway induced by APP, especially sAPPβ. We will further investigate the detailed molecular mechanism and its relationship with AD pathogenesis. In addition, we will elucidate the effect of other candidates to identify other regulators on tau secretion.

Disclosures: H. Sato: None. **K. Kasuga:** None. **N. Isoo:** None. **T. Hayashi:** None. **T. Ikeuchi:** None. **H. Yukiko:** None. **T. Tomita:** None.

Poster

PSTR400. Tau: Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR400.07/L7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA Grant AG058851
Clear Thoughts Foundation grant

Title: G protein coupled receptor kinase 2 modulates tau pathogenesis in human neurons

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Abstract: Aggregation of the hyperphosphorylated tau protein in neurofibrillary tangles (NFTs) is a pathological hallmark of Alzheimer's Disease (AD) brains. Several kinases contribute to the pathological phosphorylation of tau; however, kinase targeted therapies for AD have failed in clinical trials due to low efficacy and severe side effects. G protein coupled receptor (GPCR)

kinases (GRKs) have been implicated in neurological disorders, such as Parkinson's Disease, via phosphorylation of non-GPCR substrates, e.g., a-synuclein. Previously, we showed that GRK2 is abundantly expressed in neurons, positively correlated with soluble tau levels, and associated with NFTs in the human AD brain. Therefore, we hypothesized that GRK2 can directly modify tau phosphorylation and aggregation. To test this hypothesis, we first showed that genetic deletion of *Grk2* induces global changes in the tau phosphoproteomic profile, while GRK2 overexpression increases tau phosphorylation (pTau) at a disease-relevant site (PHF1). Furthermore, we used an inducible optogenetic system (optoTAU), which allows for control of the expression and temporal aggregation of tau, to show that optoTAU aggregation, and specifically soluble tau, is reduced in *Grk2*-deficient cells. We show that GRK2 directly interacts with tau and pathogenic species *in vitro*, which is in accordance with our previous human brain observations. Moreover, we find that GRK2 modulates pTau in a kinase activity-independent manner, via other major tau kinases (e.g., ERK, GSK3B). Specifically, ERK-mediated phosphorylation of GRK2 species at residue 670 is protective against pTau formation and is this found downregulated in AD brains. Interestingly, pharmacological inhibition of GRK2 in human directly converted induced neurons (iNs) increases both ERK activation and GRK2 s670 levels, while decreasing pTau and reactive oxygen stress in AD-derived iNs. Lastly, *Grk2* genetic deletion enhances tau ubiquitination and interaction with HSP90, which further increases tau degradation, protecting the cells from aggresome formation upon proteasome inhibition. Collectively, these studies causally implicate GRK2 as a multifactorial modulator of tau pathology through changes in phosphorylation, aggregation, and degradation of tau and support further investigation of therapeutic interventions against GRKs in AD.

Disclosures: T. Rafael Guimaraes: None. **M. MacDonald:** None. **A. Thathiah:** None.

Poster

PSTR400. Tau: Cellular Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR400.08/L8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: JSPS KAKENHI Grant Number 22K07503

Title: Characterization of tau-containing exosomes derived from Neuro2a cells

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Abstract: The stereotypical propagation of tau protein aggregates in the brain drives the progression of Alzheimer's disease. This process is involved in the cell-to-cell transmission of

pathological tau proteins between anatomically connected regions. The majority of tau proteins released from neurons into the extracellular space are membrane-free form. Recently, exosomes, a class of extracellular vesicles, were shown to contribute to the delivery of pathological tau proteins in the brain. However, reassessment of exosomal composition has revealed exosomes are heterogeneous. To elucidate the roles of exosome-dependent tau protein propagation in the disease progression, here, we developed a method for the isolation of tau-containing exosomes secreted from mouse neuroblastoma Neuro2a cell line stably expressing full-length tau with the P301S mutation. We isolated exosomes from the culture medium of these cells using differential centrifugation followed by 6%-30% iodixanol density gradient fractionation. We then analyzed distributions of tau proteins and exosomal markers in each fraction. Iodixanol density gradient fractionation separated exosomal fractions into two subpopulations. We found that a novel subpopulation of exosomes contained a substantial amount of tau proteins together with the calcium-regulated plasma membrane-binding protein Annexin A2. Annexin A2 was known to be a potential interaction partner of tau proteins in neurons. The tau-containing exosomes were distributed in distinct fractions from those with exosomal tetraspanin markers such as CD9, CD63, and CD81. Thereafter, the membranes of the isolated tau-containing exosomes were labeled by DiI, a lipophilic carbocyanine fluorescent dye. Time-lapse live cell imaging demonstrated that a large amount of the DiI-labeled, tau-containing exosomes were taken up by Neuro2a cells within twelve hours after addition to the cell culture medium. In conclusion, we established a high-resolution density gradient fractionation method to identify a novel subpopulation of exosomes containing tau proteins. These tau-containing exosomes are distinct from those expressing classical exosomal markers. Moreover, they are internalized to neuronal cells, suggesting that they might contribute to the transcellular transmission of tau proteins.

Disclosures: **N. Isoo:** None. **K. Kawata:** None. **H. Takagi:** None. **N. Iso-o:** None. **Y. Hori:** None. **T. Tomita:** None. **T. Hayashi:** None.

Poster

PSTR400. Tau: Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR400.09/M1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01 AG075092
R56 AG066782
K01 AG056673
AARF-17-505009
BrightFocus foundation A2021027S
Chronic Brain Injury Pilot Award from The Ohio State Universit

Title: Cholesterol transporter GRAMD1B expression correlates with lipid droplet formation and tau pathology.

Authors: *T. KIM[1,2], D. ACOSTA[1], N. SWEENEY[1], S. CHEN[1,2], G. E. SERRANO[3], E. BELL[4], L. S. HONIG[5], J. G. VONSATTEL[5], D. SCHARRE[4], T. G. BEACH[3], H. FU[1]; [1]Neurosci., [2]Biomed. Sci. Grad. Program, Ohio State Univ., Columbus, OH; [3]Banner Sun Hlth. Res. Inst., Sun City, AZ; [4]Dept. of Neurol., Ctr. for Cognitive and Memory Disorders, Columbus, OH; [5]Dept. of Neurol., Columbia Univ. Irving Med. Ctr., New York, NY

Abstract: Tau protein hyperphosphorylation and aggregation are pathological hallmarks of tauopathies such as Alzheimer's disease (AD) and Frontotemporal lobar degeneration (FTLD). Lipid dyshomeostasis is thought to play an important role in early AD and other tauopathies. Previous studies suggest that increased cholesterol levels were associated with tau pathology and neuronal dysfunction in AD. Recently, we identified a cholesterol transporter, GRAM domain containing 1B (GRAMD1B), was significantly increased in neural organoids derived from induced pluripotent stem cells (iPSCs) from an FTLD patient with *MAPT* R406W mutation compared to isogenic control organoids. GRAMD1B protein mediates the transport of cholesterol from the plasma membrane to the endoplasmic reticulum, contributing to cholesterol homeostasis. However, little is known about GRAMD1B levels in the brain of tauopathies, or its role in disease progression. We hypothesized that GRAMD1B may contribute to lipid droplet formation and tau pathology in the early stages of AD and other tauopathies. We performed immunofluorescence staining on the entorhinal cortex of human post-mortem brain tissues and tau mouse models to evaluate the immunoreactivity of GRAMD1B and its relationship with lipid droplets and tau pathology. Lipid droplet formation was assessed using Oil red O or LipidSpot 610 stain which measures neutral lipid stores. Interestingly, we found that lipid droplets were colocalized with increased hyperphosphorylated-tau (PHF1+, pS396/S404 tau) and GRAMD1B in early AD and FTLD, and in tau mouse models. We also found that PHF1-positive cells had a lower level of GRAMD1B than neighboring PHF1-negative cells, but GRAMD1B expression was significantly increased in PHF1-negative cells in human AD and FTLD as well as tau mouse models compared to control samples without tau pathology. In conclusion, our data suggest that there is a positive correlation between neuronal GRAMD1B, lipid droplets, and tau pathology in the early stages of tauopathies. We will further investigate the molecular mechanisms underlying GRAMD1B expression on lipid dyshomeostasis, tau pathology, and neurodegeneration.

Disclosures: T. Kim: None. **D. Acosta:** None. **N. Sweeney:** None. **S. Chen:** None. **G.E. Serrano:** None. **E. Bell:** None. **L.S. Honig:** None. **J.G. Vonsattel:** None. **D. Scharre:** None. **T.G. Beach:** None. **H. Fu:** None.

Poster

PSTR400. Tau: Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR400.10/M2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Association AARGD-16-441534
Florida Department of Health -Ed and Ethel Moore Alzheimer's disease

8AZ30
NIH/NIA R21AG055996
Thome Memorial Foundation

Title: Suppression of nutrient sensing receptor GPRC6a modifies lysosomal function and improves tau clearance in PS19 tauopathy mice

Authors: *K. CAMPBELL, H. LIANG, C. MA, J. HUNT, M. KILLION, D. LEE;
Univ. of Kentucky, Lexington, KY

Abstract: Arginine increases during the course of neurodegenerative diseases, such as Alzheimer's disease (AD) in both humans and animal models of tauopathies. The kinase complex mechanistic target of rapamycin (mTORC1) senses nutrients in eukaryotes. mTORC1 is recruited to the lysosomal surface to increase protein synthesis and cell growth during nutrient abundance. When nutrients are scarce mTORC1 fails to translocate to the lysosome and autophagy is uninhibited. During neurodegenerative disease mTORC1 signaling can become uncoupled and precipitate proteinopathies. Arginine is a potent activator of mTORC1 and signals through various protein sensors and lysosomal transporters. GPRC6a is an amino acid sensor that binds arginine with high affinity, signals to mTORC1, and increases in AD brains and tauopathy models. We hypothesize that tau pathology promotes nutrient sensing dysfunction leading to hyper mTORC1 activation. We posit that GPRC6a suppression increases activates autophagy, which rebalances proteostasis and hallmarks of tau pathology. Tau transgenic mice (*PS19*) and non-transgenic littermates were bred to mice with GPRC6a hemizygous deletion to generate four genotypes: (*nTg, GPRC6a* $^{+/-}$*, PS19, PS19/GPRC6a* $^{+/-}$). Mice were aged to 7-9 months before brains were harvested for western blot and bulk RNA-seq. Tau biochemistry was measured in detergent soluble and urea soluble fractions from the anterior and posterior cortex. Markers for mTOR activation, lysosomal function, and autophagy were also measured. Hippocampal tissue was used for bulk RNA seq Nanostring for transcriptome pathway analysis. Hemizygous deletion of GPRC6a (*PS19/GPRC6a* $^{+/-}$) decreased total tau (HT7) and various forms of phospho-tau (Ser199/202, PHF1, AT8) in soluble and insoluble fractions compared to *PS19* mice. GPRC6a suppression in PS19 mice significantly decreased phospho mTOR2448/ total mTOR ratio suggesting decreased mTORC1 activation compared to PS19 mice. Autophagy and autophagosome formation significantly increased in PS19/GPRC6a $^{+/-}$ mice, as shown by an increase in active ULK1. Both LC3-I and LC3-II increased in PS19/GPRC6a$^{+/-}$ mice compared to PS19 mice suggesting increased lysosomal biogenesis. PS19 mice showed increased transcripts associated with autophagy, neurogenesis, and proteotoxic stress compared to *nTg* littermates, and hemizygous deletion of GPRC6a (*PS19/GPRC6a*$^{+/-}$*)* normalized these signatures to that of control levels. These data indicate that GPRC6a expression regulates proteostasis of tau and could serve as a therapeutic target in tauopathies. Additionally, our data suggests that GPRC6a impacts lysosomal function and mTORC1 activation state.

Disclosures: K. Campbell: None. **H. Liang:** None. **C. Ma:** None. **J. Hunt:** None. **M. Killion:** None. **D. Lee:** None.

Poster

PSTR400. Tau: Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR400.11/M3

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Investigation of the role of complement receptors in extracellular tau clearance

Authors: *C. YOO[1,2], E. BOK[1], J. KIM[1];
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Abstract: Abnormal accumulation of misfolded tau aggregates is a key pathological hallmark of various tauopathies including Alzheimer's disease. Pathological tau spreads along anatomically connected areas in the brain through intercellular transmission and templated misfolding. The spatiotemporal spreading of tau pathology is closely correlated with cognitive decline and behavioral deficits in patients of tauopathies. Therefore, identifying the molecular mechanisms underlying the release and uptake of tau is critical to understand the pathological mechanisms of many tauopathies. The various innate immune receptors have been reported to play a role in recognition, internalization, and clearance of various toxic proteins. In particular, CR3 and CR4, two major phagocytic receptors in microglia, are known to mediate phagocytosis of fibrillar Aβ and α-synuclein, respectively. In this study, we investigated the role of CR3 and CR4 in regulating clearance of extracellular tau. In this study, we identified that CR4 selectively binds to tau fibrils but not to tau monomers using dot-blot- and immunoprecipitation assay. We further demonstrated that silencing of CR4 dramatically reduce the uptake and clearance of tau fibrils. However, silencing of CR4 does not affect the degradation of extracellular tau in the culture media. Moreover, conditioned media form CR4-silenced BV2 culture treated with tau fibrils are more potent in inducting tau aggregation in Tau RD cells compared to the controls. Taken together, our data suggest that CR4 is a novel receptor for tau fibril clearance and may play a critical role in tau spreading.

Disclosures: C. Yoo: None. **E. Bok:** None. **J. Kim:** None.

Poster

PSTR400. Tau: Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR400.12/M4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA R01AG067762
NIH/NIA R01AG044372
NIH/NIA R01NS082730

Title: Tau interactome identifies interactions associated with various cellular compartments and reveals interactions with specific tau domains

Authors: *A. ATWA[1,2], B. COMBS[1], M. M. ALHADIDY[1,2], J. LAMP[1,3], I. E. VEGA[1,2,3], N. M. KANAAN[1,2,4];
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Abstract: Tau protein aggregation is central to neuropathological alterations in Alzheimer's Disease (AD) and other neurodegenerative diseases collectively known as tauopathies. Tau was initially reported as a microtubule-associated protein involved in regulating microtubule dynamics. Multiple lines of evidence suggest that Tau plays diverse microtubule-independent functional roles regulated, in part, by direct and/or transient protein interactions. Deciphering the Tau interactome is a critical step toward better understating the physiological and pathological Tau-mediated cellular processes. In this work, we sought to map potential members of the Tau interactome using the BioID2 approach that allows for *in situ* protein labeling in living neurons. Biotin-labeled proteins are identified by biotin-targeted pulldown and mass spectrometry. We generated lentiviruses expressing fusion proteins between full-length human Tau (2N4R isoform) with BioID2 on either the N-terminus (BioID2-Tau) or C-terminus (Tau-BioID2). Tau can be subdivided into three domains: the N-terminus domain (Nterm), the microtubule binding region (MTBR), and the C-terminus domain (Cterm). To further dissect the Tau interactome, we created fusion proteins between Tau domains and BioID2 (Nterm-BioID2, MTBR-BioID2 and Cterm-BioID2). A control lentiviral construct was created to express only the BioID2 protein. Embryonic day 18 Tau knockout (TKO) primary cortical neurons were transduced on the 4th day in vitro (DIV4), and lysates were collected on DIV12 for biotin-targeted pulldown and mass spectrometry identification (n=3 biological replicates). Utilizing this approach, we identified 391 proteins as candidates of the Tau interactome among which 189 proteins interacted with the Nterm, 196 proteins with the MTBR, and 237 proteins with the Cterm. Protein interactions were further validated by either co-immunoprecipitation from adult human Tau knockin (hTKI) mouse cortical tissue (n=3) or proximity ligation assay in hTKI cortical neurons (n=3). Gene Ontology enrichment analysis mapped protein interactors associated with the somatodendritic compartment, mitochondria, cytoskeleton, synapses, ribosomes, ubiquitin-proteasome system, and the ribonucleoprotein complex. While KEGG pathways identified proteins associated with neurodegenerative diseases, including AD, Parkinson's disease, and Huntington's disease. Thus, this approach can identify potential members of the Tau interactome via *in situ* labeling. This work helps expand the growing list of Tau's potential functional roles and may advance our understanding of its biological and neurodegenerative functions.

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Poster

PSTR400. Tau: Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR400.13

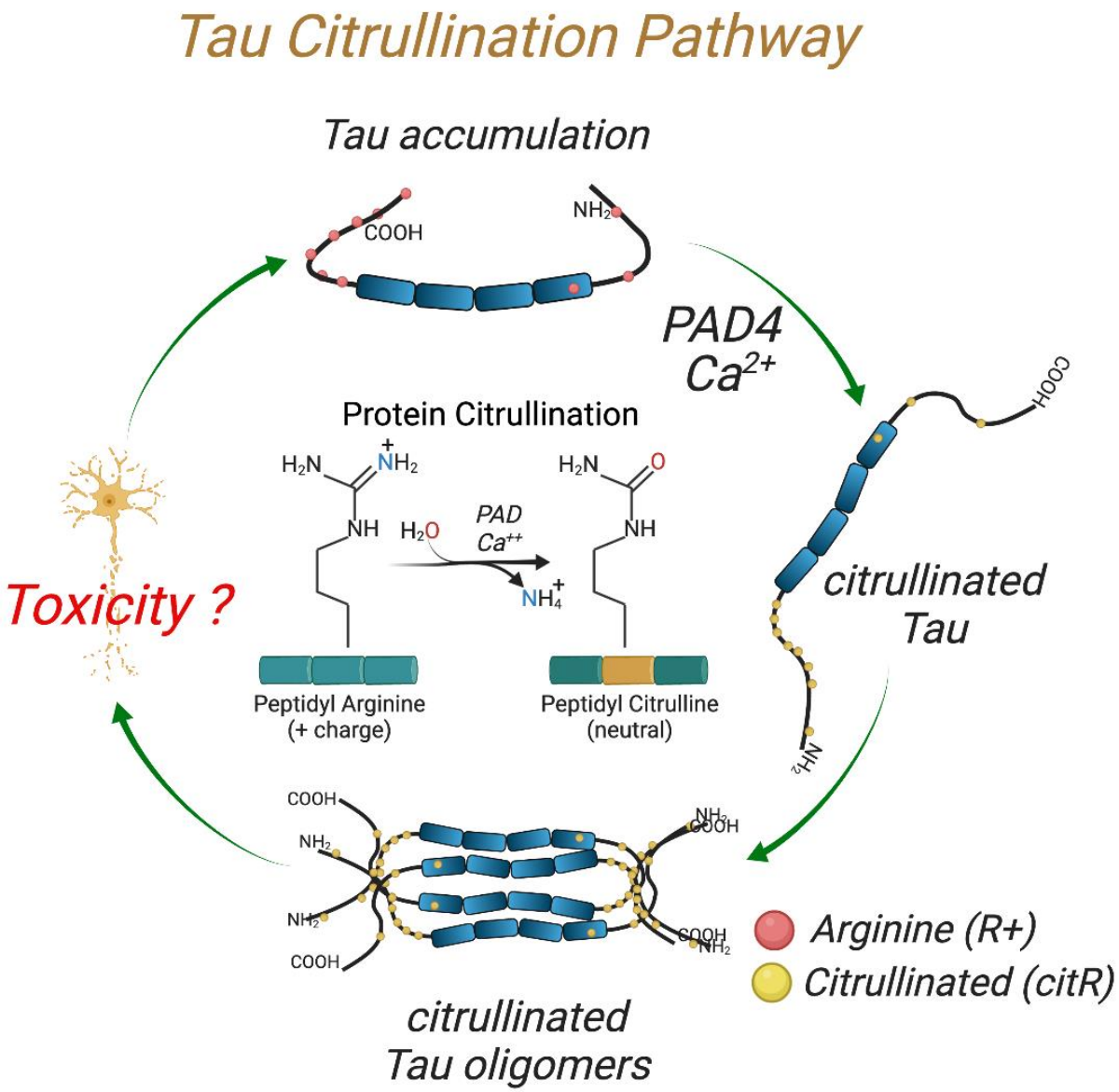
Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 1RF1AG072728-01
BrightFocus Foundation

Title: Tau Citrullination Elicits Confirmer Diversity in Various Tauopathies.

Authors: H. LIANG[1], M. SERRANO[1], M. NELSON[1], D. CRASTA[1], J. B. HUNT, Jr.[1], C. SAUNDERS[1], K. CAMPBELL[1], P. NELSON[2], M.-L. SELENICA[3], ***D. LEE**[1];
[1]Sanders-Brown Ctr. on Aging/ Neurosci., [2]Sanders-Brown Ctr. on Aging, [3]Sanders-Brown Ctr. on Aging/ Mol. Cell. Biochem., Univ. of Kentucky, Lexington, KY

Abstract: Tau deposition causes different clinical phenotypes and pathological outcomes depending on the tau strain and brain region. The pathological sequalae can stem from posttranslational modifications (PTMs) that alter structure, function, and regional susceptibility. To that end, we discovered a new modification on tau named citrullination (citR) which changes the amino acid arginine to a citrulline via the enzyme peptidyl arginine deiminases (PADs). PAD4 is primarily restricted to neurons and activated by calcium, but also harbors SNPs that increase AD (*rs16824888*) and ALS (*rs2240335*) risk. Structurally, this conversion permanently alters the target by producing loss of positive charges, changes in protein stability, and inter/ intramolecular interactions. We uncovered citR tau at 14 arginine sites via mass spec using recombinant protein and created 11-highly citR tau antibodies to the various domains. We found that citR impacts tau phosphorylation, degradation, and fibrillization. Interestingly, tau citR decreased fibrillization at the expense of accumulating oligomers, suggesting that citR promotes soluble tau aggregates. We also found increased citR tau in three different models of tauopathy mice. PAD4 overexpression increased citR tau in cellular and animal models of tau deposition. Importantly, citR tau increased in AD and tauopathies including PSP, CTE, Pick's disease, ARTAG, PART, FTLD, AGD, but not in TBI, dementia with DLB compared to control brains. Despite PAD4/ 2 being the only PADs recognized in the CNS, various tauopathies showed selective epitope specificity and regional expression of citR tau. This suggests that PAD4 may elicit 3R- versus 4R- bias for disease-related tau confirmers and regional vulnerability to coordinated calcium activation. We aim to discriminate differences in citR tau signatures that impact clinical aggressiveness of tauopathies. Collectively, we identified a common yet unknown PTM for human tauopathies that has broad implications, new biology, and provides novel therapeutic targets for the tauopathy field.



Disclosures: **H. Liang:** None. **M. Serrano:** None. **M. Nelson:** None. **D. Crasta:** None. **J.B. Hunt:** None. **C. Saunders:** None. **K. Campbell:** None. **P. Nelson:** None. **M. Selenica:** None. **D. Lee:** None.

Poster

PSTR400. Tau: Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR400.14/M5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG072458

Title: Sucrose gradient-derived Alzheimer's disease tau oligomers impair synaptic functioning and plasticity

Authors: *N. MORENO, L. FUNG, N. BHATT, A. HAQUE, C. JEREZ, A. LIMON, R. KAYED;
Neurol., Univ. of Texas Med. Br., Galveston, TX

Abstract: Alzheimer's disease (AD) is histopathologically characterized by amyloid β (Aβ) and tau accumulation as amyloid plaques and neurofibrillary tangles, respectively. Tau oligomers are thought to be the major neurotoxic species in AD, but there is currently a gap in knowledge concerning standardized methods for isolating and characterizing tau oligomers. Here, we used sucrose density gradient ultracentrifugation to isolate BDTOs from Alzheimer's disease brain tissue and performed electrophysiological recordings on oligomers from fractions 4 and 5 of the sucrose gradient to investigate the effect of these BDTOs on neuronal transmission and synaptic plasticity Our results demonstrate that fraction 4 and fraction 5 BDTOs substantially impair neuronal transmission and synaptic plasticity. Interestingly, the fraction 4 and fraction 5 sucrose density oligomers, as well as recombinant tau oligomers, appear to have dose-dependent mechanisms underlying the observed disruption of basal neuronal transmission. Ultimately, our results demonstrate that sucrose gradient ultracentrifugation can be used to isolate toxic tau oligomers from human tissue, and, while there is some variability in oligomer size and behavior between fractions, synaptic toxicity is preserved.

Disclosures: N. Moreno: None. **L. Fung:** None. **N. Bhatt:** None. **A. Haque:** None. **C. Jerez:** None. **A. Limon:** None. **R. Kayed:** None.

Poster

PSTR400. Tau: Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR400.15/M7

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Human tauopathy strains defined by phosphorylation in repeat domains of tau

Authors: *E. D. SMITH[1,2], Q. VO[1,2], B. I. GIASSON[2,1,3,4], D. R. BORCHELT[1,2,4], S. PROKOP[5,2,3], P. CHAKRABARTY[2,1,4];
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Abstract: Recent proteomic studies have identified a set of post-translational modifications (PTM) on tau protein located in the Proline-Rich Region (PRR), Microtubule Binding Region (MTBR), and C-terminal region that are found specifically in fibrillized tau isolated from Alzheimer's disease (AD) patients. In this study, our overarching aim was to determine whether

selective phosphorylation on these PTM sites will impact tau seed-induced misfolding and aggregation of tau protein. Using host tau protein that is modified on these select phosphorylation residues, we show that presence of phosphorylation mimicking substitutions in the repeat domains (S262/T263/S289/S305) substantially reduce seeding efficiency of insoluble AD-tau seeds derived from Alzheimer's disease (AD). The resultant detergent-insoluble seeded tau shows deficient phosphorylation on AT8, AT270, AT100, AT180 and PHF1 epitopes compared to parent tau carrying no phospho-mutations indicating cooperativity between phosphorylation sites located across the different structural domains of tau during the seeding process. On the other hand, PSP-tau (derived from Progressive supranuclear palsy donors) seeding efficiency is not modulated by these phospho-mimicking mutations in the repeat domains, suggesting strain-specific differences between AD-tau and PSP-tau seeds. Phospho-deficient substitutions in these selective sites do not alter seeding activity of either AD-tau or PSP-tau in our cellular assay. Finally, we identify S305 as a major determinant of the seeding efficiency of AD-tau, which suggests that modification(s) at this site could serve as a protective mechanism against templated seeding in the context of AD-tau seeds. Collectively, our data confirms the functional role of specific tau phosphorylation epitopes in determining the prion-like templated seeding properties of tau in AD and PSP.

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Poster

PSTR400. Tau: Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR400.16/M8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant RF1AG059789-01

Title: Tau pathology kinetics and features depend on the nature of the tau seed

Authors: *A. O. MATE DE GERANDO, A. KHASNAVIS, R. PERBET, L. WELIKOVITCH, B. T. HYMAN;
Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Aim: Alzheimer's disease (AD) progression has been associated with the propagation of both oligomeric and fibrillar tau species, yet their respective contribution to the pathology's dynamics is poorly understood. We now directly compare *in vivo* the spatiotemporal bioactivity of PBS soluble tau oligomers (hereby referred to as high molecular weight tau or HMW) and sarkosyl insoluble tau fibrils (SARK) derived from the same AD brain. **Methods:** HMW and SARK tau were both extracted from the parietal cortex of a Braak V AD subject. Their bioactivity was monitored in hTau mice expressing non-mutant human tau on a mouse tau null background. Mice were bilaterally injected into the dorsal hippocampus and sacrificed 1 day, 3

days, 1 week, 2.5 weeks, 1 month or 3 months after injection. The hippocampus and projection sites, e.g. entorhinal cortex, were analyzed by histology as well as by biochemistry and FRET-based *in vitro* seeding assays and data corrected to PBS-injected littermates. **Results**: *In vitro* seeding data on hippocampal homogenates show the progressive disappearance of seed-competent tau by 1 week and its return starting from 1 month after injection in parallel to the appearance of AT8-positive pyramidal neurons in both injection groups. In the entorhinal cortex, only HMW tau-injected animals start presenting seed-competency together with AT8-positive cells as early as 1 month after injection suggesting faster spreading of oligomeric than fibrillar tau species. Interestingly, newly formed tau seeds seem to replicate initial seeds as soluble seed-competent tau species are present in both HMW and SARK-injected mice but only SARK-injection triggers seed-competent sarkosyl-insoluble tau formation. **Conclusions:** Our data confirm that both HMW and SARK tau fractions contain analogous conformations to promote seeding yet reveal differential kinetics implying different contributions of these tau species to AD pathology. In addition, we show, *in vivo*, the replication of injected tau seeds characteristics, in favor of the prion-like hypothesis.

Disclosures: A.O. Mate de Gerando: None. **A. Khasnavis:** None. **R. Perbet:** None. **L. Welikovitch:** None. **B.T. Hyman:** 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.; Abbvie. F. Consulting Fees (e.g., advisory boards); Dewpoint therapeutics.

Poster

PSTR400. Tau: Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR400.17/M9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01AG062509
R21AG065606
R01AG041274
RF1070761

Title: Transmission of Alzheimer's Disease-Associated Microbiota Dysbiosis and its Impact on Cognitive Function: Evidence from Mice and Patients

Authors: *Y. ZHANG[1], Y. SHEN[1], N. LIUFU[3], L. LIU[3], W. LI[1], Z. SHI[5], H. ZHENG[5], X. MEI[5], C.-Y. CHEN[5], Z. JIANG[5], S. ABTAHI[6], Y. DONG[1], F. LIANG[1], Y. SHI[5], L. CHENG[4], G. YANG[7], J. KANG[2], J. WILKINSON[6], Z. XIE[1];
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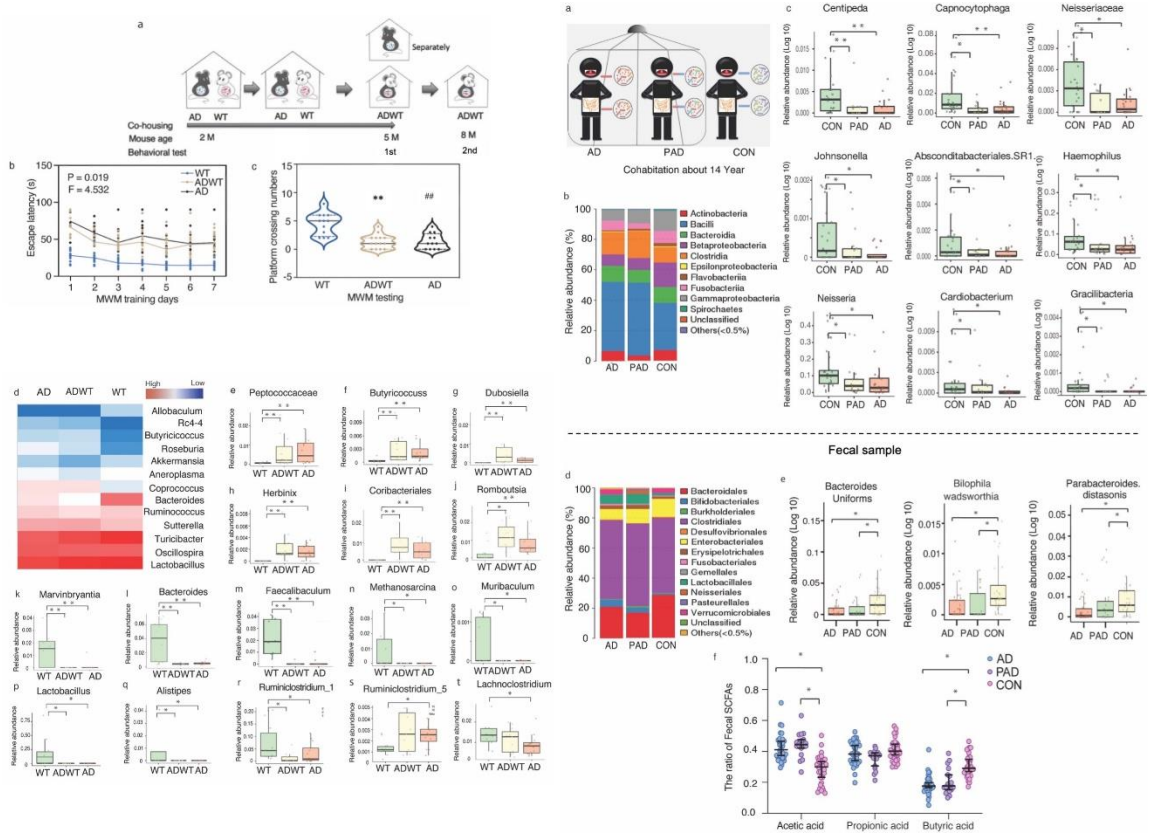
Abstract: *Background:* Spouses of Alzheimer's disease (AD) patients are at a higher risk of developing incidental dementia. However, the causes and underlying mechanism of this clinical observation remain largely unknown. One possible explanation is linked to microbiota dysbiosis, a condition that has been associated with AD. However, it remains unclear whether gut microbiota dysbiosis can be transmitted from AD individuals to non-AD individuals and contribute to the development of AD pathogenesis and cognitive impairment. *Method:* We, therefore, set out to perform both animal studies and clinical investigation by co-housing wild-type mice and AD transgenic mice, analyzing microbiota via 16S rRNA gene sequencing, measuring short chain fatty acid amounts, and employing behavioral test, mass spectrometry, site-mutations and other methods. *Result:* The present study revealed that co-housing between wild-type mice and AD transgenic mice or administrating feces of AD transgenic mice to wild-type mice resulted in AD-associated gut microbiota dysbiosis, Tau phosphorylation, and cognitive impairment in the wild-type mice. Gavage with Lactobacillus and Bifidobacterium restored these changes in the wild-type mice. The oral and gut microbiota of AD patient partners resembled that of AD patients but differed from healthy controls, indicating the transmission of microbiota. The underlying mechanism of these findings includes that the butyric acid-mediated acetylation of GSK3β at lysine 15 regulated its phosphorylation at serine 9, consequently impacting Tau phosphorylation. *Conclusion:* Pending confirmative studies, these results provide insight into a potential link between the transmission of AD-associated microbiota dysbiosis and development of cognitive impairment, which underscore the need for further research in this area.



Disclosures: **Y. Zhang:** None. **Y. Shen:** None. **N. Liufu:** None. **L. Liu:** None. **W. Li:** None. **Z. Shi:** None. **H. Zheng:** None. **X. Mei:** None. **C. Chen:** None. **Z. Jiang:** None. **S. Abtahi:** None. **Y. Dong:** None. **F. Liang:** None. **Y. Shi:** None. **L. Cheng:** None. **G. Yang:** None. **J. Kang:** None. **J. Wilkinson:** None. **Z. Xie:** None.

Poster

PSTR400. Tau: Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR400.18/M10

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Single-molecule protein analysis to explore Tau proteoform heterogeneity

Authors: S. GUHA[1], S. TAN[1], J. JOLY[1], R. WANG[1], B. NORTMAN[1], T. GILLIES[1], V. BUDAMAGUNTA[1], J. ROBINSON[1], T. RINKER[1], J. SHERMAN[1], A. ELLAHI[1], T. WEIDERHOLD[2], D. S. KIRKPATRICK[3], J. LIPKA[4], T. WENDORFF[4], N. J. PANDYA[4], A. ROHOU[4], ***G. KAPP**[1], P. MALLICK[1];
[1]Nautilus Biotech., San Carlos, CA; [2]Cell Signaling Technol., Danvers, MA; [3]Interline Therapeut., South San Francisco, CA; [4]Genentech, South San Francisco, CA

Abstract: Tau protein is modified by both mRNA transcript splicing and post-translational modification with a range of modifications (phosphorylation, acetylation, ubiquitination, and many others). The populations of specific Tau splicing variants and specific post-translational modifications have been linked to Alzheimer's disease and disease progression. However, the diversity of different Tau isoforms (proteoforms) that exists in a human sample, resulting from splicing and modification, is underexplored due to the difficulty of linking multiple modifications to a single protein molecule. It is possible to address this difficulty with analytical techniques that operate at the single-molecule level, without digesting the protein into peptide fragments. We describe a new analytical platform that leverages a set of splicing-specific and modification-specific affinity reagents to probe Tau proteins at the single-molecule level. With this single-molecule resolution, the landscape of Tau proteoforms can be investigated and specific patterns of modifications can be identified in Alzheimer's samples. The same single-molecule platform can also be used to analyze the entire set of proteins (the full proteome) in a sample using Protein Identification by Short-epitope Mapping (PrISM), which leverages proprietary multi-affinity probes designed to recognize short epitopes and a machine learning algorithm that decodes binding of hundreds of multi-affinity probes into protein quantifications. We used this new single-molecule analysis platform that combines Tau antibodies with novel instrumentation, single-molecule biochemistry, and machine learning bioinformatics to enable deep proteoform analysis. We first demonstrated that a small set of antibodies can define the combinations of modifications that are seen in both normal, healthy samples and those linked with Alzheimer's disease. Initial validation was performed with defined mixtures of recombinant proteins. Then the platform was used to analyze the Tau proteoform landscape in Tau protein enriched from induced pluripotent stem cell (iPSC)-derived neurons. Finally, the diverse

proteoform landscape of Tau in human samples, healthy and diseased, was analyzed. We focused this initial work on splice isoforms and phosphorylation, but there are many additional modifications of the Tau protein. As additional modification-specific antibodies are added to increase the analytical set, the number of identifiable proteoforms grows exponentially, enabling greater resolution into single-molecule proteoforms, any one of which may be a more precise biomarker or better therapeutic target.

Disclosures: **S. Guha:** A. Employment/Salary (full or part-time):; Nautilus Biotechnology. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nautilus Biotechnology. **S. Tan:** A. Employment/Salary (full or part-time):; Nautilus Biotechnology. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nautilus Biotechnology. **J. Joly:** A. Employment/Salary (full or part-time):; Nautilus Biotechnology. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nautilus Biotechnology. **R. Wang:** A. Employment/Salary (full or part-time):; Nautilus Biotechnology. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nautilus Biotechnology. **B. Nortman:** A. Employment/Salary (full or part-time):; Nautilus Biotechnology. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nautilus Biotechnology. **T. Gillies:** A. Employment/Salary (full or part-time):; Nautilus Biotechnology. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nautilus Biotechnology. **V. Budamagunta:** A. Employment/Salary (full or part-time):; Nautilus Biotechnology. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nautilus Biotechnology. **J. Robinson:** A. Employment/Salary (full or part-time):; Nautilus Biotechnology. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nautilus Biotechnology. **T. Rinker:** A. Employment/Salary (full or part-time):; Nautilus Biotechnology. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nautilus Biotechnology. **J. Sherman:** A. Employment/Salary (full or part-time):; Nautilus Biotechnology. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nautilus Biotechnology. **A. Ellahi:** A. Employment/Salary (full or part-time):; Nautilus Biotechnology. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nautilus Biotechnology. **T. Weiderhold:** A. Employment/Salary (full or part-time):; Cell Signaling Technology. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cell Signaling Technology. **D.S. Kirkpatrick:** A. Employment/Salary (full or part-time):; Interline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Interline Therapeutics. **J. Lipka:** A. Employment/Salary (full or part-time):; Genentech. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Genentech. **T. Wendorff:** A. Employment/Salary (full or part-time):; Genentech. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Genentech. **N.J. Pandya:** A. Employment/Salary (full or

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Poster

PSTR400. Tau: Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR400.19/N1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: FL DoH EEM 21A24
NIH F31AG082505

Title: Dnajb6 as a regulator of tau

Authors: *A. ESQUIVEL[1,2], S. HILL[1,2], L. BLAIR[1,2];
[1]Mol. Med., Univ. of South Florida, Tampa, FL; [2]USF Hlth. Byrd Alzheimer's Inst., Tampa, FL

Abstract: Tauopathies, such as Alzheimer’s Disease (AD), are a group of progressive neurodegenerative diseases marked by pathological tau. Disease severity and progression of these diseases can be linked to the pathological accumulation of tau. Recent evidence has demonstrated the prion-like properties of tau, where pathological tau templates the misfolding of native tau and propagates through synaptically connected regions of the brain. Molecular chaperones, a diverse set of proteins responsible for proteostasis, become dysregulated in AD and other tauopathies and are involved in normal and pathological tau processing. Initially, we performed an unbiased screen on approximately 60 molecular chaperones (n=2 biological replicates), across 6 different major chaperone families that identified the Hsp40s, or DnaJs, as a family enriched in regulators of tau. DnaJA2, DnaJB1, and DnaJB6 were found to significantly decrease tau seeding and their overexpression in cellular models of tau reduced intracellular tau levels. Follow up knockdown assays indicate that levels of DnaJB1 and DnaJB6 inversely correlate with tau levels. DnaJB6 was particularly potent in a more aggressive cellular model of tauopathy and was found to coimmunoprecipitate with tau. Additionally, DnaJB6 levels are decreased in PS19 mice. Additional studies are underway to identify the impact of DnaJB6 on tau *in vivo*. Overall, our data identify a role for DnaJB6 in regulating tau and support a number of studies that provide evidence for DnaJB6 as a powerful antagonist of amyloidogenic proteins.

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Poster

PSTR400. Tau: Cellular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR400.20/N2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Department of Atomic Energy, Government of India, RSI4002

Title: Igf1r interacts with β arrestins and translocates into nucleus in Alzheimer's disease

Authors: *P. SENGUPTA, D. MUKHOPADHYAY;
Biophysics & Structural Genomics, Saha Inst. of Nuclear Physics, KOLKATA, India

Abstract: The IGF1R signaling cascade forms the crux of this study due to its importance in Alzheimer's Disease (AD), crosstalk possibilities and regulatory mechanisms. IGF1R expression significantly increases in AD and several studies have shown its ablation reverses cognitive impairment. We begin our exploration by monitoring β-arrestins due to IGF1R's cytoplasmic βarr1/2 binding domain which has not been investigated in neurodegenerative scenarios before. We observed a significant upregulation in β-arr1 and downregulation in β-arr2 in SHSY5Y cells in AD like conditions (AICD is overexpressed along with extracellular introduction of Aβ) (n=4). An increase in interaction between IGF1R and β-arr1 leads to sustained MAPK signaling which is co-related with prior studies. We further probed the cAMP production (due to IGF1R's hybrid GPCR nature) through cAMP Glo assay (n=8) using various combinations of overexpression and silencing of related genes, and observed a marked reduction in cAMP in IGF1R overexpression experiments concurrent with AD like conditions. Incidentally, IGF1R-deficient cells showed relative increase in cAMP production, which further indicates that in AD, cAMP regulation is impacted by IGF1R. Furthermore, treatment with sumoylation inhibitors and phosphorylation inhibitors which potentially blocks IGF1R translocation into the nucleus, leads to increase in cAMP levels. We performed live imaging to visualize the trafficking of GFP tagged IGF1R when treated with Aβ and found that it eventually translocated to the nucleus. To ascertain that this enhanced nuclear shuttling was not an artefact of neuroblastoma, a differentiated neuronal model was generated and the cholinergic neuronal lineage was validated through an Acetylcholine assay. In the differentiated model, we further observed nuclear IGF1R in a greater number of cells treated with Aβ as compared to control sets, which was validated with fractionation studies. The study was also performed on B6C3-Tg(/APPswe,PSEN1dE9/)85Dbo/J mice brains against control brain tissue and more nuclear specs were found there as well. For the first time an altered distribution of IGF1R in AD was quantified and is gradually being understood. Thus, to conclude IGF1R interacts with β arrestin1, hyperactivates MAPK pathways at the membrane after vescicle formation, upon sumoylation travels into the nucleus in AD to regulate key pathways involved in cognition.

Disclosures: **P. Sengupta:** None. **D. Mukhopadhyay:** None.

Poster

PSTR401. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR401.01/N3

Topic: C.03. Parkinson's Disease

Support: NRF Grant 2021R1I1A1A01044890

Title: Rumex japonicus Houtt. alleviates Parkinson's disease by inhibiting mitochondrial disfunction and colonic damage in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced C57BL/6N mice

Authors: *H.-Y. KIM;
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Abstract: Parkinson's disease (PD) is common neurodegenerative disease in elderly people in the world. *Rumex japonicus* Houtt. (RJ) has been used to treat several gastrointestinal and inflammatory diseases in Korea. We recently confirmed the protective effect of RJ in colitis and colorectal cancer in mice and the neuroprotective effect of RJ in an in vitro PD model. In this study, we investigated the effects of RJ in the mitochondria of brain and the colon in MPTP-induced PD mice. C57BL/6N male mice (10-week old) were injected with MPTP (30 mg/kg, i. p.) for 5 days and treated with low or high dose of RJ (p. o.) for 14 days. RJ inhibited decrease of loss of tyrosine hydroxylase-positive cells and the mitochondrial protein expressions such as PINK1, Parkin and DJ-1 in the substantia nigra (SN). In addition, RJ reduced mitochondria-dependent apoptosis, increase of α-synuclein and inflammatory factors such as LPS, IL-1β and TNF-α in both the SN and the colon. Moreover, RJ suppressed collapse of tight junction (TJ) barrier in the colon and blood-brain barrier in the SN by regulating the expression of TJs (ZO-1, occluding and claudin-5) and/or adherens junction protein (VE-cadherin). Therefore, RJ alleviates MPTP-induced PD by protecting normal function of mitochondria and TJs in the brain and the colon.

Disclosures: **H. Kim:** None.

Poster

PSTR401. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR401.02/N4

Topic: C.03. Parkinson’s Disease

Support: MJFF Grant 15288
NIH Grant 1P20 GM130447

Title: Parkinson's disease relevant pathological features are manifested in male Pink1/Parkin deficient rats.

Authors: B. LAMBERTY, L. ESTRELLA, J. MATTINGLY, K. EMANUEL, A. TREASE, S. TOTUSEK, L. SHELDON, J. GEORGE, M. ALMIKHLAFI, T. FARMER, ***K. STAUCH**; Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Animal disease models are important for neuroscience experimentation and in the study of neurodegenerative disorders. The major neurodegenerative disorder leading to motor impairments is Parkinson’s disease (PD). The identification of hereditary forms of PD uncovered gene mutations and variants, such as loss-of-function mutations in PTEN-induced putative kinase 1 (Pink1) and the E3 ubiquitin ligase Parkin, two proteins involved in mitochondrial quality control, that could be harnessed to create animal models. However, to date, such models have not reproducibly recapitulated major aspects of the disease. Here, we describe the generation and phenotypic characterization of a combined Pink1/Parkin double knockout (dKO) rat, which reproducibly exhibits PD-relevant abnormalities, particularly in male animals. Motor dysfunction in Pink1/Parkin dKO rats was characterized by gait abnormalities and decreased rearing frequency, the latter of which was responsive to levodopa treatment. Pink1/Parkin dKO rats exhibited elevated plasma levels of neurofilament light chain and significant loss of tyrosine hydroxylase expression in the substantia nigra pars compacta (SNpc). Glial cell activation was also observed in the SNpc. Pink1/Parkin dKO rats showed elevated plasma and reduced cerebrospinal levels of alpha-synuclein as well as the presence of alpha-synuclein aggregates in the striatum. Further, the profile of circulating lymphocytes was altered, as elevated CD3+CD4+ T cells and reduced CD3+CD8+ T cells in Pink1/Parkin dKO rats were found. This coincided with mitochondrial dysfunction and infiltration of CD3+ T cells in the striatum. Altogether, the Pink1/Parkin dKO rats exhibited phenotypes similar to what is seen with PD patients, thus highlighting the suitability of this model for mechanistic studies of the role of Pink1 and Parkin in PD pathogenesis and as therapeutic targets.

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Poster

PSTR401. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR401.03/N5

Topic: C.03. Parkinson’s Disease

Support: Horizon 2020 No 848002

Title: Assessing anxiety and depression comorbidities in a primate model of Parkinson's Disease

Authors: A. SADOUN[1], Q. LI[2], E. BEZARD[3], ***E. PIOLI**[4];
[1]Motac, Bordeaux, France; [2]Motac Neurosci., Motac Neurosci., Beijing, China; [3]Inst. of Neurodegenerative Dis., Bordeaux, France; [4]MOTAC, Bordeaux, France

Abstract: Parkinson's disease (PD), in which there is widespread degeneration of the catecholamine system, is characterised by both motor and non-motor symptoms, such as impairment in cognitive performance. Anxiety and depression are also known comorbidities associated with Parkinson's disease. The study aimed to examine the anxiety/depression-like phenotype in the gold standard primate model of motor symptoms in PD, the so-called 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) macaque model. The cognitive performance was also evaluated. The anxiety / depression-like phenotype was evaluated in 11 MPTP and 6 normal macaques (*Macaca mulatta*) by ethological scan in the Human Intruder Test. The cognitive performances were assessed by the delayed matching to sample (DMTS) and the Variable Delayed Response (VDR) tasks from the Monkey CANTAB battery.The MPTP monkeys presented a passive reaction toward challenging stimuli during the intruder test, which is a sign of depressive-like individuals compared to normal animals. The MPTP monkeys showed longer learning latencies and decreased performance in the long delays in DMTS and VDR tasks. The rich behavioural repertoire of non-human primates makes them ideal translational models in which neuropsychiatric conditions can be studied. The present results that the MPTP-treated macaque replicates some depressive-like behaviour associated with PD.

Disclosures: **A. Sadoun:** A. Employment/Salary (full or part-time):; Motac. **Q. Li:** A. Employment/Salary (full or part-time):; Motac. **E. Bezard:** None. **E. Pioli:** A. Employment/Salary (full or part-time):; Motac.

Poster

PSTR401. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR401.04/N6

Topic: C.03. Parkinson's Disease

Support: ASAP-020505

Title: Effects of inflammation in the progression of parkinson's disease in a rat model overexpressing human alpha-synuclein

Authors: ***M. MASSARO CENERE**[1,3], M. TIBERI[4,1], E. PALDINO[1], S. D'ADDARIO[1,3], M. FEDERICI[1], C. GIACOMET[4,1], F. COSSA[4,1], B. ZARRILLI[4,1], A. LEDONNE[4,1,3], E. GUATTEO[1,3], N. BERRETTA[2,3], F. R. FUSCO[1], V. CHIURCHÙ[1,5], N. B. MERCURI[1,4,3];
[1]Fondazione Santa Lucia, Rome, Italy; [2]Fondazione Santa Lucia, Roma, Italy; [3]Aligning Sci.

Across Parkinson's (ASAP) Collaborative Res. Network, Chevy Chase, MD; [4]Univerity of Rome "Tor Vergata", Rome, Italy; [5]Inst. of Translational Pharmacol., CNR, Rome, Italy

Abstract: Parkinson's disease (PD) is now understood to be a multifactorial disorder involving genetic and environmental risk factors. Increasing research efforts have been made to understand how genetic and environmental factors may act in concert to impair homeostasis and elevate risk. Inflammation could be one unifying biological factor that could weaken homeostasis affecting neurodegenerative disease risk, onset, and progression. In this study, we created a double-hit animal model of PD to investigate whether overexpression of α-synuclein and inflammation accelerate the progression of PD neurodegeneration. Two months old rats overexpressing human α-synuclein (Snca) and *wild-type* littermates were intraperitoneally injected with a single dose of lipopolysaccharide (LPS, 5mg/kg) or with saline (SAL). Three months after injection, we evaluated nigrostriatal dopaminergic neurodegeneration, α-synuclein pathology, and neuroinflammation by immunohistochemistry, immunofluorescence, constant potential amperometry, and high dimensional flow cytometry analyses. We also investigated associated general locomotor and depressive-like behavior dysfunctions. The acute injection of LPS in rats caused long-lasting neuroinflammation in the *substantia nigra* (SN) and the striatum (STR). Microglia showed morphological changes, with less complex and shorter branching and increased expression of M1 activation markers. In LPS-Snca rats, macrophage and T-lymphocytes infiltration was increased into the SN and the STR. Phospho-S129 α-synuclein appeared qualitatively more inside microglial cell somas in the SN and the STR of LPS-Snca rats than in the control groups. In this context, more than 40% of nigral TH+ neurons were lost in LPS Snca rats, and these cells showed altered dendritic arborization that branches from the SN *pars compacta* (SNpc) towards *pars reticulata*. The dopaminergic neuronal loss was associated with a slight reduction in the STR-evoked dopamine release in LPS-Snca rats. However, no significant decrease in the TH+ fibers was observed in LPS-treated compared to saline-treated rats, nor behavioral changes in general locomotor activity, motor balance, and coordination. Interestingly, LPS-Snca rats preferred the 1% sucrose solution, with higher sucrose intake than the control groups. Our two-hit animal model involving a genetic mutation and an inflammatory factor reproduced some key pathogenic PD features and, most notably, accelerated the progression of PD neurodegeneration in the SNpc. Thus, it could be a valid animal model to study the mechanisms whereby α-synuclein and inflammation interactions mediate neurodegeneration in PD.

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Poster

PSTR401. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR401.05/N7

Topic: C.03. Parkinson’s Disease

Support: County Council of Västerbotten
Lars Hierta memorial foundation
Åhlen-foundation
Research foundation for Clinical Neuroscience at Umeå university hospital
Strategic research grants

Title: Nigral cell loss and neuroinflammation following a lesion to the locus coerulean noradrenergic system

Authors: *F. SHIMSHEK SHENER[1], E. HENRIKSSON[2], R. EL-HABTA[2], M. BLYBERG[2], D. PAPOVA[2], A. VIREL[2], S. AF BJERKEN[2];
[1]Umeå Univ., Umeå, Sweden; [2]Umea Univ., Umea, Sweden

Abstract: Parkinson's disease is characterized by loss of dopaminergic cells in the substantia nigra, as well as loss of noradrenergic neurons in the locus coeruleus. Neuroinflammation and microglia are thought to contribute to the progression of Parkinson's disease. Noradrenaline, aside from its neurotransmissive role, exerts anti-inflammatory effects and is hypothesized to act as a protector for dopaminergic cells in the substantia nigra.
We have recently using the N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP4) toxin-based rat model of noradrenergic denervation demonstrated a direct link between locus coerulean denervation and long-term loss of dopaminergic cells in the substantia nigra. In addition, we observed increased neuroinflammation, characterized by microglial activation, over the substantia nigra. To investigate the immunosuppressive and protective effects of noradrenaline further, we have now administered lipopolysaccharide (LPS) to DSP4-treated rats and evaluated the neuroinflammatory and degenerative process using cytokine arrays, ELISA, and stereological cell counting.
Post-mortem cell counting showed a significant reduction in tyrosine hydroxylase (TH)-positive cells in the substantia nigra of subjects treated with DSP4 or LPS compared to controls at the 6-month time point. In addition, additional dopaminergic degeneration was seen in the double treated subjects, DSP4+LPS compared to LPS only. Analysis of brain tissue revealed alterations in pro-inflammatory and anti-inflammatory cytokines, emphasizing the importance of the noradrenergic system as a modulator. Motor function and coordination assessment using the RotaRod test demonstrated a significant decrease in subjects treated with DSP4 or LPS compared to controls at both the 3-month and 6-month time points.
Our findings suggest that the substantia nigra is more susceptible to LPS-induced neuroinflammation in the absence of protection from an intact noradrenergic system.

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Poster

PSTR401. Parkinson's Disease: Animal Models

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Program #/Poster #: PSTR401.06/N8

Topic: C.03. Parkinson's Disease

Support: NIH Grant R01 ES031124

Title: Alterations in mitochondrial bioenergetics and increased phosphorylated alpha-synuclein in older mice lacking ATP13A2

Authors: *K. M. CROUCHER[1,2], J. K. LEPP[1], S. M. FLEMING[1];
[1]Pharmaceut. Sci., Northeast Ohio Med. Univ., Rootstown, OH; [2]Biomed. Sci. Grad. Program, Kent State Univ., Kent, OH

Abstract: ATP13A2 is a lysosomal protein involved in polyamine transport. Loss of function mutations in ATP13A2 have been linked to the neurodegenerative disorders Parkinson's disease (PD), Kufor-Rakeb Syndrome, Neuronal Ceroid Lipofuscinosis, and Hereditary Spastic Paraplegia. In vitro studies show loss of ATP13A2 impairs mitochondrial function and may contribute to the neurodegeneration found in these disorders. The present study sought to investigate the effect of loss of ATP13A2 function on mitochondrial function during aging in mice. In addition, given the link with PD, pathological phosphorylated alpha-synuclein was measured within the mitochondrial fraction. Male and female wildtype (WT), heterozygous (Het), and *Atp13a2* knockout (KO) mice at 3, 12, and 18 months of age were included in the study. Mitochondrial bioenergetics was measured in the prefrontal cortex, striatum, ventral midbrain, and cerebellum. Fresh mitochondrial fractions from each region were isolated and the oxygen consumption rate (OCR) of mitochondria (5ug/well) was measured in triplicate using the Seahorse XFp Flux Analyzer. OCR was measured in response to sequential injections of the substrates ADP, Oligomycin, FCCP, and Rotenone/Antimycin A in the presence of energetic substrates (glutamate, succinate, and malate). Mitochondrial bioenergetics analysis revealed a significant decrease in basal respiration in the striatum in 12 and 18 month KO mice compared to Het mice at the same age. At 12 months of age striatal OCR in KO mice was significantly reduced in response to oligomycin, FCCP, and Rotenone/Antimycin A. Further, phosphorylated alpha-synuclein in striatal mitochondria was significantly increased in 18 month KO mice. Overall, these data indicate loss of ATP13A2 in vivo disrupts mitochondrial function and promotes pathological alpha-synuclein within the striatum during aging.

Disclosures: K.M. Croucher: None. **J.K. Lepp:** None. **S.M. Fleming:** None.

Poster

PSTR401. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR401.07/O1

Topic: C.03. Parkinson's Disease

Support: NIH grant P20NS123220

Title: Characterization of a novel mouse model hLRRK2-G2019S for Parkinson's disease

Authors: *B. HUANG[1], X. LI[2], Z. YUE[3];
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Abstract: Background: Leucine-rich repeat kinase 2 (LRRK2) variant G2019S is found in both familial and sporadic Parkinson's disease (PD). Many LRRK2 G2019S mouse models have been created to investigate pathogenic mechanism of PD but have yet to show a robust Parkinson's disease phenotype. While the sequence difference between human and mouse LRRK2 (~83% homologous) may contribute to the lack of PD related symptoms, the manifestation of neuropathologies even with the expression of human LRRK2 variant could be tempered by the endogenous rodent Lrrk2. Furthermore, recent evidence from multiple reports (including ours) have shown a significant enrichment of LRRK2 expression in microglia from human tissues, yet mouse models were not able to capture that. Thus, distinct cell type expression patterns of LRRK2 between mouse and human could also underlie the difference. Therefore, we propose to establish a new mouse model of humanized LRRK2-G2019S (*h*LRRK2^{G2019S}), which may allow for better PD modeling while limiting the competing effects between human and endogenous LRRK2.Methods: We established a novel model by replacing mouse *lrrk2* with the human genomic sequence of LRRK2-G2019S. We confirmed the expression of human LRRK2 variant and lack of mouse LRRK2 in this model. Early characterizations of this model focused on male mice because of the increased severity of PD in males. We performed immunohistochemistry analysis of the kidney and brain slices obtained from *h*LRRK2^{G2019S}, Lrrk2 G2019S Knock-In (KI), LRRK2 KO, and WT mice and compared the pathologies among the mice with different genotypes. Results: In contrast to control and Lrrk2-G2019S KI mice, *h*LRRK2^{G2019S} mice show remarkable renal pathology associated with autophagy defects and an increase of pS129 α-synuclein levels. We also detected an increase of phosphorylation of rab10 and rab12 in *h*LRRK2^{G2019S} brains, compared to the control mice. Conclusions: Our ongoing study shows that the humanized LRRK2 G2019S model shows distinct pathology in peripheral tissues from the previously reported mouse KI LRRK2 G2019S and transgenic mice expressing human LRRK2 variants. We are currently investigating the humanized LRRK2 PD model in the brain and multiple peripheral tissues. Our study is expected to show better tools to understand PD and investigate peripheral molecular biomarkers due to G2019S dependent kidney dysfunction.

Disclosures: B. Huang: None. **X. Li:** None. **Z. Yue:** None.

Poster

PSTR401. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR401.08/O2

Topic: C.03. Parkinson's Disease

Support: RISE
NIH
NSF/IOS

Title: Effect of Combinations of Commonly-Used Pesticides on Organismal Toxicity in Drosophila

Authors: *A. ATHEBY, F. ABDUL-RAHMAN, H. LAWAL;
Delaware State Univ., Dover, DE

Abstract: Parkinson's Disease (PD) is a debilitating, progressive neurodegenerative disease characterized in part by the loss of dopaminergic neurons in the substantia nigra pars compacta. Epidemiological studies have established that exposure to certain pesticides and herbicides increase an individual's likelihood of developing PD. Although the full catalog of pesticides that pose risks for PD is not currently known, some potentially toxic compounds are commonly used for agricultural or gardening purposes, and may be purchased at local stores. The objective of this study therefore, was to determine whether different combinations of commercially-available pesticides and herbicides have either a synergistic or additive effect on toxicity in *Drosophila*. We first established a dose response curve in the following pesticides: acephate, atrazine, and diuron. We then exposed *Drosophila* to different combinations of the compounds at a concentration at which each alone was minimally toxic. We performed survival and locomotion ability analyses on each experimental group. We demonstrate that combinations involving atrazine and diuron show strong decreases in survival that were greater than each single pesticide alone. Moreover, we find that atrazine, which has been implicated in Parkinson's disease etiology in work by others, shows elevated toxicity. We also present data on our analysis of the effects of these compounds on reactive oxygen species generation and measures of neurotoxicity. Together, our data suggest that exposure to combinations of commonly-used pesticides such as atrazine and diuron may elevate susceptibility to environment toxins, including those that are implicated in PD.

Disclosures: A. Atheby: None. **F. Abdul-Rahman:** None. **H. Lawal:** None.

Poster

PSTR401. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR401.09/O3

Topic: C.03. Parkinson's Disease

Support: NIH R01NS115809

Title: Sex differences in novel transgenic mice with constitutively upregulated nicotinic acetylcholine receptors: implications for parkinson's disease

Authors: *G. PANDEY[1], R. SRINIVASAN[2];
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Abstract: Sex Differences in Novel Transgenic Mice with Constitutively Upregulated Nicotinic Acetylcholine Receptors: Implications for Parkinson's Disease
Gauri Pandey[1,2*], Sara M. Zarate[1], and Rahul Srinivasan[1,21]Department of Neuroscience and Experimental Therapeutics, Texas A&M School of Medicine, Bryan, TX, USA[2]Texas A&M Institute for Neuroscience, Texas A&M University, College Station, TX, USA
Parkinson's disease (PD) incidence rates predict a worldwide pandemic that will affect over 12 million people by 2040, underscoring the urgent need for neuroprotective drugs. Unfortunately, no neuroprotective drugs are currently available, and most proposed neuroprotective drugs failed clinical trials because PD is produced by a range of insults not replicated in any one animal model. For this reason, we focus on hyperactivated endoplasmic reticulum (ER) stress, a convergent apoptotic mechanism for multiple PD-related toxicities. Nicotine reduces PD risk; however, nicotine concentrations in tobacco users cannot activate neuronal nicotinic acetylcholine receptors (nAChRs), making this an unlikely mechanism for neuroprotection of dopaminergic (DA) neurons. We have previously shown that nanomolar concentrations of the nicotinic ligand cytisine rapidly chaperone β2-subunit-containing (β2*) nAChRs out of the ER. This directly reduces the ER stress response, which is critical for neuroprotection. To test this hypothesis, we created a novel transgenic mouse line named β2-mutant, with enhanced ER export of β2* nAChRs. Surprisingly, β2-mutant mice demonstrate significant increases in Sec24D ER exit sites (ERES) within substantia nigra pars compacta (SNc) DA neurons in only female but not male mice. We also induced parkinsonism in mice by unilateral injection of 6-OHDA in the dorsolateral striatum. Interestingly, the β2-mutations reduced apomorphine rotations only in female mice. This reduction corresponds with a decrease in the loss of TH+ neurons. Our data suggests the β2-mutations exert neuroprotection only in female mice. We are also testing the consequences of upregulating β2* nAChRs on striatal dopamine release using the optogenetic sensor GRABDA.
This work is supported by NIH R01NS115809.

Disclosures: G. Pandey: None. **R. Srinivasan:** None.

Poster

PSTR401. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR401.10/O4

Topic: C.03. Parkinson's Disease

Support: FRESCO Parkinson's Institute

Title: Exercise boosts dopamine release in the new parkinR275W mouse model of autosomal recessive juvenile parkinsonism

Authors: *L. ZANETTI[1], G. BASTIOLI[1], J. C. PATEL[2], M. REGONI[1], F. VALTORTA[1], J. SASSONE[1], M. E. RICE[2];
[1]Vita-Salute San Raffaele Univ., Milano, Italy; [2]NYU Grossman Sch. of Med., New York, NY

Abstract: Mutations in PARK2 gene cause Autosomal recessive juvenile parkinsonism (ARJP), an early onset familial form of Parkinson's disease characterized by early and severe dopamine (DA) neurons loss in substantia nigra pars compacta (SNc). At the moment, no neuroprotective approach exists, and a symptomatic therapy only is available. Complementing drug treatments, physical exercise has been shown to improve motor performance in individuals living with PD. However, underlying mechanisms are not yet understood. Our previous study showed that voluntary exercise for 30 days leads to an increase in evoked DA release throughout the striatum in WT mice. Based on this observation, we wanted to test the effects of exercise on DA release in pathological conditions. To fulfill this aim, we used our new, innovative mechanistic mouse model of *PARK2*-related ARJP. This model was created by taking advantage of CRISPR/Cas9 genome editing to insert the PARKIN R275W missense mutation, found with highest allelic frequency in *PARK2* patients. Characterization of this model showed it recapitulates major Parkinson's disease hallmarks, such as age-dependent loss of DA neurons in the SNc, progressive motor impairment, and decrease of DA content. Using the method of fast-scan cyclic voltammetry (FSCV), we further characterized the mouse model assessing the electrically-evoked DA release in striata slices before and after an exercise protocol. Data suggested a DA release impairment in non-runner, 6-months-old R275W mice, which worsened in aged (12-months-old) mice. Subsequently, we analyzed the effect of 30-days voluntary running protocol in 12-months old mice: we observed an increase in stimulus-evoked DA release in the striatum as well as an amelioration of motor phenotype. These findings highlight a pivotal role of exercise in treatment of PD by boosting DA release in the brain and shed light on the importance for research to learn underlying mechanistic insights.

Disclosures: L. Zanetti: None. **G. Bastioli:** None. **J.C. Patel:** None. **M. Regoni:** None. **F. Valtorta:** None. **J. sassone:** None. **M.E. Rice:** None.

Poster

PSTR401. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR401.11/O5

Topic: C.03. Parkinson's Disease

Support: KGM4562323
KFW0512311

Title: The sequential expression of T-bet, GATA3 and RORγ in basal ganglia of MPTP-induced non-human primate model with Parkinson's disease.

Authors: *J. SEO[1], K. KIM[1,2], M. KIM[1,3], Y. JUNG[1,4], S.-W. LEE[1,5], Y. LEE[1];
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Abstract: The connection between neurons and T lymphocytes, particularly the role of T helper 17 (Th17) CD4 lymphocytes, has garnered attention in the study of Parkinson's disease (PD) and potential therapeutic strategies for neurodegenerative diseases. It has been reported that Th17 lymphocytes can mediate neuronal cell death in in vitro models. However, the mechanism underlying the progression and persistence of Th17 lymphocyte infiltration into the brain following the injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in a non-human primate (NHP) model of PD remains unknown. This study aimed to investigate the long-term infiltration of Th1, Th2, and Th17 cells into the substantia nigra (SN) during the progression of PD in an NHP model after MPTP administration. The study examined the acute, sub-acute, and chronic phases of PD to understand the progression and persistence of Th1, Th2, and Th17 cell infiltration. The acute phase infiltration of Th17 cell coincides with the concurrent death of dopaminergic neurons, in contrast to the delayed infiltration of Th1 and Th2 cells. The findings of this study shed light on the infiltration progression of T lymphocytes in the NHP model of PD and provide novel insights into the pathogenesis of PD. Understanding the role of Th17 cells and their infiltration patterns in PD may contribute to the development of new therapeutic approaches for the treatment of this neurodegenerative disease.

Disclosures: J. Seo: None. **K. Kim:** None. **M. Kim:** None. **Y. Jung:** None. **S. Lee:** None. **Y. Lee:** None.

Poster

PSTR401. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR401.12/O6

Topic: C.03. Parkinson’s Disease

Support: NIH Grant NS129676

Title: An Expression based approach to assessing a Parkinson’s Disease-like state in 6-OHDA exposed Zebrafish larvae.

Authors: *A. ROMERO, M. S. EL-HALAWANY, A. K. HAMOUDA, B. R. BILL;
Univ. of Texas, Tyler, Tyler, TX

Abstract: Parkinson’s disease (PD) is a neurodegenerative disorder characterized by the loss of A-9 midbrain dopaminergic neurons in the substantia nigra pars compacta. The disorder is clinically characterized by motor symptoms including but not limited to tremor at rest, rigidity,

akinesia , and postural instability affecting the quality of life for over 10 million people worldwide. The neurotoxin 6-hydroxydopamine (6-OHDA) is a well-established methodology to chemically induce a PD-like state in animal models by damaging dopaminergic neurons. In our effort to optimize a 6-OHDA-based PD-like model in zebrafish for drug screening, we used an RNA expression-based assessment to determine optimal 6-OHDA-exposure conditions (zebrafish age at time of exposure, 6-OHDA concentration, duration of exposure). We used dopaminergic neuronal markers (e.g., *th*), PD-related genes (e.g., *pink1*), reactive oxygen species pathway genes (e.g., *sod2*) as quantitative molecular endpoints. Our preliminary results established a workflow that consists of 24 hours of 6-OHDA exposure (10-30 µM) during 3-4 day post fertilization in the presence of 0.2% ascorbic acid, 0.23 ppm methylene blue, and buffered with 40 mM HEPES (pH 7.4). RT-qPCR confirmed a statistically significant reduction in *th* and *pink1* genes expression and statistically significant upregulation of *sod2* gene expression without apparent changes in the expression of control housekeeping genes *hprt1* in zebrafish larvae treated with 30 µM 6-OHDA using the above workflow. Exposure to 30 µM 6-OHDA using this workflow resulted in no morphological or developmental differences in surviving fish as compared to control, however, 30 µM 6-OHDA exposure was associated with ~50% lethality. Further work is ongoing to improve the workflow to reduce lethality while keeping the desired quantitative molecular endpoints of selective damage in dopaminergic neuron.

Disclosures: A. Romero: None. **M.S. El-Halawany:** None. **A.K. Hamouda:** None. **B.R. Bill:** None.

Poster

PSTR401. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

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Topic: C.03. Parkinson’s Disease

Support: Aligning Science Across Parkinson's (Grant No. ASAP-020505) through the Michael J. Fox Foundation for Parkinson's Research (MJFF)
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CiberNed Intramural Collaborative Projects (Grant No. PI2020/09)

Title: Pigmentation of midbrain catecholaminergic neurons in mice following the systemic delivery of a BBB-penetrant AAV9 capsid variant

Authors: *J. LANCIEGO[1,2,3], J. CHOCARRO[1,2,3], G. ARIZNABARRETA[1,2,3], E. RODA[1,2,3], A. HONRUBIA[1,2,3], P. ARNAIZ[1], S. MARANA[1], A. CORCHO[1], A. LEON-VILLARES[1], A. VAZQUEZ[4], M. CUADRADO-TEJEDOR[1], A. GARCIA-OSTA[1], R. HERNANDEZ-ALCOCEBA[1], M. CHILLON[5,6,7,8], A. J. RICO[1,2,3];
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Investigación Biomédica en Red de Enfermedades Neurodegenerativas (Ciberned), Madrid, Spain; [3]Aligning Sci. Across Parkinson's (ASAP) Collaborative Res. Network, Chevy Chase, MD; [4]Neurosurg., Hosp. Universitario de Navarra, Pamplona, Spain; [5]Univ. Autonoma Barcelona, Univ. Autonoma Barcelona, Bellaterra, Spain; [6]Val d'Hebron Inst. de Recerca (VHIR), Barcelona, Spain; [7]Unitat de Producció de Vectors Virals (UPV), Univ. Autonoma Barcelona, Bellaterra, Spain; [8]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

Abstract: Throughout the past few years, the field of CNS gene therapy has witnessed a continuous race for the development of novel AAV capsid variants that can bypass the BBB upon systemic delivery. Several different options fulfilling this unmet need have been made available recently, these options opening a broad range of applications for both disease-modeling purposes as well as for therapeutic uses. Among existing possibilities, Voyager Therapeutics (Cambridge, MA) recently developed several AAV9 capsid variants by taking advantage of a RNA-driven screen platform known as TRACER (Tropism Redirection of AAV by Cell-type-specific Expression of RNA; see PMID: 33553485). This approach resulted in the characterization of ten individual variants showing up to 400-fold higher brain transduction over AAV9 following systemic administration. Here we took advantage of the AAV9-P31 capsid variant for modeling Parkinson’s disease in naïve, non-transgenic mice. To this aim, AAV9-P31 encoding the human tyrosinase gene (the enzymatic precursor of neuromelanin), was delivered in the retro-ocular plexus of C57BL/6 mice. One cohort of animals was euthanized four weeks post-AAV delivery, whereas another cohort was sacrificed with a follow-up time of 16 weeks. In all animals injected with AAV9-P31-hTyr, the conducted histological processing revealed an specific, time-dependent pigmentation of dopaminergic neurons located in the substantia nigra pars compacta and the ventral tegmental area, together with a somehow weaker pigmentation of noradrenergic neurons in the locus coeruleus. Moreover, preliminary results suggested the presence of intracytoplasmic inclusions positive for traditional markers of Lewy body pathology such as P62 and alpha-synuclein in pigmented neurons. Bearing in mind the well-known reciprocal association between the incidence of melanoma and Parkinson’s disease, the conducted approach enabled the development of a novel pre-clinical mice model of Parkinson’s disease mimicking the known neuropathological hallmarks of this disorder with unprecedented accuracy. By going this way, it is also worth noting that intraparenchymal delivery of AAVs will no longer be required, an issue obviously broadening the gene therapy field by allowing the implementation of a whole range of novel therapeutic strategies.

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Poster

PSTR401. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR401.14/O8

Topic: C.03. Parkinson’s Disease

Title: Znf746 undergoes liquid-liquid phase separation and poly(adp-ribose) mediated solidification

Authors: *S. PARK;
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Abstract: ZNF746 was identified as a parkin-interacting substrate (PARIS). To understand the pathophysiological properties of PARIS, we examined PARIS’s biophysical properties, showing that PARIS undergoes liquid-liquid phase separation (LLPS) and amorphous solid formation. LLPS of PARIS required the N-terminal low complexity domain 1 (LCD1), whereas the prion-like domain (PrLD, GGGGSGSGGGGGGS) at C-terminus was critical for the liquid to solid phase transition. In addition, we observed that poly (ADP-ribose) (PAR) strongly binds to the C-terminus of PARIS near the PrLD, accelerating its LLPS and solidification. MNNG-induced PAR formation led to PARIS oligomerization in human iPSC-derived dopaminergic neurons that was prevented by the PARP inhibitor, ABT-888. Furthermore, SDS-resistant PARIS species were observed in the substantia nigra (SN) of aged mice overexpressing wild-type PARIS, but not with a PAR binding-deficient PARIS mutant. PARIS solidification was also found in the SN of α-synuclein preformed fibril (α-syn PFF) injected mice and adult conditional knockout (KO) of parkin, but not in that of PARP1 KO mice injected with α-syn PFF. Herein, we demonstrate that PARIS undergoes LLPS and PAR-mediated solidification in PD models.

Disclosures: S. Park: None.

Poster

PSTR401. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

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Program #/Poster #: PSTR401.15/P1

Topic: C.03. Parkinson’s Disease

Support: NIH Grant R21NS122280

Title: SARS-CoV-2 infection enhances susceptibility in oxidative stress-induced and genetic models of Parkinson's Disease

Authors: *D. CHATTERJEE, T. RODRIGUEZ, K. CROWTHER, R. J. SMEYNE;
Farber Inst. of Neurosci. Thomas Jefferson Univ., Philadelphia, PA

Abstract: Parkinson’s disease (PD) is a neurodegenerative disease characterized by the loss of dopaminergic (DA) neurons in the SNpc of the midbrain, inflammation, and the formation of insoluble aggregates of phosphorylated alpha-synuclein. Although its causes are multifactorial, viral infections, including influenza, have been associated with the development of parkinsonism

(e.g encephalitis lethargica associated with the 1918 Spanish flu pandemic). Given the similarities in systemic responses after infection with SARS-CoV-2 (COVID-19) to those reported after the 1918 Spanish flu pandemic, we investigated, using mouse models of viral infection, whether SARS-CoV-2 infection would have the potential to elicit a similar neurological syndrome. In this study we examined if prior infection with SARS-CoV-2 increased midbrain pathology in two models of experimental parkinsonism: 1) administration of a subacute level of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and 2) mice carrying a G2019S mutation in the LRRK2 gene. Two strains of SARS-CoV-2 (WA-1 and omicron) were used. For MPTP experiments, C57BL/6J mice expressing the human ACE2 receptor (k18-hACE2) were infected with SARS-CoV-2 to induce moderate disease. After 38 days of recovery, mice were administered a subtoxic dose of MPTP and euthanized 7 days later. To examine effects in a familial mode, k18-hACE2 mice crossed with G2019S LRRK2 KI mice and were infected with the same titer of virus and euthanized 30 days later. Subsequent neuroinflammation and SNpc DA neuron loss were determined using stereology. Mice infected with SARS-CoV-2 or MPTP alone showed no significant SNpc DA neuron loss. However, in mice infected with SARS-CoV-2 (WA-1), MPTP induced a 23% or 19% greater loss of SNpc DA neurons than WA-1 alone or MPTP alone respectively ($p < 0.05$). Examination of microglial activation showed a significant increase in the number of activated microglia in the SNpc of the WA-1 + MPTP group compared with WA-1 or MPTP alone ($p < 0.01$). In k18-hACE2 X G2019S mice, WA-1 induced a 19% loss of SNpc DA neurons ($p < 0.01$), and a significant increase in the number of activated microglia ($p < 0.05$) compared to uninfected mice. Preliminary data shows that infection with the omicron variant similarly induces a 20% loss of SNpc neurons when exposed to MPTP, compared to omicron alone ($p<0.01$). Our observations have important implications for long-term public health, given the number of people who have survived a SARS-CoV-2 infection, as well as for future public policy regarding infection mitigation. Further, it will be critical to determine whether these observed effects are abrogated by vaccination or antiviral treatment.

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Poster

PSTR401. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR401.16/P2

Topic: C.03. Parkinson’s Disease

Support: DOD PD20005

Title: Abnormalities in Y-Maze Test in P+L Treated Parkinsonian Rats and its Protection with Oral Squalamine Treatment

Authors: *T. WHITE, V. PESHATTIWAR, C. SWAIN, K. LE, D. POKHAREL, T. SUBRAMANIAN;
Neurol., The Univ. of Toledo Col. of Med. and Life Sci., Toledo, OH

Abstract: Parkinson'sDisease (PD) is a chronic neurodegenerative condition with motor and non-motorclinical manifestations. One of the non-motor features that is under intenseinvestigation is cognitive decline. We previously reported a novel rat model ofPD that uses subthreshold doses of Paraquat (P) and Lectins (L) administeredorally to cause parkinsonism that simulates the environmental gut brainpathogenesis of PD with associated nigral synucleinopathy, levodopa responsiveparkinsonism and protection against PD via subdiaphragmatic vagotomy performedprior to P+L exposure. We have also reported in the past that squalamine, anatural bile salt derived from the dog fish shark when administered orallyprovides protection against parkinsonism. The Y-maze has been used extensivelyin animal models of PD to assess short-term memory. We hypothesized that animals with moresevere parkinsonian motor symptoms will exhibit a worse performance in theY-maze task as defined by spontaneous alternation. We tested this using threetreatment groups: no treatment (Normal), paraquat + lectin via oral gavage(P+L), paraquat + lectin and squalamine solution (P+L+S). Within the P+L group,animals exhibited varying degree of motor deficits as confirmed by our battery ofbehavioral tests. Twenty-fiveSprague Dawley rats were placed in a Y-Maze apparatus with an overheadautomated movement tracking system (AnyMaze). For five minutes, each rat'smovement was tracked and scored for their independent spontaneous alternation patternwith minimal distractions. The validity of the software's output was confirmedby manual scoring. Each spontaneous alternation was reported as a raw scorebased on the total of triads completed in each trial. One-way ANOVA confirmed asignificant difference ($p=0.02$) in spontaneous alternation and a valueapproaching significance ($p=0.090$) for the percent spontaneous alternationbetween treatment groups. Post-hoc analysis using student t-tests shows asignificant difference when comparing all three P+L subcategories to normalanimals. No significant difference was noted between P+L+S and normal animals.These preliminary results suggest that although motor deficits differed betweenanimals treated with P+L, the short-term memories of these animals were equallyaffected regardless of the extend of the motor deficits. When animals weretreated with oral squalamine, these cognitive deficits were not observed,suggesting that the observed phenomenon is linked to synucleinpathy.

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Poster

PSTR401. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

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Program #/Poster #: PSTR401.17/Web Only

Topic: C.03. Parkinson's Disease

Support: FRGS/1/2018/SKK08/UKM/03/5
FF-2021-177

Title: Cellular localization of Neurotrophin-3 in adult zebrafish brain and its role in Parkinson's Disease

Authors: *N. OMAR[1,2], J. MURTHY[2], S. TEOH[2];
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Abstract: Background Neurotrophin-3 (NT3) is a class of neuroprotective growth factors that are secreted in the nervous system that induces the development, maintenance, and survival of a specific neuronal population. NT3 has been sparsely studied in a zebrafish model. This study aims to present the cellular localization of NT3 in adult zebrafish brains and investigate the role of NT3 in a Parkinson's Disease (PD) model. **Methodology** Localization of NT3 expression was conducted in zebrafish brains using *in situ* hybridization to identify the neurotrophin-3 (*ntf3)* mRNA followed by fluorescence immunohistochemistry (FIHC) to visualize the co-expression of *ntf3* mRNA in neuronal and glial markers. Following that, an assessment of NT3 expression was conducted in the zebrafish PD model using the quantitative polymerase chain reaction (qPCR) method, Enzyme-Linked Immunosorbent Assay (ELISA), and NT3 IHC to investigate the alteration in NT3 expression following the induction of PD in zebrafish. **Results and Discussion** The *ntf3* mRNA-expressing cells (hereafter refers to as NT3 cells) were identified over the olfactory bulb, telencephalon, diencephalon, mesencephalon, and in rhombencephalon of the zebrafish brains. This opens the emphasis on the role of NT3 in the central nervous system apart from the previously established role in the peripheral nerve. Double labeling with the glial (GFAP) and neuronal (HuC/D) markers showed 100% overlap with the neuronal marker however with less than 20% overlap with the glial marker. This is slightly different from other neurotrophins such as the brain-derived neurotrophic marker (BDNF) where glial cells were more pronounced in expressing the proteins. Double labeling using the FIHC method was conducted for the dopaminergic marker (tyrosine hydroxylase) as well which showed 80% overlap in the region of the posterior tuberculum of the diencephalon, which is the pathognomonic location of PD in zebrafish. The *ntf3* gene expression was significantly downregulated 20 times in the PD model (0.05±0.04) compared to the control group (p=0.003). ELISA assessment of zebrafish brain was coherently dropped in NT3 protein level in the PD model (3295.32±186.11; p=0.04). The NT3 IHC assessment showed a global reduction in the intensity of the NT3 staining in the tissue section of the PD model's brain compared to the control. **Conclusion** The NT3 is actively expressed in adult zebrafish brains and showed its potential role in the pathogenesis of PD. This could offer a possibility of NT3 as part of the potential treatment of PD.

Disclosures: N. Omar: None. **J. Murthy:** None. **S. Teoh:** None.

Poster

PSTR401. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR401.18/P3

Topic: C.03. Parkinson's Disease

Support: VA Merit Review grant BX002966 (DF=PI)
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VA Merit Review grant BX001643 (CM=PI)

Title: Increased Anxiety-Related Behaviors Following Dopamine Loss Alters Glutamate Synapses in the Medial Prefrontal Cortex in an Animal Model of Parkinson's Disease

Authors: C. MOORE[1], M. HELMS[1], M. A. NIPPER[2], L. WINFREY[1], D. FINN[1,3], ***C. K. MESHUL**[4,3];
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Abstract: Anxiety is a prominent non-motor symptom of Parkinson's disease (PD). Changes in the B-spectrum recordings in PD patients of the pre-frontal cortex correlate with increased anxiety (de Hemptinne *et al.*, 2021). Loss of nigrostriatal dopamine (DA) has been resulted in a decrease in spine density in the medial prefrontal cortex (mPFC; Solis *et al.*, 2007), but no behavioral testing was carried out. We reported changes in glutamate synapses in the striatum and substantia nigra following DA loss (Meshul *et al.*, 1999; Moore *et al.*, 2020). We hypothesize that loss of DA will result in increased anxiety-related behaviors, and this will be associated with alteration in glutamate synapses. In the current study, male C57BL/6J mice (12-16 wks old) were tested for anxiety-related behavior using the elevated plus maze (Finn et al., 1997) following 4 weeks of vehicle (VEH) or treatment MPTP treatment (Moore *et al*., 2020), resulting in a 65% loss of striatal tyrosine hydroxylase and a 37% loss of DA neurons. The mice were then euthanized for histological evaluation for Golgi spine density, glutamate immunogold labeling within Layers II/III using electron microscopy, and for western blotting of various glutamate markers of the mPFC. There was an increase in anxiety-related behaviors (80% increase in closed arm entries; 100% increase in closed arm distance; 43% decrease in % open arm entries; 70% decrease in % distance in open arms) and a 78% decrease in plasma corticosterone levels in the MPTP vs VEH groups. This was associated with a 30% decrease in the density of dendritic spines in Layers II/III, and a 53% decrease in the density of glutamate immunogold density within vesicular glutamate transporter 1 (Vglut1; cortico-cortical connections) labeled nerve terminals and their associated spines, with no change within vesicular glutamate transporter 2 (Vglut2; thalamo-cortical connections) positive terminals/spines in the MPTP vs VEH groups. This decrease in glutamate terminal density suggests an increase in glutamate release (Meshul *et al*., 1999). There was a 15% decrease in the width of the synaptic cleft and a 23% increase in the length of the contact between the pre- and postsynaptic membranes in the MPTP vs VEH groups. There was an increase in protein expression of Vglut1 (40%) and Vglut2 (37%), a decrease in GLT-1 (50%) and EAAC1 (51%), and an increase in GLAST (225%) in the MPTP vs VEH groups within the mPFC. These data suggest that nigrostriatal DA loss results in a decrease in dendritic spines within the mPFC, which may be due to increased glutamate release/extracellular glutamate levels (decrease in glutamate transporters) and an associated change in anxiety-related behavior.

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Poster

PSTR401. Parkinson's Disease: Animal Models

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Program #/Poster #: PSTR401.19/P4

Topic: C.03. Parkinson's Disease

Support: ASAP-020505

Title: Behavioral and mitochondrial characterization of a rat model that overexpresses human tyrosinase in substantia nigra

Authors: *S. D'ADDARIO[1,2,3], M. MASSARO CENERE[2,1], S. SCARICAMAZZA[1], V. NESCI[1,2], A. FERRI[1,4], C. VALLE[1], R. VENTURA[2,5], M. VILA[6,2,7], A. LEDONNE[8,2,1], N. B. MERCURI[1,2,8];
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Abstract: BACKGROUND: Neuromelanin (NM) is a catecholamine-based polymer pigment derived from dopamine (DA) and DA precursors. During the lifespan NM is synthetized and then accumulated in a large part of DA neurons, particularly in the substantia Nigra pars compacta (SNpc) and in noradrenergic neurons of locus coeruleus. Over the years, several studies tried to shed light on the exact mechanism underlying NM synthesis and accumulation. Still, only a few works investigated if the selective accumulation of NM could induce behavioral and/or cellular dysfunctions. Notably, some of these works suggested that the accumulation of oxidized DA and NM could cause mitochondrial dysfunction in Parkinson's disease (PD) patient-derived neurons.Epidemiological observations, functional magnetic resonance study, and postmortem brain analysis have supported the central role of neuromelanin in PD pathogenesis.METHODS: In the present work, to selectively induce accumulation of NM, we injected into SN of male and female rats an Adeno-Associated Virus able to over-express human tyrosinase AAV9-CMV-htyr-GFP (AAV-htyr) or AAV9-CMV-null-GFP (AAV-null). Two months-old rats were injected, and after 4 weeks, they were behaviorally tested (motor, anxiety, and compulsive domain). The mitochondrial metabolic activity was directly assessed on SNpc punches through Seahorse XF96. Data were analyzed, keeping gender separate, to evidence sex differences.RESULTS: Animals tested in grip strength show a hypertonic response independently from sex, but when the same animals undergo anxiety characterization, AAV-htyr

males are more anxious than AAV-null. This difference was not found in females. Moreover, to verify sensitivity to psychostimulants, we administered 2.5 mg of amphetamine i.p., and we measured locomotion pre and post-injection; our results suggest that AAV-htyr males are more vulnerable to amphetamine effects on locomotion compared to AAV-null. No marked difference was found in females. Regarding mitochondrial activity, our data show that both sexes are affected. Taken together, our results suggest that in the initial stages of neuromelanin formation both sexes are affected with slight differences. Future studies should investigate if this trend remains the same after more time from the injection of AAV-htyr.

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Poster

PSTR401. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR401.20/P5

Topic: C.03. Parkinson’s Disease

Support: Transpharmation Ltd
Merck & Co., Inc.

Title: Neurodegeneration in the systemic rotenone model of Parkinson’s disease is associated with neuroinflammation, oxidative stress and gliosis

Authors: *J. PRENDERVILLE[1], R. WINTERS[1], W. LAU[2], D. FENG[2], J. KAHN[3], E. MCECHERN[3], T. ROSAHL[3], D. ZWILLING[3], J. BROWNLEES[4], G. HIGGINS[2]; [1]Transpharmation Ltd., Dublin, Ireland; [2]Transpharmation Ltd., Fergus, ON, Canada; [3]MRL Neuroscience, Merck & Co., Inc., West Point, PA; [4]MSD Neurosci. MSD (UK) Limited, London, United Kingdom

Abstract: Parkinson’s disease (PD) is the most common movement disorder, estimated to affect more than 8.5 million individuals globally. Motor symptoms of PD include bradykinesia, tremor and postural instability, and non-motor symptoms include cognitive impairment. The primary pathophysiological feature of PD is degeneration of dopaminergic neurons in the substantia nigra (SN). This neurodegenerative process is associated with phosphorylation of alpha-synuclein and accumulation of protein aggregates, neuroinflammation, oxidative stress and gliosis. This study characterised the systemic rotenone rat model of PD based on Cannon et al 2009, investigating the temporal progression of motor symptoms and neuropathology.
Male Lewis rats (n=8/group) received daily administration of rotenone by intraperitoneal injection (3.25mg/kg) for 1, 5 or 10 days. Behavioural assessments (locomotor activity, postural instability and beam walk tests) were performed at baseline and on the final day of treatment (day 1, 5 or 10) prior to tissue collection. Tyrosine hydroxylase (TH) positive cells were

quantified in the SN by immunohistochemistry. Immunofluorescence quantification of phosphorylated (P129) alpha-synuclein and microglia (Iba1 positive cells) was performed. Neuroinflammatory markers (TNF-alpha, IL-6, IL-1beta) in brain homogenate were analysed. Both 5- and 10-days rotenone treatment resulted in motor deficits, including reduced rearing counts (Day 10: Veh: 144$\pm$23; Rot: 13$\pm$10; $p<0.05$) and postural instability (Day 10: Veh: 4.2$\pm$0.1; Rot: 7.3$\pm$0.5; $p<0.05$). A 50% reduction in TH positive cells in the dorsolateral SN was observed following 10-day rotenone treatment ($p=0.057$). The number of microglia in the dorsolateral SN was increased with 10-day treatment ($p<0.05$) and evidence of alpha-synuclein accumulation was also observed. TNF-alpha and IL-6 were increased in whole brain homogenate at 10 days ($p<0.05$). Dopamine levels were reduced in 1-, 5- and 10-days treatment by 44%, 50% and 62%, respectively, compared to vehicle treated control.
There is an unmet clinical need for new PD therapeutics with improved efficacy. The establishment of robust preclinical models effectively modelling the behavioural and pathophysiological features of PD is required to advance drug discovery. This study reports that the systemic rotenone rat model of PD is associated with disease relevant motor symptoms and dopaminergic neurodegeneration in the SN. Furthermore, neurodegeneration in this model is associated with temporal changes in markers of oxidative stress, neuroinflammation and gliosis, and a useful approach to studying treatments that may modify these processes.

Disclosures: **J. Prenderville:** A. Employment/Salary (full or part-time):; Transpharmation Ltd. **R. Winters:** A. Employment/Salary (full or part-time):; Transpharmation Ltd. **W. Lau:** A. Employment/Salary (full or part-time):; Transpharmation Ltd. **D. Feng:** A. Employment/Salary (full or part-time):; Transpharmation Ltd. **J. Kahn:** A. Employment/Salary (full or part-time):; Merck & Co., Inc. **E. McEchern:** A. Employment/Salary (full or part-time):; Merck & Co., Inc. **T. Rosahl:** A. Employment/Salary (full or part-time):; Merck & Co., Inc. **D. Zwilling:** A. Employment/Salary (full or part-time):; Merck & Co., Inc. **J. Brownlees:** A. Employment/Salary (full or part-time):; MSD (UK) Limited. **G. Higgins:** A. Employment/Salary (full or part-time):; Transpharmation Ltd.

Poster

PSTR401. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

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Program #/Poster #: PSTR401.21/P7

Topic: C.03. Parkinson’s Disease

Support: Ron and Pratima Gatehouse Fund
Anne M. and Phillip H. Glatfelter, III Family
Department of Defense NETP GRANT13204752, NIDDK R01 DK124098, NINDS R01 NS104565

Title: Nigrovagal chemogenetic inhibition effects on paraquat+lectin model and electrophysiology of the hemiparkinsonian substania nigra

Authors: *J. A. KALBUS, C. SWAIN, K. LE, T. SUBRAMANIAN, K. VENKITESWARAN; Univ. of Toledo Col. of Med., Toledo, OH

Abstract: We tested the hypothesis that unilateral chemogenetic inhibition of the nigrovagal pathway will provide contralateral protection and cause ipsilateral hemiparkinsonism. Adult Sprague Dawley (SD) rats were stereotactically injected with AAV2- Ef1a-eYFP-IRES-WGA-Cre into the DMV and AAV8-hSyn-DIO-hM4Di-mCherry into the SNpc on the left side. This allowed chemogenetic inhibition of the left nigrovagal pathway when P+L was given with chemogenetic activator JHU in the water supply. Control animals received inactive vectors. In a second experiment DAT-Cre transgenic rats received AAV8-hSyn-DIO-hM4Di-mCherry into the SNpc on the left side followed by P+L oral gavage for a week while on JHU. All test animals developed stable left hemiparkinsonism and control animals developed bilateral parkinsonism as per Vibrissae and stepping tests. Histology showed unilateral expression of hM4Di and mCherry exclusively in the left nigrovagal pathway in the wild type SD rats and ipsilateral SNpc neuronal preservation with all its projections expressing hM4Di and mCherry in the transgenic rats. Contralateral SNpc demonstrated >50% neuronal degeneration. We show that maladaptive nigrovagal neuronal hyperactivity plays a critical role in accelerating synucleinopathy and SNpc degeneration. In our experiments, we observed that after 7 days of P+L exposure some adult Sprague Dawley rats naturally became hemiparkinsonian even without nigrovagal inhibition. Previous experiments have demonstrated that neuronal firing in the subthalamic nucleus (STN) increases with PD progression (Remple et al., Mov Disord., 2011). We wanted to see if significant differences in neuronal firing and burst between the pathologic and nonpathologic Substancia Nigra pars compacta (SNpc) and reticulata (SNr) are present. In vivo electrophysiology was done and showed significant difference between the affected side and unaffected side in various region of the basal ganglia, with the unaffected side being similar to normal animals. Recordings in the affected STN of hemiparkinsonian animals showed significantly higher firing rates (40-60 Hz) compared to normal animals (8-20 Hz), while the unaffected side did not have such difference. The SNpc firing rate was decreased in the affected side and the SNr had an abnormal firing pattern. While these results are from naturally hemiparkinsonian rats, we expect that further investigation will show that chemogenetically protected hemiparkinsonisn rats will have similar electrophysiological findings.

Disclosures: J.A. Kalbus: None. **C. Swain:** None. **K. Le:** None. **T. Subramanian:** 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.; Department of Defense, UCB pharma, Bukwang and BlueRock. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); honoraria for study section service from the National Institutes of Health. F. Consulting Fees (e.g., advisory boards); Scientific advisory board for Teva, Neurocrine and Supernus. **K. Venkiteswaran:** None.

Poster

PSTR401. Parkinson's Disease: Animal Models

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Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR401.22/P8

Topic: C.03. Parkinson's Disease

Support: NIH R01NS115809

Title: Effects of 17-β-estradiol depletion in cytisine treated female mice using a 6-OHDA mouse model of Parkinson's disease

Authors: *R. C. GARCIA[1], G. PANDEY[2], S. ZARATE[1], R. SRINIVASAN[1];
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Abstract: Parkinson's disease (PD) is characterized by the progressive loss of dopaminergic neurons in the substantia nigra and the two greatest risk factors for PD are advanced age and male sex. While there is a nearly two-fold higher prevalence of PD in men than women, currently there are no neuroprotective treatments for either sex, underscoring an unmet need for the development of neuroprotective treatments. Interestingly, chronic tobacco use has been shown to reduce the risk of PD by 50% and our studies have shown that low doses of nicotine (100 nM) attenuate apoptotic endoplasmic reticulum (ER) stress in DA neurons. Thus, we rationalized that nicotine and nicotinic agonists could reduce ER stress. Using a 6-OHDA model of PD we found that alternate day i.p. injections of 0.2 mg/kg cytisine, a partial nicotinic acetylcholine receptor agonist, was sufficient to reduce parkinsonian motor deficits and decrease 6-OHDA induced neurodegeneration of DA neurons only in female mice. In contrast, cytisine treated male mice showed no change in motor deficits or DA neuron loss but rather showed a pathological increase in nuclear expression of the apoptotic ER stress protein CHOP. To test whether cytisine and estrogen could work in combination to exert neuroprotection in female mice, we exposed primary DA cultures to 10 nM 17-β-estradiol, 200 nM cytisine, or 17-β-estradiol and 200 nM cytisine. We found that 17-β-estradiol alone reduced expression of CHOP and that 200 nM cytisine reduced two additional ER stress proteins, ATF6 and XBP1. To characterize the combined effects of cytisine and 17-β-estradiol in vivo, we are testing three methods of estrogen depletion in female mice (1) surgical depletion of 17-β-estradiol via ovariectomy (OVX) (2) chemical inhibition of aromatase activity, and (3) depletion of 17-β-estradiol in reproductively senescent female mice. In the model of OVX we have found that intact cytisine treated females had significantly less apomorphine induced rotations than intact saline treated females; however, there was no difference in the average number of rotations between intact cytisine females and OVX cytisine female mice. Additionally, we found no differences in SNc TH intensity or GFAP reactivity after 6-OHDA lesion for either the intact cytisine or OVX cytisine groups but found decreased TH intensity and increased GFAP reactivity in the intact saline group. These data suggest sex specific mechanisms in cytisine pharmacology and distinct roles for cytisine and 17-β-estradiol in the neuroprotective effect of cytisine in female mice.

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Poster

PSTR401. Parkinson's Disease: Animal Models

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Program #/Poster #: PSTR401.23/Web Only

Topic: C.03. Parkinson's Disease

Support: Indian Council of Medical Research (ICMR, Government of India) Grant - GAP410
Science and Engineering Research Board-Department of Science and Technology (SERB-DST, Government of India) Grant - GAP413.

Title: Calcineurin inhibition rescues dopamine induced cytotoxicity comprehensively and attenuates behavioral deficits in a Parkinson's disease model.

Authors: *R. MONDAL[1,2], C. BANERJEE[1,2], S. NANDY[1], M. ROY[1,2], J. CHAKRABORTY[1,2];
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Abstract: Parkinson's disease (PD), a progressive neurodegenerative disorder, is caused by dopaminergic neuronal cell death in substantia nigra region of the brain. To explain this site specific neuronal loss, several studies suggest that dopamine (DA) itself can induce cytotoxicity. The current available therapy for PD is L-DOPA (LD), a precursor for DA synthesis. Whether L-DOPA therapy can accelerate neurodegeneration in PD patients or not is still inconclusive. However, lessening the detrimental effects induced by DA is anticipated to prolong the efficacy of the therapy. Our study determines the role of (i) Monoamine oxidase mediated DA degradation and (ii) dopamine-quinones, the two major governing factors of DA toxicity, on cell survival and mitochondrial homeostasis. Methods including neuronal cytotoxicity assays, confocal imaging and immunoblotting were employed to explore the shared druggable pathways. Our *in vitro* data demonstrate that both these factors enhance Calcineurin (CaN) activity, thereby resulting in mitochondrial translocation of DRP1 which leads to apoptotic mitochondrial fragmentation. Inhibition of CaN-DRP1 axis can prevent DA toxicity as a whole. Further, evaluation of the impact of DA exposure on PD progression in MPTP-induced C57BL/6 mice model (male, 8-10 weeks) of sporadic PD reveal that LD mediated behavioral improvement diminishes with time, mostly because of continued DAergic neuronal death and dendritic spine loss at striatum. CaN inhibition alone, or in combination with LD, can provide prolonged behavioral protection. This protective effect is observed when specifically CaN-DRP1 axis is hindered, whereas inhibiting interaction between CaN and other substrates, including proteins involved in neuro-inflammation(e.g. NFAT), remained ineffective when LD is co-administered. In this study, we conclude that CaN inhibition can mitigate DA toxicity and can rescue PD related behavioral abnormalities by preserving neuronal architecture at striatum. We propose that CaN inhibitors might extend the therapeutic window of LD therapy.

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Poster

PSTR401. Parkinson's Disease: Animal Models

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Program #/Poster #: PSTR401.24/P9

Topic: C.03. Parkinson's Disease

Support: NIH R21 AG072811
NIH RF1 AG079199

Title: Dopaminergic specific accumulation of neuromelanin in mice promotes neuronal damage and leads to parkinsonian-like motor impairment

Authors: *A. DRUMOND-BOCK[1], K. CARTER[1], H. E. BLANKENSHIP[1,2], E. TROYANO-RODRIGUEZ[1], D. WEINSHENKER[3], M. J. BECKSTEAD[1,4];
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Abstract: Intracellular accumulation of the pigment neuromelanin (NM) in dopaminergic neurons of the substantia nigra pars compacta (SNc) is a natural process occurring in the human brain. Although NM could potentially have a protective function neutralizing excessive dopamine (DA), metals and other protein aggregates into lipid rich organelles, its buildup in catecholaminergic areas in aging individuals correlates with neurodegeneration associated with Parkinson's disease (PD). While NM synthesis is well documented in humans and other species, there are no reports of visible aggregation of the pigment in the brains of rats or mice, making it challenging to study this process in a laboratory animal context. To overcome this issue, a recent study promoted age-dependent accumulation of NM in neurons of the SNc through adenoviral-induced expression of human tyrosinase (hTyr), the rate-limiting enzyme for melanin synthesis in peripheral tissues. The consequent increase of NM in the SNc promoted PD tissue phenotype similar to the observed in patients, including nigrostriatal degeneration. We are adapting this technique, inducing dopaminergic-specific expression of hTyr by performing injections of adenovirus containing a floxed *hTyr* gene into the SNc of $DAT^{IREScre}$ mice (transgenic mice expressing *Cre recombinase* gene under the dopamine transporter (*Dat*) gene promoter). Immunofluorescence images revealed large expression of hTyr exclusively in the DA neurons of the SNc 7 days after adenoviral injection and a posterior loss of hTyr and tyrosine hydroxylase (TH) starting at 14 days, culminating with the complete absence of TH-positive cells by 35 days. The progressive loss of TH-positive dopaminergic neurons occurred in parallel with formation of NM granules, observed in unstained brain sections and after Fontana-Masson stain. Patch-clamp recordings of these neurons detected disruption of pacemaker firing as early as 14 days after *hTyr* induced-expression. Finally, behavioral studies confirmed locomotor impairment in these mice, which lost the ability to maintain rotarod balance, spent less time exhibiting ambulatory and exploratory behavior, and spent more time resting. Overall, our results suggest that dopaminergic-specific aggregation of NM in the SNc affects neuronal function and electrophysiological properties and promotes parkinsonian locomotor phenotypes. This tool will enable a further investigation into the interaction between NM accumulation and the progression of PD.

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Poster

PSTR401. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR401.25/P10

Topic: C.03. Parkinson's Disease

Support: NIH Grant ZIAAG000945

Title: Determining the Efficacy of a Novel Daglb-knockout mouse Model for Parkinson's Disease

Authors: D. BASU, *L. WANG, H. CAI;
Natl. Inst. on Aging, Bethesda, MD

Abstract: Our lab previously found that a rare genetic missense mutation of the 2-arachidonoyl-glycerol (2-AG) synthase diacylglycerol lipase β (DAGLB), which is enriched in nigral dopaminergic neurons (DANs), contributes to early onset of Parkinson's Disease (PD). Viral knockdown of *Daglb* in nigral DANs of mice induced a reduction of substantia nigra (SN) 2-AG levels and impaired locomotor skill learning. To further model DAGLB-deficiency in PD, the present study generated a conditional knockout (cKO) model of the same *Daglb* enzyme in mice by crossing a dopamine transport (DAT)-Cre strain with a novel *Daglb* flox/flox strain. The *Daglb* cKO model was evaluated through a series of behavior tests which assessed locomotion, motor skill learning, and non-motor behaviors. Additionally, fiber photometry was used to measure the release of 2-AG in the nigrostriatal pathway. In our results, we were able to verify that the *Daglb* cKO mice had a decreased level of *Daglb* in nigral DANs compared to littermate controls via RNA Scope. However, this reduction did not translate into any further differences between groups. Both *Daglb* cKO and controls performed similarly in open field tests (which measured locomotion) and the rotarod task (which measured motor skill learning). Both groups also performed similarly in non-motor behavioral assays which measured anxiety levels. Furthermore, fiber photometry revealed that *Daglb* cKO mice and control mice had an equivalent level of 2-AG production in SN, meaning the decrease of *Daglb* levels in the cKO did not induce a decrease in nigral 2-AG. Overall, we did not observe any behavioral or physiological phenotypes in this new line of *Daglb* cKO mice. We suspect that the discrepancy of results between *Daglb* cKO mice and our previous viral knockdown approach might be due to a compensatory mechanism through the other diacylglycerol lipase, *Dagla*. Presence of *Dagla* in the mouse nigral DANs was verified in our RNA scope data. Future experiments will use AAV vectors to deliver Cre selectively in the nigral DANs of adult *Daglb* floxed mice and *Dagla* floxed mice. The resulting mouse models may better capture the disease phenotypes.

Disclosures: **D. Basu:** None. **L. Wang:** None. **H. Cai:** None.

Poster

PSTR401. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR401.26/Q1

Topic: C.03. Parkinson’s Disease

Title: Maresin 1 changes reactive microglia from pro-inflammatory to pro-survival states in 6HODA model of Parkinson’s Disease

Authors: *T. E. MPOFU, T. CHANEY, J. M. CALANDRIA, S. KALA BHATTACHARJEE; Neurosci. Ctr. of Excellence, LSUHSC New Orleans, New Orleans, LA

Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disorder that affects the dopaminergic neurons in the substantia nigra. Microglia, the resident macrophage-like cells located in the central nervous system, mediates synaptic pruning, perform phagocytosis of cellular depositions and waste, and release pro and anti-inflammatory responses contributing to neurodegeneration or neuroprotection. Microglia become reactive upon stimulus from neurons or astrocytes determining the type of phenotype these cells acquire. These phenotypes modulate defensive or neuroprotective efforts to modulate neuroinflammation. The pro-inflammatory phenotypes exhibit cytokine responses, while the pro-survival ones are accompanied by high LC3-associated phagocytosis (LAP) that scavenges debris and unfolded fibrillar subproducts like alpha-synuclein fibrils. These two phenotypes corelates with morphological changes. Here we propose that DHA derivative Maresin 1 (Mar1) induce the second step in the polarization to enhance the activation of the LAP phagocytic state, leading to a decrease in the inflammatory signals and a change in the morphology of the microglia in the Sustantia nigra. This hypothesis was tested “in vivo” in a 6- hydroxydopamine (6-HODA) toxicity rat model and “in vitro”, in adult rat brain cultures of microglial cells treated with Tumor necrosis factor (TNF) and interferon alpha (IFNα) or alpha-synuclein (α-syn) fibrils to induce classical activation. In rat culture microglial cells, we used immunocytochemistry to detect p65 nuclear translocation and LC3 vesicles. Mar1 induced a decrease in p65 translocation, elicited LC3-phagocytosis as it was observed by the increase in the LC3-positive vesicles. To confirm these findings, we recorded the changes in phagocytic activity of α-synuclein fibrils tagged with pHRodo, a molecule that changes fluorescent wavelength emission when it reaches the lysosomal lower pH, using Incucyte real-time imaging system. In the 6HODA toxicity model, immunohistochemistry using IBA1 to detect microglial cells in different areas of the rat brain showed that microglia were more abundant, and the shape resembles more to pro-inflammatory when rats treated with saline than when treated intranasally with Mar1.Altogether these data points to a pro-survival role of Mar1 in the polarization of microglia that lay to road to future therapeutical developments for PD.

Disclosures: T.E. Mpofu: None. **T. Chaney:** None. **J.M. Calandria:** None.

Poster

PSTR401. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR401.27/Q2

Topic: C.03. Parkinson's Disease

Support: F99NS124179
R01NS124037

Title: Parkinson'S disease risk gene, cd38, regulates astrocyte gene expression and bioenergetics

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Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disease that affects over 10 million people worldwide. Currently, it is estimated that 80-90% of all PD cases manifest with no identifiable cause. Recent research efforts have worked to pinpoint risk genes and environmental factors that increase risk of PD development. Single-nucleotide polymorphisms in the cluster of differentiation 38 (CD38) gene locus have been associated with increased risk of PD in multiple GWAS studies. CD38 is an ectoenzyme that converts NAD+ to NAM and contributes to maintaining cellular bioenergetics. In the CNS, CD38 is highly expressed in motor regions affected by PD pathology (midbrain). Our own studies further determined that CD38 enrichment is highest in astrocytes and microglia. In the work presented herein, we describe how CD38 influences astrocytic gene expression and mitochondrial function. We further describe how CD38 expression regulates NAD+ and NAM levels in the midbrain. Astrocyte samples for RNA-sequencing were prepared from midbrains of one-year old male C57BL/6J CD38 wild-type (WT), heterozygous (HET), and knockout (KO) mice using our magnetic-activated cell sorting (MACS) method to acquire astrocyte isolates. RNA-sequencing revealed numerous DEGs in pairwise comparisons of CD38 KO to WT mice, with moderate differences between CD38 HET and WT mice. Interestingly, mitochondrial DEG and GO analysis data suggested mitochondrial dysfunction in both CD38 HET and KO mouse astrocytes. In a subsequent set of experiments, mitochondrial function was evaluated in primary astrocyte cultures from CD38 WT, HET, and KO mice utilizing the Seahorse XF platform. CD38 HET and KO astrocytes displayed altered mitochondrial function compared to WTs. In addition to astrocyte-specific evaluations, midbrain tissues from one-year old CD38 WT, HET, and KO were measured for NAD+ and NAM content. Relative to WTs, CD38 KO samples had increased NAD+ and decreased NAM, further suggesting bioenergetic dysfunction. These findings serve to provide direction for future studies

to evaluate the relationship between CD38 function, aging, and vulnerability of neuronal populations compromised in Parkinson's disease.

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Poster

PSTR401. Parkinson's Disease: Animal Models

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Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

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Topic: C.03. Parkinson's Disease

Support: DOD Grant W81XWH-19-0771
DOD Grant W81XWH-19-0772

Title: Long-term alterations in mitochondrial function in the alpha-synuclein preformed fibril rat model of Parkinson's disease

Authors: ***J. K. LEPP**[1], K. M. CROUCHER[1], A. C. STOLL[2], C. J. KEMP[2], J. R. PATTERSON[2], K. C. LUK[3], C. E. SORTWELL[2], S. M. FLEMING[1];
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Abstract: The alpha-synuclein (aSyn) preformed fibril (PFF) model of Parkinson's disease (PD) is widely used to understand the mechanisms that contribute to progressive neurodegeneration in the PD. Mitochondrial dysfunction is well established in the brain and periphery in PD and is known to interact with aSyn. When aSyn PFFs are injected into the striatum, aSyn pathology is found in the cortex and amygdala in addition to the nigrostriatal pathway. However, how aSyn PFFs impact mitochondrial bioenergetics in these regions is not clear. In the present study, the effect of intrastriatal aSyn PFFs on mitochondrial bioenergetics in multiple brain regions was investigated in rats receiving unilateral injections of PBS (n=10) or aSyn PFFs (16 μg; n=10). At approximately 18 months post injection, brains were removed, and mitochondria were isolated from the ipsilateral and contralateral prefrontal cortex (n=5), striatum (n=5), amygdala (n=5), and ventral midbrain (n=5). Oxygen consumption rate (OCR) was measured in the presence of the substrates ADP, Oligomycin, FCCP, and Rotenone/Antimycin A for each sample in triplicate (5ug/well of mitochondria). In the PFF rats there was a significant decrease in basal OCR (pmoles O_2/minute/ug protein) in the ipsilateral striatum and ventral midbrain compared to the contralateral side. In the ipsilateral striatum OCR was significantly lower in response to Rotenone/Antimycin A compared to the contralateral side. In the ipsilateral ventral midbrain OCR was significantly lower compared to contralateral ventral midbrain. There was no difference in OCR between ipsilateral and contralateral sides in the PBS-treated rats. There was

also no effect of PFFs in the prefrontal cortex or amygdala. Protein analysis measuring aSyn, phosphorylated aSyn, and mitochondrial proteins is ongoing. These data indicate that intrastriatal aSyn PFFs alter mitochondrial bioenergetics specifically within the nigrostriatal system despite aSyn pathology in extranigral regions.

Disclosures: **J.K. Lepp:** None. **K.M. Croucher:** None. **A.C. Stoll:** None. **C.J. Kemp:** None. **J.R. Patterson:** None. **K.C. Luk:** None. **C.E. Sortwell:** None. **S.M. Fleming:** None.

Poster

PSTR402. Therapeutic Strategies: Small Molecule Therapeutics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR402.01/Q4

Topic: C.03. Parkinson's Disease

Support: Michael J Fox Foundation Grant (MJFF-021969) to LM and RGS

Title: Evaluation of the pharmacological properties and therapeutic efficacy of the FDA-approved 5HT1F-receptor agonist Lasmiditan in Parkinsonian mice

Authors: *A. ISHII[1], M. J. CORENBLUM[1], J. MEREDITH[2], P. WENE[2], N. MENAKURU[2], J. JANDA[3], R. SCHNELLMANN[3,4], L. MADHAVAN[5,3,4];
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Abstract: Parkinson's disease (PD) is a relentlessly progressive neurodegenerative disorder characterized by debilitating motor deficits and neurocognitive decline over the course of aging. Although symptomatic therapies are available, a major issue faced by PD patients is the lack of treatments that can deter the progression of the disease. Mitochondrial abnormalities are a known feature of PD and many studies indicate an early role for mitochondrial dysfunction in the disease. In this context, we have previously identified 5 hydroxytryptamine 1F (5-HT1F) receptors as novel mediators of mitochondrial biogenesis (MB). We have also demonstrated, in an acute 6-hydroxydopamine (6-OHDA) mouse model, that the administration of a specific 5-HT1F receptor agonist LY344864 enhanced MB and improved locomotor activity. Based on these data, we are currently assessing the therapeutic potential of the FDA-approved 5-HT1F receptor agonist, Lasmiditan, in a more chronic and progressive mouse model of PD (lin61 Thy1-ASyn mice). Firstly, we studied the pharmacokinetic (PK) and pharmacodynamic (PD) properties of Lasmiditan in wild-type and PD mice. Robust levels of both total and unbound Lasmiditan were seen in both plasma and brain, with a Tmax was around 0.5 hours in plasma and just over 1 hour in the brain. Additionally, total brain to plasma ratios were generally >2 and unbound brain to plasma ratios were >1, indicating good brain penetration of Lasmiditan. Examination of protein markers of MB (such as TFAM, NDUFS1, NDUFB8) via western blot, indicated that Lasmiditan was able to significantly induce MB in PD-relevant brain regions such as the striatum and substantia nigra. It was found that 0.3 mg/kg and 1 mg/kg doses were most

efficient at MB induction, with effect saturation usually occurring at the 1mg/kg dose. Given this, we treated 4 mos old WT and Thy1-ASyn mice with Lasmiditan (1mg/kg, IP, alternate dats for 1.5 mos) and are assessing several motor and cognitive behaviors in the mice, as well as analyzing the mouse brains at cellular and molecular levels. Ongoing experiments will also additionally examine 10-month-old aged mice to evaluate the efficacy of Lasmiditan as the first molecular pathogenesis-based therapy for PD.

Disclosures: A. Ishii: None. **M.J. Corenblum:** None. **J. Meredith:** None. **P. Wene:** None. **N. Menakuru:** None. **J. Janda:** None. **R. Schnellmann:** None. **L. Madhavan:** None.

Poster

PSTR402. Therapeutic Strategies: Small Molecule Therapeutics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR402.02/Q5

Topic: C.03. Parkinson's Disease

Support: Michael J Fox

Title: Muscarinic M5 Receptor as a Therapeutic Target to Decrease Motor Symptoms in Mouse Models of Parkinson's Disease

Authors: *D. HALL[1], N. E. CHAMBERS[2], D. NABERT[2], P. WAGNER[1], M. MILLETT[3], M. S. MOEHLE[2];
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Abstract: Parkinson's disease (PD) is a neurodegenerative movement disorder associated with debilitating motor deficits such as bradykinesia and akinesia. Loss of nigral dopamine neurons and the development of Lewy bodies in the brain precedes these motor symptoms. Past research has investigated the importance of acetylcholine receptors as modulators of Parkinsonian motor symptoms; however the contributions of the M5 muscarinic acetylcholine receptor (M5) has not been investigated for therapeutic efficacy. M5 has unique expression profile largely rectricted to dopaminergic cells, and are present on nigrostriatal DA terminals the striatum and cell bodies in the substantia nigra. Data from drug dependency studies suggest that the net activity of M5 is pro-dopaminergic and suggests that M5 positive allosteric modulators (PAM) may be beneficial in Parkinson's disease.. To test this hypothesis we used two Parkinsonian mouse models. In the first set of experiments we used mice with a unilateral medial forebrain bundle 6-OHDA lesion to model severe Parkinson's disease with over 95% loss of unilateral DA neurons. Three weeks after lesion surgery we performed a motor battery consisting of Erasmus ladder, cylinder test, and forehand adjusting steps test. Consistent with our expectations, 6-OHDA lesioned mice showed deficits in the use of the lesioned forehand and show bradykinesia in the Erasmus ladder compared to normal age-matched controls. Using a within-subjects design, mice received M5 PAM or NAM, both with and without L-DOPA. There were no effects of M5 PAM and NAM on Parkinsonian motor deficits, likely due to the large extent of DA lesion. Additionally, we tested

the effects of M5 PAM on L-DOPA-induced dyskinesia. In the second set of experiments we used the preformed fibril model (PFF) of Parkinson's disease, in which 50-70% of DA neurons remain, and monomer controls. To investigate how the progression of DA loss may play a role in M5's ability to modulate motor symptoms, we tested PFF animals at 3 and 6 months post-injection. Following recovery from surgery, animals were tested for their baseline motor phenotype in the Erasmus ladder. Excitingly, PFF animals showed deficits vs. monomer controls at both 3 and 6 months. These deficits were modified by treatment with M5 PAM, suggesting that increasing striatal DA release with M5 PAM may be a viable treatment strategy in early and mid-stage Parkinson's disease.

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Poster

PSTR402. Therapeutic Strategies: Small Molecule Therapeutics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR402.03/Q6

Topic: C.03. Parkinson's Disease

Support: Weston Brain Institute (WBI)

Title: Positive allosteric modulation of $mGlu_2$receptors with AZD8529 alleviates L-DOPA-induced dyskinesia and psychosis-like behaviours in the MPTP-lesioned marmoset

Authors: *J. SHAQFAH[1], I. FROUNI[1,2], C. KWAN[1], D. BÉDARD[1], S. G. NUARA[3], J. C. GOURDON[3], A. HAMADJIDA[1], P. HUOT[1,2,4,5];
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Abstract: AZD8529 is a highly selective metabotropic glutamate 2 ($mGlu_2$) receptor positive allosteric modulator (PAM) that has undergone clinical trials for schizophrenia and smoking cessation. In previous studies, we demonstrated that the selective $mGlu_2$ PAMs LY-487,379 and CBiPES alleviated L-3,4-dihydroxyphenylalanine (L-DOPA)-induced dyskinesia and psychosis-like behaviours (PLBs) in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned marmoset model of Parkinson's disease (PD). However, neither drug has clinically relevant pharmacological properties, contrary to AZD8529, which could be repurposed for PD if efficacious in pre-clinical studies. To assess the effect of AZD8529 on L-DOPA-induced dyskinesia and PLBs in the MPTP-lesioned marmoset, marmosets (n=6) were rendered parkinsonian by repeated MPTP injections followed by a recovery period to ensure development of a stable phenotype. Dyskinesia and PLBs were elicited by daily oral administration of L-DOPA/benserazide (L-DOPA/vehicle) for a minimum of 30 days. On experimental days,

marmosets were injected with L-DOPA (15/3.75 mg/kg subcutaneously [s.c.]) along with vehicle or varying concentrations of AZD8529 (0.1, 0.3, 1, and 10 mg/kg s.c.). These doses were selected based on the pharmacokinetic profile of AZD8529 in the marmoset, which will be reported separately. After treatment, marmosets were individually recorded in observation cages for 6h, and this footage was analysed for dyskinesia, PLBs, and parkinsonism by an experienced, blinded, rater. The results showed a significant reduction in global dyskinesia severity (by 45%, 46%, 61%, 70%, respectively; $P<0.001$ for all doses), and on-time with disabling dyskinesia (by 83%, 86%, 99%, and 97%, respectively; $P<0.001$ for all doses) when compared to L-DOPA/vehicle. Similarly, there was a significant reduction in global PLB severity (by 44%, 45%, 58%, and 64%, respectively; $P<0.001$ for all doses), and on-time with disabling PLBs (by 82%, 83%, 97%, 94%, respectively; $P<0.001$ for all doses) when compared to L-DOPA/vehicle. Additionally, AZD8529 significantly increased the duration of the anti-parkinsonian action of L-DOPA at doses of 0.3 mg/kg and above (by 29%, 21%, and 26%, respectively; $P<0.05$ for all doses). Our results demonstrate the potential of AZD8529 and $mGlu_2$ positive allosteric modulation for alleviating L-DOPA-induced dyskinesia and PLBs and amplifying L-DOPA anti-parkinsonian effects.

Disclosures: **J. Shaqfah:** None. **I. Frouni:** None. **C. Kwan:** None. **D. Bédard:** None. **S.G. Nuara:** None. **J.C. Gourdon:** None. **A. Hamadjida:** None. **P. Huot:** 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.; Montreal Neurological Institute - McGill University.

Poster

PSTR402. Therapeutic Strategies: Small Molecule Therapeutics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR402.04/Q7

Topic: C.03. Parkinson’s Disease

Support: R15NS130532-01 (RKL)
1R03NS088395-01A1 (RKL)
1R15NS093539-01 (RKL)
1R21NS107960-01 (RKL)

Title: Microglia/macrophage repopulation with a 14-day pulse of PLX5622 is associated with better spatial reference memory, fewer inclusions, lower insoluble alpha-synuclein, and larger-sized inclusions in fibril-infused aged mice

Authors: ***M. ABBAS**[1], T. N. BHATIA[1], A. S. JAMENIS[1], R. N. CLARK[1], K. M. MINER[1], M. N. CHANDWANI[1], L. O'DONNELL[1], K. C. LUK[3], J. CHEN[4], X. HU[5], R. K. LEAK[2]; [1]Grad. Sch. of Pharmaceut. Sci., [2]Duquesne Univ., Duquesne Univ., Pittsburgh, PA; [3]Univ. of Pennsylvania, Univ. of Pennsylvania, Philadelphia, PA; [4]Univ. of Pittsburgh Sch. of Med., Univ.

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Abstract: Alpha-synucleinopathies such as dementia with Lewy bodies (DLB) are characterized by pathologically phosphorylated, insoluble alpha-synuclein as well as microglia/macrophage reactivity. We have modeled the limbic Lewy-related pathologies of DLB with injections of preformed alpha-synuclein fibrils (PFFs) in the bulbar anterior olfactory nucleus (OB/AON) of 18-month-old, nontransgenic mice. These mice develop impairments in spatial reference memory in the Y-maze and Lewy-like pathology across the limbic rhinencephalon. In patients with DLB, microglia/macrophages display dystrophic and gnarled morphologies associated with cellular senescence. Therefore, we tested the hypothesis that depleting and repopulating microglia/macrophages with transient dietary delivery of PLX5622, an inhibitor of the colony-stimulating factor receptor 1 (CSF1R), may hold therapeutic potential in our experimental model. We infused mice with PFFs at 18 months of age, and one week later, we administered 1200 mg PLX5622 per kg chow for 14 days. After an additional seven weeks, we noted more entries in the novel arm of the Y maze in the microglia/macrophage-repopulated group, suggesting superior spatial reference memory, particularly in female mice. Male mice treated with PLX5622 became slightly hyperactive, suggesting that caution must be exercised with use of CSF1R inhibitors. When we counted $Iba1^+$ and $pSer129^+$ objects with AI/Deep Learning, we found that $pSer129^+$ inclusion counts were only lowered by PLX5622 withdrawal in male mice. In OB/AON tissue extracts subjected to ultracentrifugation of the Triton X-insoluble fraction, levels of nonionic detergent-insoluble alpha-synuclein were also lowered by transient PLX5622 in males. Unexpectedly, the average sizes of the $pSer129^+$ inclusions were increased by microglia/macrophage repopulation in both males and females. Spatial reference memory was negatively correlated with $pSer129^+$ inclusion counts and with $Iba1^+$ hydraulic radii (a measure of microglia/macrophage reactivity), but positively correlated with average inclusion size. We therefore conclude that depletion and repopulation of microglia/macrophages modifies limbic alpha-synucleinopathy by lowering inclusion counts and detergent-insoluble alpha-synucleinopathy but also raising inclusion sizes, albeit in sex-biased manners. Further studies are warranted to identify how repopulated microglia/macrophages increase inclusion sizes while lowering inclusion counts, and to test if fewer but larger inclusions are less toxic than smaller, more abundant inclusions.

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Poster

PSTR402. Therapeutic Strategies: Small Molecule Therapeutics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR402.05/Q8

Topic: C.03. Parkinson’s Disease

Support: Michael J Fox Foundation

Title: Exploratory omics study reveals potential roles of sigma-2 receptor modulators in AAV1/2A53T-aSyn rat model of Parkinson's disease

Authors: *A. REAVER[1], B. N. LIZAMA[1], K. PANDEY[2], D. M. DUONG[3], N. SEYFRIED[3], A. O. CAGGIANO[1], M. E. HAMBY[1];
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Abstract: Synucleinopathies, including Lewy body dementia (DLB) and Parkinson's disease (PD) comprise the second most common neurodegenerative disease worldwide. In synucleinopathies, alpha-synuclein (αSyn) oligomers cause neuronal dysfunction. The sigma-2 receptor (S2R) is involved in pathways associated with age-related disease including autophagy, oxidative stress and intracellular trafficking. We have shown that S2R modulation *in vitro* reverses αSyn oligomer-induced deficits in neuronal vesicle trafficking. Given this data and S2R's functional overlap with pathways affected in PD, we hypothesized that S2R modulation would alter PD-related transcripts and pathways *in vivo*. Mutations in the αSyn gene, such as A53T, cause familial PD. Ectopic expression of human A53T αSyn *in vivo* causes production of toxic αSyn oligomers, resulting in neuroinflammation and loss of dopaminergic neurons. Using this model, we assessed the impact of two chemically distinct S2R modulators, CT1812, an investigational therapeutic currently in Phase 2 clinical trials for DLB and AD, and CT2168 for their ability to modify disease-relevant pathways at the transcriptional and protein level. Human A53T-αSyn (AAV1/2-hA53T-aSyn) or AAV1/2 empty vector was administered unilaterally into the right substantia nigra of female Sprague Dawley rats. hA53T-αSyn AAV-injected animals were dosed with vehicle, CT1812, or CT2168 (3mg/kg) and empty vector-injected animals dosed with vehicle (N=14 per group) for ~45 days. Ipsilateral striatal tissue was harvested for proteomics and RNAseq. Differential expression and pathway analyses identified transcripts, proteins, and pathways significantly altered by mutant αSyn as compared to control. Significant changes included increases in inflammatory pathway-related genes and proteins ($p \leq 0.05$) and decreases in dopamine pathway-related proteins ($p \leq 0.01$), demonstrating that the A53T αSyn *in vivo* model recapitulates key features of synucleinopathies. To investigate the role of S2R, we next analyzed the effect of CT1812 and CT2168 on the striatal transcriptome and proteome in the disease model. Both compounds altered transcripts and proteins involved in signal transduction and the glutamatergic pathway, and downregulated inflammation-related proteins and transcripts that were upregulated in the disease model ($p \leq 0.05$). In sum, these omics analyses suggest that S2R modulators may impact key pathways disrupted in synucleinopathies, including neuroinflammation and stress response. These findings support further development of S2R modulators for synucleinopathies. This research was supported by the Michael J. Fox Foundation.

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Poster

PSTR402. Therapeutic Strategies: Small Molecule Therapeutics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR402.06/R1

Topic: C.03. Parkinson's Disease

Support: NIH Grant RO3NS095063
NIH Grant RO1NS102337

Title: Potent Antioxidant and Mitochondrial-protect Effects of ATH434, a Novel Inhibitor of α-Synuclein Aggregation with Moderate Iron-binding Affinity

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Abstract: Iron is essential for supporting energy metabolism, mitochondrial function, and maintaining cellular redox potential. Excess labile iron can generate reactive oxygen species in mitochondria which, if unchecked, can lead to sustained oxidative stress and eventual cell death. Parkinson's disease (PD) and Multiple System Atrophy (MSA) are neurodegenerative conditions characterized by regional excess brain iron and resultant oxidative stress in areas of pathology, leading to clinical trials of iron binding small molecules for their treatment. ATH434, a small molecule drug candidate with moderate ferric iron affinity (K_d 10^{-10}, Finkelstein 2017), promotes cellular iron efflux, reduces excess brain iron and aggregated α-synuclein, improves neuronal survival, and restores motor performance in murine PD and MSA models. ATH434 is currently in phase 2 MSA trials. Deferiprone (DFP) is a high ferric iron affinity drug (K_d 10^{-21}, Hider 2014) approved for treating systemic iron-overload disorders. Because DFP is designed to reduce cellular iron stores, it has potential for maladaptive pharmacological effects in healthy cells. DFP has also demonstrated efficacy in preclinical PD models. The required doses, however, are higher than expected given its ready brain access and high ferric iron affinity, suggesting that ATH434 may possess unique beneficial properties.In this study, we investigated the efficacy of ATH434 and DFP as potential antioxidants and mitochondrial protectants using a menadione-induced model of oxidative stress in the glutamatergic neuronal HT22 cell line. ATH434 restored the reduced mitochondrial membrane potential after menadione-stressed neurons, demonstrating an ability to preserve mitochondrial function. ATH434 also demonstrated in-solution antioxidant capacity in an ABTS radical scavenging assay. DFP was ineffective in both of these assays. The chemical antioxidant effect and cellular effects with an ATH434 analog possessing dramatically reduced iron binding affinity suggests a direct interaction between ATH434 and free radicals. Superoxide production in menadione-stressed neurons, as determined by changes in the indicator MitoSOX, was not reduced by ATH434 or iron chelators such as DFP, suggesting that superoxide is not the interactant. Reductions in peroxide and hydroxyl radicals may therefore underlie the direct antioxidant potential of ATH434. Together, these results suggest that antioxidant activity may be an important contributor to the efficacy of ATH434 in neurodegenerative disorders characterized by excess labile central iron, thus enhancing the efficacy of its moderate iron binding.

Disclosures: **D. Bailey:** 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.; Alterity Therapeutics. **R. Nihlawi:** 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.; Alterity Therapeutics. **M.J. Bradbury:** A. Employment/Salary (full or part-time):; Alterity Therapeutics. **D.J. Kosman:** 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.; Alterity Therapeutics.

Poster

PSTR402. Therapeutic Strategies: Small Molecule Therapeutics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR402.07/R2

Topic: C.03. Parkinson's Disease

Title: Inhibition of the Y-chromosome gene, SRY, attenuates neuroinflammation and disease progression in acute and progressive rat models of Parkinson's disease.

Authors: *D. KIM[1,3], P. PINARES-GARCIA[3,2], E. JACKSON[1], M. MICHALAS[1], J. THOMPSON[1], T. SIMPKINS[1], J. LEE[1,3,2];
[1]Psychiatry, [2]Anat. and Developmental Biol., Monash Univ., Clayton, Australia; [3]Ctr. for Endocrinol. and Metabolism, Hudson Inst. of Med. Res., Clayton, Australia

Abstract: Parkinson's disease (PD) is characterized by loss of dopamine (DA) neuron in the substantia nigra pars compacta (SNc). Whilst the cause(s) of PD is unknown, increasing evidence points to the male-sex as a strong risk factor. Men are twice as likely to be diagnosed with PD than women with an earlier age of onset and faster disease progression. We previously showed that aberrant upregulation of the Y-chromosome gene, *SRY*, mediates a novel male-specific mechanism of DA cell loss (Lee et al., 2019, PNAS). Here, we assessed the i) cellular relationship between SRY expression and disease progression and ii) consequence of SRY suppression on neuroinflammation and mitochondrial function in pre-clinical rat and cellular models of PD. Time-course studies in the acute 6-OHDA and progressive rotenone rat PD models revealed progressive losses in nigral TH neurons, which was paralleled by increase in activated OX42+ microglia with disease progression in both models. SRY and OX42 co-immunofluorescence revealed an increase in SRY-positive, but not SRY-negative, activated microglia with disease progression in both PD models. In contrast, SRY-positive TH neurons were not affected by disease progression in both PD models, suggesting DA neurons that expressed SRY are more resilient. Remarkably, suppressing SRY expression in male rats, via nigral SRY antisense oligonucleotide (ASO) infusions, at 4 weeks post rotenone treatment reversed the progression of motor deficits and nigral degeneration, and attenuated microglial activation ($P<0.001$ vs sense control, two-way ANOVA). Moreover, qRT-PCR analysis revealed

that rotenone induced up-regulation of pro-inflammatory cytokine or anti-oxidant genes in male rat SNc were all diminished by ASO infusion. SRY siRNA pre-treatment in the human male neuronal cell line, M17, diminished rotenone-induced upregulation of SRY, SOD2 and BAX protein, without altering oxygen rate consumption or TOM20 expression and content, suggesting that SRY regulates antioxidant capacity but not mitochondrial function in rotenone-injured neurons. Collectively, these results demonstrate a positive relationship between SRY and neuroinflammation and suggest that microglial SRY inhibition may present a novel disease-modifying strategy for male PD patients.

Disclosures: D. Kim: None. **P. Pinares-Garcia:** None. **E. Jackson:** None. **M. Michalas:** None. **J. Thompson:** None. **T. Simpkins:** None. **J. Lee:** None.

Poster

PSTR402. Therapeutic Strategies: Small Molecule Therapeutics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR402.08/R3

Topic: C.03. Parkinson's Disease

Support: NIH/NINDS R56 NS109608
NIH R01 NS122805-01
Arizona Biomedical Research Commission Grant ADHS18-198846

Title: In a hemi-lesioned model of L-DOPA-induced dyskinesia neuronal firing was reduced in the un-lesioned striatum and ketamine reduced burst-like firing in striatal neurons bilaterally

Authors: *A. VISHWANATH[1,2], M. J. BARTLETT[3,4], A. KEENER[2], T. FALK[3,5], S. L. COWEN[2];
[2]Psychology, [3]Neurol., [4]Surgery, [5]Pharmacol., [1]The Univ. of Arizona, Tucson, AZ

Abstract: Parkinson's Disease (PD) is a neurodegenerative disorder associated with the loss of dopamine producing neurons in the substantia nigra pars compacta. While levodopa (L-DOPA) is the gold standard treatment for PD, long-term use often results in L-DOPA Induced Dyskinesia (LID), a debilitating condition associated with aberrant facial and limb movement and dystonia. LID symptoms are also correlated with a fine-tuned 80 Hz oscillation in the primary motor cortex (M1) and sub-regions of the basal ganglia. LID and PD have been shown to increase firing rates and burst firing in cortical and striatal neurons. Sub-anesthetic doses of ketamine, a N-methyl-D-aspartate receptor antagonist, have been shown to reduce LID symptoms in preclinical models and reduce burst firing in cortical neurons. This study sought to identify changes in neural activity associated with LID and the treatment of LID with ketamine. **We hypothesized that burst firing of M1 and striatal neurons would be associated with LID and that ketamine would reduce burst activity along with behavioral measures of dyskinesia.** Methods: An animal model of LID was produced using the 6-hydroxydopamine hemi-lesioned rat model of PD. PD animals were administered L-DOPA (12 mg/kg >10

consecutive days) to establish stable LID. Dyskinetic and sham-lesioned rats were implanted bilaterally with 16-tetrode hyperdrives (M1: AP: 1.5, ML: ±2.2, DV: <2mm, striatum: AP: 1.5, ML: ±2.2, DV: >3mm). The LID (n = 5) and sham-lesioned (n = 5) rats were administered L-DOPA (12 mg/kg, *i.p.*) or saline followed by ketamine (20 mg/kg, *i.p.*). Local-field potentials and single-unit activity (M1: n >1000; striatum: n = 198 neurons) were recorded. Contrary to our predictions, the data indicated that LID did not change the firing rates of medium spiny neurons (MSNs) in the lesioned hemisphere, but reduced MSN firing in the un-lesioned hemisphere. This suggests a mechanism whereby neural activity in the dopamine-intact hemisphere compensates for persistent dopamine depletion in the lesioned hemisphere. We also observed that ketamine reduced striatal and M1 burst firing in the lesioned and un-lesioned hemispheres. Future analyses will investigate interactions between M1 and striatum by measuring cross-correlations between regions and spike-field coupling with LID-associated oscillatory activity.

Disclosures: A. Vishwanath: None. **M.J. Bartlett:** None. **A. Keener:** None. **T. Falk:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); TF has a patent for the use of ketamine as a novel treatment for levodopa-induced dyskinesia associated with Parkinson's disease, that has been licensed to PharmaTher Inc.. **S.L. Cowen:** None.

Poster

PSTR402. Therapeutic Strategies: Small Molecule Therapeutics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR402.09/R4

Topic: C.03. Parkinson's Disease

Title: Neuroprotective effects of caffeine, camellia sinensis and telfairia occidentalis in MPTP-induced PD in female Swiss mice

Authors: *A. O. IMAM-FULANI, A. O. SHUAIB, J. INYANG, H. AFOLABI, H. OLORUNOJE, O. ALIMI, O. BABATUNDE, O. ADEKUNLE, O. ONOSHI, E. A. MAKANJUOLA, T. E. ESO, T. T. OGUNSESAN;
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Abstract: L-dopa is the most commonly used drug for managing Parkinson's Disease (PD). However, most L-dopa-treated patients develop complicated motor fluctuations and dyskinesia. Also, Adenosine slows movement and becomes more active as Parkinson's progresses, so targeting A_2A receptor may help improve movement when combined with dopamine-replacement therapy such as L-dopa. Caffeine has been linked to dopamine pathways, although there are still controversies on this. Epigallocatechin gallate (EGCG) in Camellia Sinensis (CS) have also been implicated in neuroprotection especially in AD. However, Telfeiria Occidentalis (TO), despite its widely explored systemic functions, there is still sparsity of information on the neuroprotective effect. Research on natural dopamine supplements is not as comprehensive considering the global significance. We therefore studied the effects of caffeine, CS and TO

saponins on antioxidant, inflammatory parameters and motor activity in MPTP-induced PD in female Swiss mice. 40 mice (20-30g) were used for the study, 35 were injected MPTP (ip) to induce PD. Mice were grouped into 8 (n=5); Control, were healthy mice administered 0.2ml of vehicle, Groups 2-8 were MPTP-induced PD administered; 0.2ml vehicle; 10mg/kg L-Dopa; 10mg/kg caffeine; 5mg/kg caffeine; 400mg/kg CS; 200mg/kg CS and 25mg/kg TO respectively for 3 weeks. Behavioral tests were performed using pole test, beam test and open field maze for motor activity and anxiety. Brain, liver and heart tissues were collected for antioxidant (SOD, CAT, GSH), inflammatory (TNFα, IL1-α), oxidative stress (MDA) markers, and serum lipid profile (TC, TG, HDL) assays. Brain tissues were also collected for IHC. TH and α-synuclein (α-syn) were assessed to account for dopamine loss. Results on brain showed CAT and GSH loss in PD mice, and was restored by caffeine, CS and TO administration. No change occurs for SOD across all groups. MDA was elevated in PD but was significantly reduced with the treatments. TNFα and IL1-α elevated in PD mice was also reduced close to normal in treated groups. Motor activity in the PD was impaired compared to control and treated groups with enhanced activity. Biochemical analysis showed that caffeine, CS, TO showed anti-inflammatory and antioxidant properties in the brain and other tissues. All treated groups showed enhanced serum lipid profile compared to PD model. More importantly, the IHC showed that TH was significantly increased in the treated groups and α-syn expression showed significant reduction in treated groups. Collectively, this study revealed that there was enhance motor activity and neuroprotection with caffeine, CS and TO treatment in MPTP-induced PD.

Disclosures: A.O. Imam-Fulani: None. **A.O. Shuaib:** None. **J. Inyang:** None. **H. Afolabi:** None. **H. Olorunoje:** None. **O. Alimi:** None. **O. Babatunde:** None. **O. Adekunle:** None. **O. Onoshi:** None. **E.A. Makanjuola:** None. **T.E. Eso:** None. **T.T. Ogunsesan:** None.

Poster

PSTR402. Therapeutic Strategies: Small Molecule Therapeutics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR402.10/R5

Topic: C.03. Parkinson’s Disease

Support: Michael J. Fox Foundation

Title: Unbiased proteomic and transcriptomic analysis of sigma 2 receptor modulation in an in vivo model of synucleinopathy

Authors: *B. N. LIZAMA[1], A. REAVER[1], K. PANDEY[3], D. M. DUONG[4], N. T. SEYFRIED[4], A. O. CAGGIANO[2], M. E. HAMBY[1];
[1]Cognition Therapeutics, Inc., Pittsburgh, PA; [2]Cognition Therapeutics, Inc., Purchase, NY; [3]Emtherapro, Atlanta, GA; [4]Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Synucleinopathies, which include Parkinson’s disease and dementia with Lewy bodies (DLB), comprise the second most prevalent neurodegenerative disease worldwide. Alpha

synuclein (αSyn) oligomers are the toxic form of αSyn protein contributing to neurodegeneration. αSyn oligomers can bind to synapses and spread trans-synaptically to anatomically connected regions. Small molecule modulators of the sigma 2 receptor (S2R) block αSyn oligomers from binding to neurons and reverse oligomer-mediated trafficking deficits *in vitro*. To elucidate the mechanisms by which the S2R impacts aspects of synucleinopathies, S2R modulators CT1812 and CT2168 were tested in an *in vivo* preformed fibril (PFF) model of pathological αSyn spreading. Using proteomics and transcriptomics, we assessed proteins, transcripts, and pathways in the PFF model that were altered by S2R modulators.
aSyn PFF or monomer was injected into the striatum of female Sprague Dawley rats. PFF-treated rats were dosed once daily *p.o.* (3mg/kg) with vehicle, CT1812, or CT2168, and monomer-treated rats dosed with vehicle, for ~60 days. Striatal tissue was harvested for proteomics (TMT mass spectrometry), and substantia nigra was harvested for RNAseq (N=10 per group), followed by differential expression and pathway analyses (Metacore, STRING).
Compared to monomer, αSyn PFF significantly decreased tyrosine hydroxylase (TH) and dopamine-related signaling proteins and transcripts ($p \leq 0.05$). αSyn PFF increased proteins and transcripts related to the immune response and significantly enriched pathways related to synapses and vesicle cycling ($p \leq 0.05$). We next analyzed the effects of CT1812 and CT2168 in the disease model. While a subset of significant proteins/transcript changes were unique to each compound, we observed overlapping changes related to cholesterol transport, metabolism, and cell survival ($p \leq 0.05$). All significantly differentially expressed proteins (47) and transcripts (74) in common between the two compound treatments exhibited the same directionality of change, including an increase ($p \leq 0.05$) in mRNA expression of cerebral dopamine neurotrophic factor (*Cdnf*), a neuroprotective protein. Given that each compound is chemically distinct, these data suggest that these common changes are driven by S2R.
Ongoing work includes mechanistic studies to further elucidate the mechanism of action of S2R modulation. These findings demonstrate that S2R ligands can modulate pathways associated with synucleinopathy and support further clinical development with CT1812, currently in Phase 2 clinical trials for DLB (NCT05225415). This work was supported by the Michael J. Fox Foundation.

Disclosures: **B.N. Lizama:** A. Employment/Salary (full or part-time):; Cognition Therapeutics, Inc. **A. Reaver:** A. Employment/Salary (full or part-time):; Cognition Therapeutics, Inc.. **K. Pandey:** None. **D.M. Duong:** None. **N.T. Seyfried:** None. **A.O. Caggiano:** A. Employment/Salary (full or part-time):; Cognition Therapeutics, Inc. **M.E. Hamby:** A. Employment/Salary (full or part-time):; Cognition Therapeutics, Inc..

Poster

PSTR402. Therapeutic Strategies: Small Molecule Therapeutics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR402.11/R6

Topic: C.03. Parkinson’s Disease

Support: NIH Grant NS109608
NIH Grant NS122805-01
Arizona Biomedical Research Commission (ABRC) Grant ADHS18-198846

Title: Pravastatin sensitizes parkinsonian rats to L-DOPA and blocks the long-term anti-dyskinetic activity of sub-anesthetic ketamine

Authors: *C. J. STOPERA[1], M. J. BARTLETT[5], S. L. COWEN[2], S. J. SHERMAN[3], T. FALK[4];
[1]Neurosci. GIDP, [2]Dept. of Psychology, [3]Dept. of Neurol., [4]Dept. Of Neurol., The Univ. of Arizona, Tucson, AZ; [5]Dept. of Neurol., Col. of Med., Tucson, AZ

Abstract: Our lab has shown that sub-anesthetic ketamine-treatment (10-hrs; 5 x 20 mg/kg *i.p.*; 2-hrs apart) both attenuates and provides long-term reduction of L-DOPA-induced dyskinesia in a rat 6-hydroxydopamine (6-OHDA) Parkinson's disease (PD) model. Additionally, our group also found that the long-term anti-dyskinetic effects of ketamine were brain-derived neurotrophic factor (BDNF)-dependent. HMG-CoA reductase inhibitor Pravastatin, a polar compound, has been shown by other groups to block the long-term anti-depressive activity of ketamine by interfering with the direct binding of ketamine to the receptor for BDNF, the TrkB receptor. Therefore, we tested if two types of statins, Pravastatin and Lovastatin that differ in polarity, also interfere with the anti-dyskinetic activity of ketamine. Unilateral 6-OHDA lesioned male Sprague-Dawley rats (n=12-17 per group) were pre-treated for 14 days with either vehicle, pravastatin (10 mg/kg; *s.c.*), or lovastatin (10 mg/kg; *i.p.*), and then maintained with daily injections of the treatment for an additional 14 days. During the treatment phase, rats were also simultaneously primed with daily L-DOPA injections (6 mg/kg; *i.p.*). The rats were treated with either vehicle or ketamine (20 mg/kg; *i.p.*) via the 10-hr treatment protocol on days 0 and 7 of the study, with no additional treatment on day 14. Limb, axial, and orolingual abnormal involuntary movements (AIMs) were scored every 3-4 days by a blinded investigator. On Days 0, 7, and 14, ketamine by itself and ketamine combined with lovastatin (the non-polar statin) significantly attenuated AIMs ($p<0.05$) compared to the vehicle treated group. These scores were not significantly different when comparing ketamine by itself to ketamine combined with lovastatin. Additionally, lovastatin by itself provided temporary reduction of AIMs score compared to vehicle ($p<0.01$) that did not persist long-term on Day 14. In contrast, on Day 0, pravastatin by itself significantly increased AIMs score compared to the vehicle treated group ($p<0.05$). When ketamine was combined with pravastatin (the polar statin) on Day 14, pravastatin blocked the long-term effects of ketamine, causing a significant increase in the AIMs score. This data suggests that the polar statin drug pravastatin sensitized the parkinsonian rats upon first exposure of L-DOPA and further interferes with the long-term anti-dyskinetic effects of ketamine, whereas the non-polar statin drug lovastatin does not interfere, and actually provides some anti-dyskinetic effects on its own. This data provides further support for the use of non-polar statin drugs when required by a patient with Parkinson's disease taking L-DOPA.

Disclosures: C.J. Stopera: None. **M.J. Bartlett:** None. **S.L. Cowen:** None. **S.J. Sherman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SJS and TF have a patent for the use of ketamine as a novel treatment for levodopa-induced dyskinesia associated with Parkinson's disease, that has been licensed to PharmaTher Inc.. **T. Falk:** E. Ownership Interest (stock, stock options, royalty,

receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SJS and TF have a patent for the use of ketamine as a novel treatment for levodopa-induced dyskinesia associated with Parkinson's disease, that has been licensed to PharmaTher Inc...

Poster

PSTR402. Therapeutic Strategies: Small Molecule Therapeutics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR402.12/R7

Topic: C.03. Parkinson's Disease

Title: Deep phenotyping of Polg (D257A) mitochondrial DNA mutator mouse - a mechanistic model of primary mitochondrial dysfunction.

Authors: J. KAHN[1], C. QUINN[1], Q. XU[1], L. YAO[1], N. G. HATCHER[1], K. HAWKINS[3], E. MCEACHERN[1], W. WEI[3], J. RICHARDSON[3], J. BROWNLEES[3], D. HENZE[1], *R. DESAI[2], ***R. DESAI**[3];
[1]Merck Res. Labs., West Point, PA; [2]Merck Res. Labs., London, United Kingdom; [3]MSD Discovery Centre, UK, London, United Kingdom

Abstract: Mitochondrial dysfunction underlies deficits in bioenergetics and degeneration of neurons in several neurodegenerative diseases. The Polg mutator ($Polg^{D257A}$ knock-in mutant) mouse model and cultured mouse embryonic fibroblasts (MEFs) from this model recapitulate the pathological elements of age-related somatic mitochondrial dysfunction observed in neurodegenerative and metabolic muscle diseases; and therefore could prove to be a valuable model in which to test drug efficacy and establish quantifiable biomarkers of mitochondrial dysfunction. $Polg^{D257A}$ mice demonstrate increasing locomotive deficits with age compared to littermate controls in open field locomotion and accelerating rotarod measures. In 8-10 month old $Polg^{D257A}$ mice, there was significant elevation of plasma lactate, 3-hydroxybutyrate, TCA metabolites (e.g. fumarate, malate, succinate and 2-ketoglutarte) and amino acids such as alanine, threonine, and isoleucine. In CSF, there were decreases in ascorbate, myo-inositol, amino acids, and TCA metabolites. Concurrent with elevated plasma alanine, we observed elevated levels of downstream 1-deoxysphingolipids in brain extracts from $Polg^{D257A}$ mice by LC-MS/MS quantification. Specifically, alanine-derived deoxydihydroceramides (doxDHCers) and deoxyceramides (doxCers) were elevated in Polg brain extracts and exhibited marked age-dependent increases compared to age-matched wild type controls. Furthermore, $Polg^{D257A}$ mutant MEFs show reduced oxidative consumption rate and increased glycolytic respiration when compared to wild type controls as measured by Seahorse mitochondrial stress test. High-passage $Polg^{D257A}$ MEFs (passage 16+) exhibit increased lactate production, decreased mitochondrial membrane potential and increased mtDNA copy number, suggestive of deficits in mitochondrial quality control. Further omics evaluation of the model is planned to enable an unbiased approach towards identifying a deficit signature for the mouse. Establishing quantifiable biochemical endpoints in both *in vitro* and *in vivo* systems against a common genotype and mitochondrial

deficit, enabled identification and validation of early and sensitive biomarkers for testing the efficacy of novel mitochondrial-targeted therapeutics.

Disclosures: **J. Kahn:** None. **C. Quinn:** None. **Q. Xu:** None. **L. Yao:** None. **N.G. Hatcher:** None. **K. Hawkins:** None. **E. McEachern:** None. **W. Wei:** None. **J. Richardson:** None. **J. Brownlees:** None. **D. Henze:** None. **R. Desai:** None. **R. Desai:** None.

Poster

PSTR402. Therapeutic Strategies: Small Molecule Therapeutics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR402.13/R8

Topic: C.03. Parkinson's Disease

Support: NIH/NINDS R56 NS109608
R01 NS122805-01
Arizona Biomedical Research Commission (ABRC) grant ADHS18-198846

Title: Naloxone partially blocks the anti-dyskinetic and enhances the antiparkinsonian effects of sub-anesthetic ketamine

Authors: *R. PARMAR[1], C. STOPERA[3], M. J. BARTLETT[4], A. ESQUEDA[5], S. J. SHERMAN[6], T. FALK[2];
[2]Dept. Of Neurol., [1]Univ. of Arizona, Tucson, AZ; [3]The Univ. of Arizona, Tucson, AZ; [4]Univ. Of Arizona, Tucson, AZ; [5]Yale Univ., New Haven, CT; [6]Dept. of Neurol., Univ. of Arizona Col. of Med., Tucson, AZ

Abstract: We have previously demonstrated that treatment with sub-anesthetic ketamine can reduce L-DOPA-induced dyskinesia (LID) in 6-hydroxydopamine (6-OHDA)-lesioned male rats and is acutely antiparkinsonian. Other studies have shown that ketamine is a multifunctional ligand with similar binding affinity to both *N*-methyl-D-aspartate (NMDA) and opioid receptors. Through NMDA receptor antagonism ketamine modulates neuroplasticity. Ketamine has agonist activity on μ- and κ-opioid receptors. In addition, the striatum is rich in both μ- and δ-opioid receptors and long-term L-DOPA therapy changes the levels of opioid peptides, and recent data indicates that opioid agonism is required for the anti-depressive activity of ketamine. We hypothesize that ketamine may also activate opioid receptors in the basal ganglia, contributing to either anti-dyskinetic or antiparkinsonian action. In a 1st study, we used the pan-opioid receptor antagonist naloxone to investigate if opioid receptor activation is necessary for reducing LID. Unilateral 6-OHDA lesioned male Sprague-Dawley rats were treated with 6 mg/kg of L-DOPA for 21 days to establish a pre-clinical model of LID. Once abnormal involuntary movements (AIMs) were stable over three testing days, the rats (n = 10-11/group) were treated on Day 0 with either vehicle, ketamine (20 mg/kg), or ketamine (20 mg/kg) + naloxone (3 mg/kg or 5 mg/kg) using a 10-hour treatment protocol where all animals received 5 treatments (i.p.) 2 hours apart.

Finally, L-DOPA was administered immediately following the 10-hour protocol and AIMs were assessed. All animals received L-DOPA (6 mg/kg) every 3-4 days to maintain the LID and AIMs were scored once a week. Similar to our published data, ketamine alone significantly reduced AIMs following the treatment ($p < 0.05$ vs. vehicle). Naloxone at the 3 mg/kg dose did not prevent the effect of ketamine ($p < 0.05$ vs. vehicle) to reduce LID. However, the addition of naloxone at the 5 mg/kg dose had a partial antagonistic effect and the anti-dyskinetic effect of ketamine was reduced by ~50%. This data suggests that opioid receptor activation may be contributing to the anti-dyskinetic effects of ketamine. In a 2nd study, we investigated if the antiparkinsonian activity of ketamine is dependent on opioid receptor activation in unilateral 6-OHDA-lesioned rats, using the forelimb adjusting steps (FAS) test in groups treated with ketamine or ketamine + naloxone (3 mg/kg or 5 mg/kg; n = 10/group). At the 3 mg/kg dose, naloxone did not change the efficacy of the antiparkinsonian activity of ketamine, whereas at 5 mg/kg dose, naloxone enhanced the antiparkinsonian effect of ketamine ($p< 0.05$ vs. ketamine alone).

Disclosures: **R. Parmar:** None. **C. Stopera:** None. **M.J. Bartlett:** None. **A. Esqueda:** None. **S.J. Sherman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SJS has a patent for the use of ketamine as a novel treatment for levodopa-induced dyskinesia associated with Parkinson's disease, that has been licensed to PharmaTher Inc.. **T. Falk:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); TF has a patent for the use of ketamine as a novel treatment for levodopa-induced dyskinesia associated with Parkinson's disease, that has been licensed to PharmaTher Inc.. S. J..

Poster

PSTR402. Therapeutic Strategies: Small Molecule Therapeutics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR402.14/S1

Topic: C.03. Parkinson's Disease

Title: A structural perspective on the interaction between glucocerebrosidase and the positive allosteric modulator BIA 28-6156

Authors: J. M. DANTAS, N. M. F. S. CERQUEIRA, ***V. L. BATALHA**, L. KISS, T. KAROLI, J. HOLENZ;
BIAL - Portela & Cª. S.A., Trofa, Portugal

Abstract: BIA 28-6156 is a Phase II clinical candidate for the treatment of patients with Parkinson`s Disease (PD) who have a mutation in the Glucocerobrosidase (GBA1) gene (GBA-PD). Heterozygous GBA1 pathogenic variants are the most common genetic risk factor for PD, accounting for 5-15% of PD patients. Usually, GBA-PD patients present an earlier age of onset, may progress more rapidly, and may experience earlier and more significant cognitive impairment as compared with idiopathic PD patients. Here we describe the proposed structural

interaction of BIA 28-6156 with human recombinant glucocerebrosidase protein (hrGCase) and the impact of this interaction in presence of saposin C (Sap C), a key activator protein for glucocerebrosidase (GCase) in the lysosome. BIA 28-6156 increases *in vitro* wt hrGCase activity as determined using the 4-methylumbelliferyl-β-D-glucopyranoside (4-MUG) assay. Key structural interactions between BIA 28-6156 and an allosteric binding pocket of hrGCase were investigated using ligand- and protein-based NMR experiments. The binding site and binding pose of BIA 28-6156 at hrGCase were identified and characterized by molecular docking using glide and molecular dynamic simulations using the Desmond force field. The interaction between GCase with BIA 28-6156 and Sap C was modelled using HADDOCK. NMR experiments mapped the epitopes of BIA 28-6156 interacting with hrGCase and showed that this molecule influences the interaction between hrGCase and Sap C. Molecular modelling studies indicate that BIA 28-6156 binds to GCase in a shallow binding pocket, near the long α-helix of domain III and close to TRP312. This residue is located in an unfolded loop of GCase structure that undergoes a conformational change and is stabilized by BIA 28-6156. A model for GCase with BIA 28-6156 in complex with Sap C is reported suggesting a role played by BIA 28-6156 in the interaction between both proteins and provides insights on the mechanism underling the observed positive allosteric modulation of GCase.

Disclosures: **J.M. Dantas:** A. Employment/Salary (full or part-time):; BIAL - Portela & Cª. S.A. **N.M.F.S. Cerqueira:** A. Employment/Salary (full or part-time):; BIAL - Portela & Cª. S.A. **V.L. Batalha:** A. Employment/Salary (full or part-time):; BIAL - Portela & Cª. S.A. **L. Kiss:** A. Employment/Salary (full or part-time):; BIAL - Portela & Cª. S.A. **T. Karoli:** A. Employment/Salary (full or part-time):; BIAL - Portela & Cª. S.A. **J. Holenz:** A. Employment/Salary (full or part-time):; BIAL - Portela & Cª. S.A..

Poster

PSTR402. Therapeutic Strategies: Small Molecule Therapeutics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR402.15/S2

Topic: C.03. Parkinson’s Disease

Support: Parkinson's Disease Foundation Postdoctoral Award

Title: Investigating the impact of gut bacterial levodopa metabolism on Parkinson's disease outcomes

Authors: *C. OLSON, J. STANSIL, M. SANDY, K. SPITLER, E. BROWN, C. TANNER, A. NELSON, S. GOLDMAN, P. TURNBAUGH;
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Abstract: Several studies report that the gut microbiota affects pathogenesis in Parkinson’s disease (PD) murine models. However, precise mechanisms are difficult to identify, and whether microbiome-based findings in PD models are clinically relevant is unclear. Our laboratory’s

prior collaborative work demonstrates that a common gut bacterium *Enterococcus faecalis* catabolizes levodopa through tyrosine decarboxylase. In preliminary findings in mice, antagonizing *E. faecalis* worsened whole blood levodopa pharmacokinetics. Through transcriptomic, sequencing, and pharmacokinetic approaches, we are evaluating which host and/or bacterial pathways are responsible for suppressed levodopa absorption. In a paired double-blind clinical trial of PD patients taking levodopa while consuming a mild antibiotic, we are evaluating whether particular gut bacterial composition, metabolism, or metabolites correspond with patient behavioral outcomes and responsiveness to levodopa. Combining a sequencing and targeted metabolomic approach may help identify gut bacterial pathways contributing to levodopa drug response and broader patient outcomes impacting quality of life.

Disclosures: C. Olson: None. **J. Stansil:** None. **M. Sandy:** None. **K. Spitler:** None. **E. Brown:** None. **C. Tanner:** None. **A. Nelson:** None. **S. Goldman:** None. **P. Turnbaugh:** None.

Poster

PSTR403. Alpha-synuclein: Mechanisms and Transmission

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR403.01/S3

Topic: C.03. Parkinson’s Disease

Support: HMRF 15163051

Title: Investigating the role of free radicals and inflammation on the spread of pathological factors in Parkinson's disease

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Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disease worldwide. Loss of dopaminergic neurons within the Substantia nigra pars compacta (SNpc) and levy body deposits within cells are two major pathological characters of the PD. It has been shown that the spread of α-synuclein and tau protein, from the brainstem to limbic and neocortical structures, is one of the most important pathological factures of the PD dementia. Free radicals and inflammation play an important role in the development of PD, while their exact effect on the spread of pathological factors and mechanisms remains unclear. This study aims to investigate the effect of free radicals on the spread of pathological factors in PD mice model and evaluate their potential mechanisms. 6-OHDA is stereotactic injected into the median brain bundle of C57BL/6N mice to construct PD model. Our results show that at 3 weeks after the injection, there was significant motor function impairment and TH^+ cell loss (80.34 ± 2.48%) in the ipsilateral side of SNpc in 6-OHDA groups, compared with control group. At 7 weeks

after the injection, there was no significant cognitive impairment, neuronal loss, increased expression of mRNA for cytokines, and oxidative stress in the ipsilateral hippocampus in 6-OHDA groups by behavioral tests, qPCR and immunohistochemical analysis. In addition, no significant change of phosphorylate α-synuclein and tau was found in the ipsilateral hippocampus in 6-OHDA groups. These results may help us to understand whether oxidative stress is a key factor promoting spreading of pathological factors in PD pathogenesis.

Disclosures: P. Xiao: None. **J.Y. Ho:** None. **R. Chang:** A. Employment/Salary (full or part-time):; The Univ. of Hong Kong.

Poster

PSTR403. Alpha-synuclein: Mechanisms and Transmission

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR403.02/S4

Topic: C.03. Parkinson's Disease

Support: NINDS grant NS101134t

Title: Leucine carboxyl methyltransferase 1(LCMT-1) Mitigates alpha-Synuclein Mediated Toxicity in Mice

Authors: *S. C. MADDILA[1], J. LIU[1,2], J. ZHANG[1], K. HASSANZADEH[1], E. JUNN[1], R. E. NICHOLLS[2], M. MOURADIAN[1];
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Abstract: Accumulation of aggregated alpha-Synuclein (α-Syn) fibrils in Lewy bodies and Lewy neurites is a characteristic feature of Parkinson's disease (PD) and Dementia with Lewy Bodies (DLB). Phosphorylation of α-Syn at Ser129 is considered a marker of pathological α-Syn in synucleinopathies. Characterizing the factors that regulate this phosphorylation state can elucidate the molecular pathogenesis of these disorders and potentially identify new therapeutic strategies. We previously found that the B55α containing protein phosphatase 2A (PP2A) isoform is the main enzyme that dephosphorylates α-Syn at Ser129. The assembly and activity of this trimeric holoenzyme are regulated by reversible carboxyl methylation of the catalytic C subunit, a process governed by the opposing activities of a PP2A-specific leucine carboxyl methyltransferase (LCMT-1) and a PP2A-specific methylesterase (PME-1). Notably, our postmortem studies have shown that LCMT-1 expression is decreased while PME-1 expression is increased in PD and DLB affected brains. In this study, we aimed to determine if over-expressing LCMT-1 can mitigate α-Syn mediated pathology in a mouse model. Preformed fibrils (PFF) of α-Syn or PBS were injected in the right striatum of mice that overexpress LCMT-1 in forebrain neurons under the control of the calcium calmodulin kinase IIα (*CaMKIIα*) gene promoter as well as in control mice. At six months post-PFF injections, LCMT-1 over-

expressing mice exhibited better performance on the rotarod and nesting behavior compared with PFF injected control mice. Immunohistochemical stains showed that the ipsilateral striatum in LCMT-1 over-expressing mice had a smaller decrease in tyrosine hydroxylase (TH) levels and a smaller increase in the microglial marker Iba1 compared to PFF injected control mice. Additionally, LCMT-1 over-expressing mice exhibited less abundant phospho-S129-α-Syn labeled cells in the ipsilateral striatum and substantia nigra pars compacta compared to PFF injected control mice. These findings demonstrate that LCMT-1 over-expression mitigates the pathogenicity of α-Syn and its tendency to propagate in the brain. They also suggest that the reduced levels of LCMT-1 in human synucleinopathy brains contribute to the pathogenicity of these diseases through reduced phosphatase activity leading to increased α-Syn phosphorylation state. Thus, efforts to enhance PP2A activity have the potential for disease modification in synucleinopathies. Funding support: NINDS grant NS101134t

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Poster

PSTR403. Alpha-synuclein: Mechanisms and Transmission

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR403.03/S5

Topic: C.03. Parkinson's Disease

Support: Ro1NS108810

Title: Activation of Toll Like Receptors by Pathogenic Alpha-Synuclein (PFFs) and HIV-TAT proteins

Authors: *M. P. NELSON[1], K. A. MAGUIRE-ZEISS[2], L. ROSARIO[3], L. HORAN-PORTELANCE [4];
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Abstract: In studying, age-related neurodegenerative proteinopathies, how microglia detect and react to disease-relevant proteins is not fully understood. We hypothesize that Toll-Like Receptors (TLRs), a subtype of pattern recognition receptors found on many types of innate immune cells including microglia, preferentially recognize and respond to pathogenic proteins like α-synuclein preformed fibrils (PFFs) and the HIV-1 protein Tat. Ligand binding to TLRs promotes the nuclear translocation of NF-κB, a transcriptional regulator of proinflammatory molecules. In this study we exposed HEK-TLR5 reporter cells to PFFs, Tat, or negative &

positive controls. The reporter, secreted alkaline phosphatase (SEAP) is under the control of NF-κB and SEAP activity, a surrogate for TLR activation, was measured in the cell culture supernatant. When compared to vehicle, both PFF- and Tat-exposed cells resulted in significant activation of TLR5 signaling (p<0.0001). Furthermore, inhibition of NF-kB signaling broadly via bindarit and of TLR5 more specifically using the novel antagonist (TH1020) caused significant reduction of SEAP activity. Next, we exposed primary microglia cultures to PFFs and showed an increase in TLR5 RNA expression (p<0.0009) and an increase in expression of the proinflammatory cytokines IL-1ß (p<0.0001) and IL-6 (p<0.0001). The gene expression of additional inflammatory modulators, matrix metalloprotease-9 (MMP; p< 0.0001), MMP-3 (p=0.0004); MMP-13 (p<0.0001) was also increased. Using ELISA and zymography, we confirmed significant increases in TNF-α (p=0.0002), IL-1ß (p=0.001), and MMP-9. However, when TLR5 antagonists or NF-kB inhibitors were applied to primary culture there were no significant changes to these proinflammatory molecules. Taken together, these data support the hypothesis that PFFs and TAT activate TLR5 promoting an inflammatory profile and suggest that attenuation of this signaling pathway may decrease NF-κB signaling. Future studies will test combinatorial drug approaches.

Disclosures: M.P. Nelson: None. **K.A. Maguire-Zeiss:** None. **L. Rosario:** None. **L. Horan-Portelance :** None.

Poster

PSTR403. Alpha-synuclein: Mechanisms and Transmission

Location: WCC Halls A-C

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Program #/Poster #: PSTR403.04/S7

Topic: C.03. Parkinson's Disease

Support: NS108686
NS112540
NS092093
NS086074
ASAP-000592

Title: Exogenous alpha-synuclein fibrils cause postsynaptic deficits in Lewy body dementia

Authors: *S. C. VERMILYEA[1], C. PEREZ DE NANCLARES[1], R. SCHLICHTE[1], J. MEINTS[1], H. CLARK[1], D. LIAO[1], A. ARAQUE[1], M. K. LEE[1,2];
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Abstract: Alpha-synuclein (αS) is currently the primary pathological protein implicated in Parkinson's disease dementia (PDD) and dementia with Lewy bodies (DLB). Previously we have shown that mutant A53T αS transgenic mice (TgA53T) have tau-dependent age-related cognitive deficits. Further, A53T expressing neurons have GSK3β-dependent tau mislocalization to

dendritic spines leading to synaptic dysfunction. However, in PDD/DLB, most cases are linked to a known genetic mutation. Because dementia in PDD/DLB is associated with high neuritic αS pathology in CA2, likely from afferents of the entorhinal cortex (EC), without overt neurodegeneration, we hypothesize that in sporadic DLB, the neurites from EC neurons are releasing toxic αS, impacting hippocampal dendrites, leading to tau mislocalization and synaptic deficits that leads to cognitive deficits. Analysis of hippocampal sections from sporadic PD cases confirm neuritic αS pathology within the CA regions of the hippocampus. Significantly, while PD cases were devoid of pathological tau (e.g. AT8), hippocampal neurons exhibit tau mislocalization to the somatodendritic compartment in sporadic PD cases compared to controls. To directly test our hypothesis, we exposed primary cultures of hippocampal neurons to αS preformed fibrils (PFF). The neurons were transfected to express DsRed and Tau-eGFP to monitor the neurites/spines and tau, respectively. Live cell imaging at 24 hours post-PFF treatment shows that αS PFF, even at very low doses (0.05-4.0 υg/mL; 3.5-280 nM), causes tau mislocalization. Treatment of αS monomer (4.0 υg/mL) had no effect. We also show that αS PFF dependent mislocalization of tau occurs independent of endogenous αS but requires GSK3β activity. Longer term analysis shows that αS PFF does not impact the integrity of the spines within 72 hours. However, αS PFF causes significant loss of spines by 14 days post-PFF treatment, and both pre- and post-synaptic markers synapsin and PSD95 respectively, are significantly reduced. Finally, to define the in vivo significance of our hypothesis, we show that WT mice stereotactically injected with αS PFF to the CA1 region of the hippocampus have mEPSC frequency and amplitude deficits 48 hours after injection, as well as a lack of LTP following high frequency stimulation. We conclude that in PDD/DLB, Lewy neuritic pathology may be releasing toxic αS species leading to tau mislocalization and synaptic deficits that leads to circuit dysfunction and cognitive deficits.

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Poster

PSTR403. Alpha-synuclein: Mechanisms and Transmission

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR403.05/S8

Topic: C.03. Parkinson’s Disease

Title: Blocking the aerobic/energy source in neurons via SGLTs inhibits/delays α synuclein transmission/propagation

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Abstract: Progressive loss of dopaminergic neurons in the substantia nigra (SN) pars compacta (SNpc) and the formation of cytoplasmic inclusions containing misfolded α-synuclein (α-syn), Lewy bodies (LBs), are two major neuropathological hallmarks of Parkinson Disease (PD),

which is the second most common neurodegenerative disease. PD and Diabetic mellitus (DM) share potential contributing factors and have overlapping pathology. Studies have unveiled a crucial connection between PD and DM. DM is characterized by impaired glucose metabolism and subsequent hyperglycemia. The elevated risk of developing cognitive abnormalities in individuals with impaired glucose metabolism has been well-documented. Hyperglycemia, a common pathogenesis in T2DM, has been shown to contribute to the onset of α-syn pathology in neurons and oligodendrocytes. Phosphorylated α-syn inclusions have been found in pancreatic β cells of T2DM subjects, indicating the existence of PD-related peripheral pathology in DM. But the complicated effect of glucose transporter on PD induced dopaminergic degeneration is still not well understood. Here, we demonstrate that α-syn fibrils induce active glucose energy transporter SGLTs activation in A53T alpha-synuclein overexpressing cells, which increase the uptake of α-syn into neurons. Furthermore, the inhibition of SGLTs inhibits the α-syn uptake and the formation of Lewy body-like inclusions. These results demonstrated a new therapeutic approach for PD.

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Poster

PSTR403. Alpha-synuclein: Mechanisms and Transmission

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR403.06/S9

Topic: C.03. Parkinson's Disease

Support: MJFF

Title: Elevated levels of Gut Microbiome- derived Trimethyl Amine-N-Oxide (TMAO) in Parkinson's disease

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Abstract: Gut microbiome dysbiosis is closely associated with neurodegenerative diseases and the changes in the concentration of gut microbial metabolites act as a proof of concept for gut microbiome dysbiosis and potential prognostic biomarker in PD. Recent research has shown gut microbiome dysbiosis in PD patients that can lead to increased gut permeability and systemic inflammation. Recent studies have shown the circulating trimethylamine-n-oxide (TMAO) can cross the blood brain barrier (BBB) and associated with the progression of neurological diseases. Furthermore, emerging evidence suggests that gut microbial metabolites such as trimethylamine

(TMA) are elevated in PD patient bloods and associated with faster disease progression. In this study, we aimed to measure and compare the circulating levels of TMAO in healthy (n= 44) and PD (n= 46) patients. We also performed high resolution metagenomic shotgun analyses on healthy (n= 60) and PD (n= 17) patients to link the metabolites to altered gut microbial metabolism. Using mass spectrometry, we found levels of plasma TMAO were significantly higher in PD patients compared to controls. In consistent with this, we found an elevated levels of TMAO by- product, formaldehyde (FA) in plasma of PD patients. Interestingly, the shotgun metagenomic analysis of fecal sample showed altered diversity in the microbiota between PD and HC with an increase in the TMA-producing bacterial species and enzymatic pathways. Consistent with the published literature our data confirmed increased in the circulating levels of TMAO and metagenomics data confirmed key changes in microbiota composition such as *E. coli* and *Clostridium sp* between healthy individuals and PD patients. This suggests an increased propensity for production of pathogenic microbial metabolites such as TMA in the gut of PD patients due to gut dysbiosis causing an elevation in the levels of TMAO which can cross the blood- brain barrier (BBB), contributing to PD pathology and progression.

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Poster

PSTR403. Alpha-synuclein: Mechanisms and Transmission

Location: WCC Halls A-C

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Topic: C.03. Parkinson's Disease

Support: JP22dm0207072
JP21dk0207046
JP22dk0207061

Title: Identification of novel regulators of disease-specific α-synuclein aggregation by genome wide screening using the CRISPR/Cas9 system

Authors: *I. TOMIZAWA[1], Y.-W. CHIU[1], T. MANO[2,3], M. ONO[4], M. HIGUCHI[4], T. TODA[2], A. IWATA[2,5], Y. HORI[1], T. TOMITA[1];
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Abstract: Synucleinopathies, including Parkinson disease and multiple system atrophy (MSA), are a group of neurodegenerative diseases characterized by the intracellular accumulation of α-synuclein (α-syn). Recently, cryo-EM analysis demonstrated that α-syn fibrils in the brain of patients have disease-specific structures, although they shar the same cross-β-sheet structure. The structure of fibrils generated *in vitro* from recombinant α-syn (preformed fibril, PFF) also differs from that of the patient-derived fibrils. The difference in the structure of α-syn fibrils contribute to the difference in the disease-specific interactors that regulate the intracellular aggregation of α-syn. However, the exact molecules are still unknown. In this study, we aimed to identify disease-specific regulators of α-syn aggregation and performed a genome wide screening based on the CRISPR/Cas9 system. We edited the genomes of a monoclonal HEK293A cell line co-expressing Cas9 and α-syn with a genome-wide gRNA library and induced intracellular α-syn aggregation using PFF and MSA patient-derived fibrils. The intracellular α-syn fibrils were visualized by a fluorescent probe and cells were isolated based on fluorescence intensity using flow cytometry. Among 19,114 genes included in the gRNA library, 92 genes for MSA-derived fibrils were identified as disease-specific candidate genes. Gene ontology analysis of these candidates revealed disease-specific pathways related to α-syn aggregation. Our study suggests that disease-specific regulators and pathways may regulate intracellular α-syn aggregation, and that these may explain the difference in the pathogenesis of synucleinopathies.

Disclosures: I. Tomizawa: None. **Y. Chiu:** None. **T. Mano:** None. **M. Ono:** None. **M. Higuchi:** None. **T. Toda:** None. **A. Iwata:** None. **Y. Hori:** None. **T. Tomita:** None.

Poster

PSTR403. Alpha-synuclein: Mechanisms and Transmission

Location: WCC Halls A-C

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Topic: C.03. Parkinson’s Disease

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CONICET

Title: Exploring the impact of alpha synuclein on intracellular trafficking: insights into neurodegenerative disease mechanisms

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Abstract: The regulation of intracellular trafficking in neurons is crucial since this cell type has a unique architecture, and the proper functioning of the exocytic pathway plays a fundamental role for its survival. Alpha synuclein (AS), is a widely studied protein for its involvement in various neurodegenerative diseases, including Parkinson's disease and synucleinopathies.

Despite being the focus of several studies, its normal function and its role in neuronal death remains partially understood. One intriguing hypothesis suggests that AS may be affecting intracellular trafficking, thus affecting neuronal survival. Our research focuses on unravel the effects of AS on this crucial process in mammalian neurons and how it affects their structure and function. We use an innovative exocytic pathway synchronization system which allows coordinated release of transmembrane proteins from the endoplasmic reticulum (ER) using a specific drug. This system enables us to analyze alterations in protein trafficking dynamics and vesicle flow. Through this synchronization system, we found that AS selectively affects protein trafficking from the Golgi apparatus to neuronal processes. Interestingly, AS modulates the trafficking of p75NTR and its vesicle size, while leaving the Transferrin Receptor unaffected. Furthermore, cofilin expression rescues AS-induced trafficking defects and neurodegeneration, as assessed by Sholl analysis. We propose that AS may alter the dynamics of the actin cytoskeleton, thus affecting the fission machinery of p75NTR vesicles. This result could explain the reduced p75NTR trafficking observed after the expression of AS. Our findings suggest that AS could induce neurodegeneration, at least partially, by affecting protein trafficking.

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Poster

PSTR403. Alpha-synuclein: Mechanisms and Transmission

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Topic: C.03. Parkinson’s Disease

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CiberNed Intramural Collaborative Projects (Grant No. PI2020/09)

Title: Location of Lewy body pathology drives dopaminergic cell vulnerability in pigmented non-human primates

Authors: *J. CHOCARRO[1,2,3], A. J. RICO[1,2,3], G. ARIZNABARRETA[1,2,3], E. RODA[1,2,3], A. HONRUBIA[1,2,3], P. ARNAIZ[1], S. MARANA[1], A. CORCHO[1], A. LEON-VILLARES[1], A. VAZQUEZ[4], J. LANCIEGO[1,2,3];
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Abstract: Although dopaminergic neurons located in the ventral tier of the substantia nigra pars compacta (SNCv) are known to be more vulnerable to degenerate than those located in the dorsal

tier (SNCd), the underlying mechanisms sustaining such a differential vulnerability still are poorly understood. In this regard, the content in calbindin, a calcium-buffering protein specific for SNCd neurons and not found in SNCv neurons, has often been claimed to play a major role. Here we took advantage of a cohort of four non-human primates (NHPs) being injected with an adeno-associated viral vector encoding the human tyrosinase gene (AAV-hTyr) to further enhance pigmentation of dopaminergic neurons in both tiers of the SNC. Pigmented dopaminergic neurons exhibited Lewy body pathology made of endogenous alpha-synuclein, later on leading to a time-dependent degeneration of dopaminergic neurons, together with a pro-inflammatory scenario. Immunohistochemistry for calbindin and ALDH-1 was used to disclose between neurons in the SNCd and SNCv respectively, together with P62 as a marker for Lewy body pathology. Obtained data showed a preferential location for Lewy bodies within SNCv neurons (identified as ALDH-1+), this distribution accounting for more than 90% of P62+ intracytoplasmic inclusions observed in pigmented neurons throughout the whole rostrocaudal extent of the SNC. Lewy bodies were also found in SNCd neurons, although to a much lower extent than those observed in SNCv. In summary, here we provide evidence showing that the location of Lewy body pathology, instead of the lack of calbindin, may be taken as the driving force sustaining the susceptibility of dopaminergic SNCv neurons to neurodegeneration.

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Poster

PSTR403. Alpha-synuclein: Mechanisms and Transmission

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Program #/Poster #: PSTR403.10/T3

Topic: C.03. Parkinson’s Disease

Support: KAKENHI Grant Number JP21K07299

Title: Differential expression profiles of α-synuclein in rodent and primate brain

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Abstract: α-Synuclein (αSyn), one of major components of Lewy bodies (LBs) and Lewy neurites (LNs), is physiologically expressed in presynapses and is involved in synaptic function. Abnormal intracellular aggregation of αSyn is observed as LBs and LNs in neurodegenerative disorders such as Parkinson’s disease or dementia with Lewy bodies. Accumulated evidence suggests that abundant expression of αSyn is one of the risk factors for neurodegeneration. Therefore, it is important to know the endogenous expression profiles of αSyn. Our previous

studies demonstrated brain region-dependent differential expression patterns of αSyn using wild-type mice. Synaptic expression of αSyn is mostly accompanied by the expression of vesicular glutamate transporter-1 (vGluT-1), an excitatory presynaptic marker protein. In contrast, expression of αSyn in GABAergic inhibitory synapses is different among brain regions. For examples, in the cerebral cortex and hippocampus, αSyn is not observed in inhibitory synapses. On the other hand, αSyn is clearly expressed in inhibitory synapses of the globus pallidus and substantia nigra pars reticulata. These results indicate that expression of αSyn is regulated in cell-type or brain-region dependent manner. Here, we studied expression profiles of αSyn in human and common marmoset brain. In the cerebral cortex of the primate brain, αSyn was mainly expressed in the excitatory synapses expressing vGluT-1, as happens in mouse brain. Interestingly, there were some αSyn-positive synapses co-expressing glutamic acid decarboxylase in those brains. These results suggested that expression profile of αSyn in inhibitory synapses was different between rodents and primates. Precise characterization of these αSyn-positive inhibitory neurons is under progress. Further studies of the differential expression of αSyn among different species will provide new insights for understanding physiological function of αSyn.

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Poster

PSTR403. Alpha-synuclein: Mechanisms and Transmission

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR403.11/T4

Topic: C.03. Parkinson's Disease

Title: Phosphoproteomic changes as an early stage predictive indicator of cortical synaptic dysfunction in synucleinopathies

Authors: *A. SCOTT[1], S. DUTTA[2], H. KHAN[1], R. FERREIRA[1], L.-A. ROSSITTO[3], U. ARAYAL[1], K. JAYANT[1], X. CHEN[3], J.-C. ROCHET[1];
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Abstract: Cortical dysfunction plays a critical role in non-motor symptoms associated with Parkinson's disease (PD) and other synucleinopathies. Recent studies have reported functional changes in cortical circuitry in pre-clinical models of PD, but with limited mechanistic insight. Therefore, in search of causative or predictive factors, we hypothesized that aSyn aggregation leads to alterations of the cellular phosphoproteome. To address this hypothesis, we utilized an *in vivo* model of aSyn aggregation involving the injection of aSyn preformed fibrils (PFFs) in the mouse cortex and striatum to study the downstream effects of aggregation. We analyzed phosphoproteomic profiles of the cortical homogenates of the PFF injected vs monomer injected mice. In this model, we showed that PFF injection leads to the presence of aSyn aggregates that

stain positive for the phosphorylated form of serine residue 129 (pSer129) in the sensorimotor cortex, SNpc, and other anatomically connected brain regions. Phosphoproteomic analysis of the sensorimotor cortex revealed significant differences between the PFF and monomer groups 3 months post-injection. Gene ontology analysis of the phosphoproteomic changes suggested that aSyn PFF administration led to perturbations in modulation of chemical synaptic transmission and synaptic signaling. Moreover, we identified phosphosites that were previously not reported and distinct from sites reported for acute synaptic excitation. Further, motif enrichment analysis and kinase prediction show that the majority of differentially abundant motifs are predicted to be phosphorylated by cyclin dependent kinase 5 (CDK5) or casein kinase II (CKII). The phosphoproteomic changes suggest a possible mechanism of synaptic dysfunction in the sensorimotor cortex of PFF-injected mice. Collectively, these findings deepen our understanding of the molecular underpinnings of synucleinopathy disorders, particularly the prodromal phase, laying the groundwork for developing well-tailored intervention strategies.

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Poster

PSTR403. Alpha-synuclein: Mechanisms and Transmission

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Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

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Topic: C.03. Parkinson's Disease

Support: R15NS130532-01
1R03NS088395-01A1
1R15NS093539-01
1R21NS107960-01

Title: Partial Demyelination Worsens a-Synucleinopathy in the Preformed Fibril Model

Authors: *R. N. CLARK[1], R. E. LANDES[1], M. ABBAS[1], A. JAMENIS[1], K. MINER[1], K. C. LUK[2], X. HU[3], R. LEAK[1];
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Abstract: Lewy body disorders are characterized by deposition of aggregated alpha-synuclein protein into insoluble inclusions. In Parkinson's disease, long and unmyelinated axons (*e.g.*, nigrostriatal fibers) are more likely to harbor alpha-synuclein^{+} inclusions, compared to sturdier, myelinated fibers (*e.g.*, cerebellar tracts), but it is not known if this relationship is causal. To address this problem in an experimental model of Lewy body disease, we infused preformed fibrils into the anterior olfactory nucleus of the caudal olfactory bulb (OB/AON) in 9 to 11-month-old mice of both sexes. Partial demyelination was elicited by dietary administration of cuprizone (0.3%), an established oligodendrocyte-targeting, copper chelating agent, for 8 weeks.

Compared to control diet mice, cuprizone-fed animals of both sexes displayed the expected decrease in two major protein constituents of the myelin sheath, myelin basic protein (MBP) and proteolipid protein (PLP) in the OB/AON. However, no additional loss of MBP and PLP was observed in fibril-treated mice, suggesting that cuprizone and fibril exposure act neither additively nor synergistically on myelin-associated proteins, perhaps because they impinge on the same physiological processes in white matter. However, cuprizone exposure increased the fraction of Triton-insoluble alpha-synuclein that was phosphorylated at Serine 129 (pSer129) in the amygdala and piriform cortex, revealing an exacerbation of insoluble Lewy-like pathology with forced demyelination. Insoluble pSer129 levels were negatively correlated with PLP levels in fibril-infused mice under control diet conditions (r=-0.7026; two-tailed p=0.0187), and this negative correlation was abolished by cuprizone exposure (r=-0.1067; two-tailed p=0.7692). Thus, higher levels of myelin-related protein PLP are associated with lower Lewy-like pathology as expected, but forced demyelination uncouples this link. Similarly, we discovered a negative correlation between PLP and Triton-insoluble pSer129 alpha-synuclein levels in amygdala tissues from men (but not women) with Lewy body disease (Pearson r=-0.9026, two-tailed p=0.0054), and a trend towards a negative correlation between MBP and insoluble pSer129 in the human amygdala samples (Pearson r=-0.7233, two-tailed p=0.0662). Finally, we noted that Lewy body disease was associated with lower MBP levels in the olfactory bulbs of men compared to women. Further investigations into oligodendrocyte precursor cells and pre- and post-myelinating oligodendrocytes are now warranted, to understand the impact of myelination status on Lewy body disease.

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Poster

PSTR403. Alpha-synuclein: Mechanisms and Transmission

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR403.13/T6

Topic: C.03. Parkinson’s Disease

Support: NIH/NINDS R21 NS121393

Title: Synucleinopathy decreases the expression and membrane localization of the complement regulator CD55 prior to neurodegeneration.

Authors: N. C. KUHN[1], J. R. PATTERSON[1], C. J. KEMP[1], A. C. STOLL[1], K. STEECE-COLLIER[1], K. C. LUK[2], C. E. SORTWELL[1], ***M. J. BENSKEY**[1];
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Abstract: Parkinson's disease (PD) is characterized by loss of midbrain dopamine neurons, accumulation of fibrillar alpha synuclein (a-syn) in Lewy bodies, and neuroinflammation.

Neuroinflammation occurs in early-stage PD patients, suggesting it may contribute to neurodegeneration. The complement system is a division of the innate immune system that coordinates the clearance of debris, synapses, and cells in the central nervous system. Activated complement proteins label Lewy body containing neurons in the PD brain, indicating complement may mediate phagocytic removal of synucleinopathy affected neurons. However, activation of complement in PD and associated models has only been documented after neurodegeneration has occurred, making it impossible to determine if complement activation occurs in response to cell death or prior to cell death, where it may drive to neurodegeneration. We aimed to determine if complement activation occurs in the substantia nigra pars compacta (SNc) prior to neurodegeneration in a model of synucleinopathy. Intra-striatal injection of recombinant a-syn pre-formed fibrils (PFFs) in rats results in aggregation of endogenous a-syn and gliosis that peaks 2 months post injection, followed by nigral degeneration at 6 months post injection. To determine if synucleinopathy causes complement activation prior to neurodegeneration we used ddPCR to quantify transcripts representing different parts of the complement cascade. We observed significant increases in transcripts from the classical (C1qa, C4b) and alternative activating pathways (CFd, Cfb), anaphylatoxin receptors (C3aR, C5aR), phagocytic receptor CD11b (ITGAM) and the complement regulator, C1 Inhibitor (Serping1). We observed significant decreases in transcripts for the complement regulators CD55 and CD59. We performed immunofluorescence (IF) in the SNc at the same time point to determine if the decrease in CD55 occurred in nigral neurons. CD55 immunoreactivity was decreased in nigral neurons of PFF injected rats, where neurons containing serine 129 phosphorylated a-syn (pSyn) were almost devoid of CD55 immunoreactivity. Using the proximity ligation assay we observed interaction between a-syn and CD55 in the rat SNc, and this interaction was increased between CD55 and pSyn. We performed similar analyses in postmortem human PD tissue and observed decreased CD55 in nigral neurons, interaction between pSyn and CD55 in the SNc, as well as CD55 in the core of Lewy bodies. Taken together, these data demonstrate synucleinopathy causes a robust activation of the complement system and a decrease in the expression of membrane bound complement regulators in nigral neurons.

Disclosures: **N.C. Kuhn:** None. **J.R. Patterson:** None. **C.J. Kemp:** None. **A.C. Stoll:** None. **K. Steece-Collier:** None. **K.C. Luk:** None. **C.E. Sortwell:** None. **M.J. Benskey:** None.

Poster

PSTR403. Alpha-synuclein: Mechanisms and Transmission

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR403.14/T7

Topic: C.03. Parkinson’s Disease

Title: Alpha-synuclein and cytokine assessment in aged Parkinson's Disease mouse model via push-pull or open-flow microdialysis

Authors: L. YU[1], M. VAN DER HART[2], C. CHAU[1], A. LEUNG[1], G. DUENAS[1], I. T. Y. CHENG[1], T. AMARLKHAGVA[1], S. YELLAI[1], H. A. KOOIJKER[1], *M. A. BOWMAN[1], J.

ROESER[1], T. H. I. F. CREMERS[2];
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Abstract: Neuroinflammation has been implemented as a confounding variable in the pathology and progression of neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's (PD). However, methods in capturing and concurrently measuring these pathological markers (such as alpha-synuclein) and their affected systems (cytokine readouts) in a singular animal are limited by collection methods and analytical viability. The benefits of simultaneous profiling for immune-markers and disease biomarkers may elucidate pathways and therapeutic windows that can alter and/or ameliorate disease progression. To address this limit, we set out to test the concurrent sampling methods of large molecule microdialysis, push-pull (semi-porous membrane with 1-3 MDa cutoff) versus open flow (unfiltered, theoretically no limit). We acquired a cohort of 22-month aged PD transgenic (TG) mice that overexpressed human wild-type alpha-synuclein (JAX# 017682), their age-matched non-carrier (WT) littermates, and stereotaxically implanted a bilateral guide in the hippocampus (A/P -3.1mm to bregma, M/L +/- 2.8mm to midline). After a two-week recovery period, a push-pull probe (PP PE 6/3, CRL-GRO, the Netherlands) was inserted in the left hippocampus (D/V -4.1mm to dura), and an open flow sampling stylet (cOM-8, BASi, USA) in the right hippocampus (D/V -3.6mm to dura). A dynamic-no-net-flux (DNNF) microdialysis was performed in awake, freely behaving animals at four varying flow rates from 0.5 µl/min to 1.25 µl/min. Probes and stylets were perfused with aCSF (147 mM NaCl, 3.0 mM KCl, 1.2 mM CaCl2 and 1.2 mM MgCl2) containing 1% BSA and 2mM glucose. Samples were collected and aliquoted for alpha-synuclein protein analysis and a proinflammatory panel assay from Meso Scale Discovery (MSD# K15048). All samples were measured via ELISA/EIA kits acquired from MSD®. As expected, probe recoveries for absolute concentrations of the various analytes were higher from the open flow conditions when compared to the push-pull method. Surprisingly, the flow rate affected the recovery of analytes in the "unfiltered" open flow stylet, like the push-pull condition where higher flow rate samples measured lower analyte concentrations. Furthermore, BSA additives for non-specific binding during collection and analysis also showed differing analyte concentrations (*in vitro* results). Therefore, generating accurate biomarker profiles in disease pathology from large molecule dialysis must be sensitive to technical conditions and analytes of interest in neuropathology.

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Poster

PSTR403. Alpha-synuclein: Mechanisms and Transmission

Location: WCC Halls A-C

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Program #/Poster #: PSTR403.15/T8

Topic: C.03. Parkinson's Disease

Support: Michael J. Fox Foundation
NIH/NINDS R03NS108229
Branfman Family Foundation
Indiana Clinical and Translational Sciences Institute (CTSI)

Title: Impact of post-translational modifications on membrane-induced alpha-synuclein aggregation in synucleinopathy disorders

Authors: *M. GUZMAN SOSA[1], O. ALI[1], S. DONZELLI[3], S. DUTTA[1], A. SADEK[3], P.-M. E. IVEY[1], K. J. WEBB[1], H. A. LASHUEL[4], J.-C. ROCHET[2];
[2]Medicinal Chem. &Molecular Pharmacol, [1]Purdue Univ., West Lafayette, IN; [3]Ecole Polytechnique de Lausanne, Lausanne, Switzerland; [4]Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland

Abstract: Alpha-synuclein (aSYN) is a presynaptic protein that forms aggregates in the brains of individuals with Parkinson's disease (PD) and other synucleinopathy disorders. aSYN is a natively unfolded protein in solution but can adopt an alpha-helical structure upon binding to phospholipid membranes, and this interaction is thought to play a role in vesicle trafficking and the release of neurotransmitters. Data from our group and others suggest that the aggregation of membrane-bound aSYN plays a central role in the protein's neurotoxicity in PD via a mechanism involving vesicle disruption. Furthermore, increasing evidence suggests that the mechanism of membrane-induced aggregation is distinct from the more extensively studied process of aSYN aggregation in the absence of membranes. Although various post-translational modifications (PTMs) of aSYN identified in cell culture, animal models, or human brain have been shown to modulate aSYN aggregation in the absence of lipids, little is known about the effects of PTMs on membrane-induced aSYN self-assembly. We hypothesize that certain PTMs promote the protein's aggregation at the surface of phospholipid membranes, resulting in enhanced aSYN neurotoxicity, based on evidence that some PTMs alter the conformation of membrane-bound aSYN and/or perturb aSYN-membrane interactions. To address this hypothesis, aSYN variants with N- and C-terminal truncations previously identified in the human brain were characterized in terms of their relative propensities to undergo membrane-induced self-assembly and elicit vesicle disruption. We found that aSYN variants with C-terminal truncations underwent more extensive oligomerization and accelerated fibrillization compared to WT aSYN when incubated with phospholipid vesicles at a low pH mimicking the acidic conditions of endocytic compartments. Moreover, the rate of aSYN-mediated vesicle disruption at pH 7 (relevant to cytosolic conditions) increased with the extent of the C-terminal truncation. In general, N-terminal aSYN truncation mutants formed oligomers or fibrils more rapidly than WT aSYN in the presence of vesicles at low pH but had a reduced ability to elicit membrane permeabilization at pH 7. Current efforts are focused on characterizing our panel of aSYN truncation variants in terms of aggregation propensity in neuronal cell culture models using fluorescence lifetime imaging microscopy (FLIM) analysis. The results of these studies will provide new insights into the role of aSYN PTMs and membrane-induced aggregation in the pathology of synucleinopathy disorders, setting the stage for developing therapies targeting aSYN in the brains of patients.

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Poster

PSTR403. Alpha-synuclein: Mechanisms and Transmission

Location: WCC Halls A-C

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Topic: C.03. Parkinson's Disease

Support: Michael J. Fox Foundation
NS110436
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P41GM136463
Intramural Research Programs of NIDDK and NHLBI

Title: Structure of alpha-synuclein fibrils derived from human Lewy body dementia tissue

Authors: *D. D. DHAVALE[1], A. M. BARCLAY[3], C. G. BORCIK[4], K. BASORE[2], D. BERTHOLD[3], I. R. GORDON[1], J. LIU[1], M. MILCHBERG[4], J. Y. O'SHEA[1], M. J. RAU[2], Z. SMITH[1], S. SEN[3], B. SUMMERS[2], J. SMITH[3], O. A. WARMUTH[4], Q. CHEN[3], J. A. FITZPATRICK[2], C. SCHWIETERS[5], E. TAJKHORSHID[3], C. M. RIENSTRA[4], P. T. KOTZBAUER[1];
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Abstract: The defining feature of Parkinson disease (PD) and Lewy body dementia (LBD) is the accumulation of alpha-synuclein (Asyn) fibrils in Lewy bodies and Lewy neurites. We developed and validated a novel method to amplify Asyn fibrils extracted from LBD postmortem tissue samples and used solid state nuclear magnetic resonance (SSNMR) studies to determine atomic resolution structure. Amplified LBD Asyn fibrils comprise two protofilaments with pseudo-2_1 helical screw symmetry, very low twist and an interface formed by antiparallel beta strands of residues 85-93. The fold is highly similar to the fold determined by a recent cryo-electron microscopy study for a minority population of twisted single protofilament fibrils extracted from LBD tissue. These results expand the structural landscape of LBD Asyn fibrils and inform further studies of disease mechanisms, imaging agents and therapeutics targeting Asyn.

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Poster

PSTR403. Alpha-synuclein: Mechanisms and Transmission

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Program #/Poster #: PSTR403.17/T9

Topic: C.03. Parkinson's Disease

Support: NIH R01 NS100090
NIH RO1 NS124226.

Title: Caffeic Acid Inhibits α-Synuclein Seeding Activity in Real-time Quaking-Induced Conversion (RT-QuIC) Seed Amplification Assay: Relevance to Translational Research in Synucleinopathies

Authors: *A. GEORGE[1,2], C. JANARTHANAM[2], P. PADHI[2], J. THOMAS[2], H. JIN[2], V. ANANTHARAM[2], A. KANTHASAMY[2], G. PHILLIPS[3], A. KANTHASAMY[2];
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Abstract: Parkinson's Disease (PD) and other synucleinopathies, including Dementia with Lewy Bodies (DLB) and Multiple System Atrophy (DLB), are characterized by the accumulation of proteinaceous amyloid fibrils primarily composed of α-synuclein (αSyn) in the brain. The currently available treatment options for PD mainly target its symptoms but do not alter αSyn seeding activity and disease progression. Recently, we and others have developed an ultra-sensitive αSyn real-time quaking-induced conversion (RT-QuIC) seed amplification assay termed αSyn SAA for detecting αSyn seeding activity in multiple biomatrices including brain, CSF, submandibular gland and skin. To extend this versatile αSyn SAA platform for translational drug discovery, we tested whether it can be adapted to identify compounds intervening in the seeding activity. As proof of principle, we tested caffeic acid (CA), which is a well-known phytochemical enriched in many foods and drinks including coffee. CA has been shown to possess diverse biological functions, such as anti-inflammatory and antioxidant properties. Our initial experiments tested various CA concentrations ranging from 50nM to 100μM range in αSyn SAA. The addition of CA to the RT-QuIC reaction mixture containing human αSyn synthetic fibers exhibited a dose-dependent inhibition on the formation of αSyn fibrillar seeds, with complete suppression observed at low μM concentrations. Next, we prepared human brain homogenates obtained from autopsy-confirmed PD cases to test in the αSyn SAA in the presence and absence of different concentrations of CA. We observed a consistent dose-dependent inhibition of αSyn seeding activities upon the addition of CA, with complete suppression at low μM concentrations. In line with the RT-QuIC findings, dot blot analysis of the RT-QuIC end-products obtained from PD brain homogenates using an αSyn filament-specific antibody also demonstrated a dose-dependent inhibitory effect of CA against αSyn seeding *in vitro*. Collectively, these findings indicate that CA exerts an inhibitory effect on the aggregation of αSyn and suggest that CA and its related analogs should be further explored for the development of anti-αSyn pharmacotherapy for treating synucleinopathies. Our data also demonstrate that the RT-QuIC-based αSyn SAA could be exploited as an *in vitro* drug discovery platform for screening anti-synucleinopathy agents.

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Poster

PSTR403. Alpha-synuclein: Mechanisms and Transmission

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Program #/Poster #: PSTR403.18/T10

Topic: C.03. Parkinson's Disease

Support: Aligning Science Across Parkinson's (ASAP)

Title: Altered cortical cellular and network dynamics in a PFF-based model of Parkinson's Disease

Authors: *P. A. PATEL, K. A. FERGUSON, J. A. CARDIN, M. J. HIGLEY;
Neurosci., Yale Univ., New Haven, CT

Abstract: Parkinson's disease (PD) is characterized by progressive decline of motor function and tremor primarily resulting from loss of dopaminergic cells in the substantia nigra. However, prodromic and progressive cognitive symptoms also suggest neocortical dysfunction, a structure for which relatively little is known about PD-associated cellular- or circuit-level changes. To explore this question, we adopted a pre-formed α-synuclein fibrillar (PFF) seeding model to drive pathogenesis in cortical projection neurons. We found that injection of PFFs into the dorsal striatum robustly drives intracellular accumulation of α-synuclein phosphorylated at serine 129 (pS129) in pyramidal neurons (PNs) of the ipsilateral somatosensory and motor cortices. To monitor neural activity, we carried out 2-photon calcium imaging of GCaMP6s-expressing PNs in these cortical areas of awake, head-fixed mice placed on a freely-moving running wheel. We used animals of both sexes over the course of pathological progression. Control experiments included imaging the cortex contralateral to the injection site as well as imaging mice injected with a non-pathogenic α-synuclein monomer. Individual neurons were tracked across multiple imaging sessions spanning several weeks. At the conclusion of the final imaging session, brains were collected for pS129 immunostaining to identify cells with pathological aggregates. These images were then aligned post-hoc with *in vivo* imaging data to determine the single-neuron relationship between dysfunction and the presence of inclusions. Our initial results suggest pathological cells exhibit elevated levels of resting cytosolic calcium. Furthermore, there were significantly reduced amounts of spontaneous activity and blunted modulation in response to fluctuations in behavioral state, measured via variation in pupil diameter, whisking, and locomotion. Finally, using immunohistochemistry to probe activated microglia via increased IBA1 and CD68 immunoreactivity as well as astrocyte activation by elevated GFAP, we found evidence of non-cell autonomous glial inflammatory activity surrounding the areas of altered neuronal activity. Together, these results provide novel insight into cellular and circuit mechanisms underlying the progression of neocortical α-synuclein pathology.

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Poster

PSTR403. Alpha-synuclein: Mechanisms and Transmission

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Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR403.19/U1

Topic: C.03. Parkinson's Disease

Support: JPB Foundation award

Title: Regulation of L-type Ca^{2+} channels by alpha-synuclein

Authors: *S. CHOI[1,2], M. BEN-JOHNY[3], M. SOMAYAJI[4], E. KANTER[4,2], D. SULZER[4,2], E. V. MOSHAROV[4,2];
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Abstract: α-Synuclein (α-Syn) plays a central role in sporadic and familial Parkinson's Disease (PD) via a toxic gain-of-function mechanism, and a variety of experimental strategies are under development to decrease α-Syn levels in PD patients. α-Syn deficiency has been shown to alter the kinetics of dopamine release, but there has been relatively little analysis of changes in endogenous physiological responses in neurons expressing normal levels of the protein. We studied the effects of α-Syn deficiency on intrinsic electrophysiological properties in mouse mesencephalic neuron in culture and acute midbrain slices. Although substantia nigra (SN) dopaminergic neurons from α-Syn deficient cells showed overall normal electrophysiological properties, they had significantly lower L-type Ca^{2+} channels (LTCC) activity than cells from wild-type animals. This effect was likely related to a decrease in surface expression of LTCCs with no alteration in mRNA or single channel open probability and conductance. The decreased activity-dependent Ca^{2+} flux resulted in deficient pCREB/cFos induction - a central step in the regulation of immediate early genes expression required for synaptic plasticity. We conclude that strategies for α-Syn reduction should examine possible changes in LTCC activity and downstream second messenger systems that regulate synaptic plasticity and learning.

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Poster

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Program #/Poster #: PSTR403.20/U2

Topic: C.03. Parkinson's Disease

Support: NIH Grant

Title: Role of Norepinephrine in the spread and aggregation of Lewy pathology in an α-synuclein PFF mouse model.

Authors: *R. MIRABEL;
Univ. of Florida, Gainesville, FL

Abstract: Specific Aims
Role of Norepinephrine in the spread and aggregation of Lewy pathology in an α-synuclein PFF mouse model.
Background: Parkinson's disease (PD) is a neurodegenerative disorder prevalent in ~1% of the population above 60 years, and is characterized by severe motor deficits as well as non-motor symptoms such as loss of smell, constipation and cognitive disability. Sporadic PD is characterized by widespread alpha-synuclein (α-syn) positive inclusions called Lewy bodies and neurites in the brain, along with loss of dopamine neurons of the S*ubstantia nigra pars compacta* (SNpc). However, the underlying mechanisms of α-syn mediated pathogenesis, progression, and neurodegeneration are still not clear. Consequentially, strategies for treating pathogenesis and progression of sporadic PD have not been well developed. Previous studies and our preliminary data suggest that the norepinephrine transporter (NET) is a potential therapeutic target for α-syn pathology in PD. Our overall **hypothesis** is that genetic deletion or pharmacological inhibition of NET will reduce α-syn propagation, neurodegeneration, neuroinflammation and behavioral deficits. We will test our hypothesis by injecting pre-formed fibrils (PFFs) of α-syn in a genetic or pharmacological mouse model of NET inhibition.**Method**: 5 μg of α-syn PFFs or 0.8 μl of PBS (control) were injected unilaterally into the dorsal striatum of WT and NET KO mice. 3- or 6- months after injection, brains from WT and NET KO mice were isolated, sectioned, and stained for phosphorylated serine 129 (pSer129) of α-syn, tyrosine hydroxylase (TH) and NET. A separate cohort of WT mice seeded with PFFs were also administered desipramine (30mg/kg/day) in their drinking water after PFF injections. All mice also underwent behavioral analyses for motor function prior to immunostaining.**Results**: pSer129 α-syn aggregates and neurites were observed throughout the cortex, basal ganglia and midbrain in WT mice at 3- and 6-months post-injection. In the midbrain, pSer129 α-syn labeling was partially colocalized with TH labeling. pSer129 α-syn labeling was reduced in NET KO mice compared to WT mice in all brain regions at 3 and 6-month timepoints.**Conclusion**: Genetic or pharmacological inhibition of NET reduces the spread and aggregation of α-syn in a PFF seeding mouse model, and has a reduced propensity to cause motor dysfunction.

Disclosures: R. Mirabel: None.

Poster

PSTR403. Alpha-synuclein: Mechanisms and Transmission

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR403.21/U3

Topic: C.03. Parkinson's Disease

Support: NIH Grant 5R01NS111978-04
APDA Post-Doctoral Fellowship P11
Parkinson's Foundation Launch Award 1046253

Title: Serine-129 phosphorylation of α-synuclein is an activity-dependent trigger for physiologic protein-protein interactions and synaptic function

Authors: *L. PARRA-RIVAS[1], K. MADHIVANAN[2], B. AULSTON[4], L. WANG[5], D. PRAKASHCHAND[1], N. P. BOYER[1], V. SAIA-CEREDA[1], K. BRANES-GUERRERO[1], D. PIZZO[1], Y. OGAWA[6], P. RANGAMANI[1], S. ROY[3];
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Abstract: Phosphorylation of α-synuclein at the Serine-129 site (α-syn Ser129P) is an established pathologic hallmark of synucleinopathies, and also a therapeutic target. In physiologic states, only a small fraction of α-syn is phosphorylated at this site, and most studies have focused on pathologic roles of this post-translational modification. We found that unlike wild-type (WT) α-syn that is widely expressed throughout the brain, the overall pattern of α-syn Ser129P is restricted, suggesting intrinsic regulation. Surprisingly, preventing Ser129P blocked activity-dependent synaptic attenuation by α-syn - thought to reflect its normal function. Exploring mechanisms, we found that neuronal activity augments Ser129P, which is a trigger for protein-protein interactions that are necessary for mediating α-syn function at the synapse. AlphaFold2-driven modeling and membrane-binding simulations suggest a scenario where Ser129P induces conformational changes that facilitates interactions with binding partners. Our experiments offer a new conceptual platform for investigating the role of Ser129 in synucleinopathies, with implications for drug-development.

Disclosures: L. Parra-Rivas: None. **K. Madhivanan:** None. **B. Aulston:** None. **L. Wang:** None. **D. Prakashchand:** None. **N.P. Boyer:** None. **V. Saia-Cereda:** None. **K. Branes-Guerrero:** None. **D. Pizzo:** None. **Y. Ogawa:** None. **P. Rangamani:** None. **S. Roy:** None.

Poster

PSTR403. Alpha-synuclein: Mechanisms and Transmission

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR403.22/U4

Topic: C.03. Parkinson's Disease

Support: Department of Defense PD210045 W81XWH-22-1-0545
Medical College of Georgia at Augusta University Start-Up Fund

Title: Age-dependent motor and cognitive decline following neurotoxic α-synuclein exposure in new Parkinson's disease models

Authors: M. CHEN[1], K. SZELONG[1], J. VINCENT[1], A. EZEANII[1], S. WAKADE[2], S. YERIGENAHALLY[1], **D. E. MOR**[1];
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Abstract: Parkinson's disease (PD) is a debilitating neurodegenerative disorder characterized by both motor and cognitive symptoms and the formation of pathological inclusions containing aggregated α-synuclein protein. The gut-to-brain hypothesis of PD suggests that α-synuclein aggregation may originate in the gastrointestinal tract, potentially due to environmental toxin exposures, and thereafter spread to the central nervous system in a prion-like fashion. While rodent models have demonstrated that gut-to-brain α-synuclein spreading can occur, the precise mechanisms of α-synuclein transmission and resulting neurotoxicity remain largely unknown. Animal models that are amenable to rapid, high-throughput investigation are needed in order to facilitate the discovery of disease mechanisms and potential therapeutic targets. To this end, we have developed new 'gut-to-brain' PD models using the small model organism, *C. elegans. C. elegans* have a well-defined nervous system with highly conserved neurotransmitter signaling, a diverse set of motor and cognitive behaviors, and high genetic tractability. To initiate α-synuclein spreading from the gut, worms are exposed to an exogenous source of wild-type human α-synuclein pre-formed fibrils (PFFs). We have shown that PFFs are ingested, spread to body tissues, and induce age-dependent motor dysfunction that is dependent on host expression of human α-synuclein in neurons or muscle, consistent with prion-like mechanisms. PFF feeding also promotes dopamine neuron degeneration and the aggregation of host α-synuclein, recapitulating key features of PD. Furthermore, PFF exposure causes age-dependent loss of learning and memory function, offering new models of cognitive decline in PD. To identify mechanisms by which gut-derived α-synuclein may enter neurons and cause toxicity, we conducted a targeted RNAi screen and showed, for the first time *in vivo*, that heparan sulfate proteoglycans regulate disease phenotypes caused by α-synuclein PFFs. The results of this study offer new tools and potential targets for PD therapeutics aimed at halting the neurotoxic spread of α-synuclein from the gut to the brain.

Disclosures: M. Chen: None. **K. Szelong:** None. **J. Vincent:** None. **A. Ezeanii:** None. **S. Wakade:** None. **S. Yerigenahally:** None. **D.E. Mor:** None.

Poster

PSTR403. Alpha-synuclein: Mechanisms and Transmission

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR403.23/U5

Topic: C.03. Parkinson's Disease

Support: Adrienne Helis Malvin Medical Research Foundation

Title: Reactive astrocyte-derived neurotoxins are responsible for the neurodegeneration in a model of Parkinson's disease

Authors: J.-J. SONG, H. PARK, Y. CHOI, J. WANG, J. SHIN, S.-C. CHOU, A. JHALDIYAL, V. DAWSON, T. DAWSON, ***T.-I. KAM**;
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Abstract: Activation of microglia can lead to the conversion of astrocytes to neurotoxic species of reactive astrocyte that are observed in post-mortem tissues of various neurodegenerative diseases including Parkinson's disease (PD) and Alzheimer's disease (AD). However, it is not clear whether and how reactive astrocytes contribute to neurodegeneration of PD. We have recently developed a mouse model of sporadic α-synucleinopathy in which α-synuclein (α-syn) preformed fibrils (PFFs) were injected into the duodenum and the pylorus muscularis layer in the stomach that are innervated by the vagal nerve. In this gut-brain α-syn model, the α-syn pathology spreads in the brains and the mice exhibit a non-motor behavior including cognitive deficits as well as motor deficits. Here, we showed genetic depletion of neurotoxic reactive astrocytes in the gut-brain α-syn mouse prevented the loss of neurons and behavioral deficits, suggesting that neurotoxic reactive astrocytes play an important role in neurodegeneration induced by pathologic α-syn. Using secretome analysis, we isolated the neurotoxins that are secreted from the α-syn-induced reactive astrocytes and induce the neurotoxicity. Inhibition of secretion of neurotoxins in astrocytes or its receptor in neurons suppressed the reactive astrocyte-mediated neurotoxicity in cultures. Moreover, pharmacological inhibition or genetic depletion of neurotoxin receptor prevented pathologic α-syn-induced neurodegeneration and movement and cognitive deficits in the gut-brain α-syn mouse. Thus, strategies aimed at inhibiting reactive astrocyte-derived neurotoxins and its neuronal receptor could hold promise as a disease-modifying therapy to prevent the loss of neurons in α-synucleinopathies.

Disclosures: J. Song: None. **H. Park:** None. **Y. Choi:** None. **J. Wang:** None. **J. Shin:** None. **S. Chou:** None. **A. Jhaldiyal:** None. **V. Dawson:** None. **T. Dawson:** None. **T. Kam:** None.

Poster

PSTR403. Alpha-synuclein: Mechanisms and Transmission

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR403.24/U6

Topic: C.03. Parkinson's Disease

Support: MOST 111-2320-B-182-004-
CMRPD1K0512

Title: Alpha-synuclein-induced cognitive impairment and disruption of glutamate and complement systems in the hippocampus and striatum of mice

Authors: *Y.-T. HUANG[1], Y.-Y. LEE[1], H.-Y. LI[3], J.-C. CHEN[2];
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Abstract: Parkinson's disease (PD) belongs to a neurodegenerative disease with characteristic phenotype of movement disorder, while some patients show non-motor symptoms of anxiety, depression and dementia (so called PDD). Clinically, abnormal aggregation of α-synuclein (α-Syn) was found in patient's brain, whereas α-Syn-enriched Lewy bodies signal a neuronal death. So far, the cause of PDD is rarely explored and molecular alterations linking hippocampal synucleinopathy to dementia requires further investigation. To mimic the symptoms the PDD, mice were injected with AAV-α-Syn bilaterally into the substantia nigra (SN). Mice exhibited motor dysfunction after 6 months viral injection, along with anxiety, depression-like behavior and cognitive-related deficits. Biochemical analyses revealed decreased gene expression of glutamatergic receptors (NR2A, NR2B, mGluR5, AMPA1) in the SN, striatum (STR) and hippocampus (HIP). The microglia markers, Iba1 and complement C3 were also decreased in the STR and HIP. At the protein level, Syn211 expression increased in the SN, STR, and HIP, and phosphorylated Ser129 of α-Syn was observed in the SNc, along with activated GFAP and Iba1. To understand synucleinopathy-induced dementia, mice were injected α-Syn preformed fibrils (PFFs) into the hippocampus bilaterally. After 8 months of PFF injection, there was a significant increase in amount of α-Syn/pSer129. The mice displayed cognitive deficits in recognition memory, social memory, working memory, and long-term memory. In addition, RNA-sequencing analysis revealed pathway of transforming growth factor beta (TGFβ) were enriched in the PFF-injected mice. The TGFβ-related genes were increased in the HIP during the progress of synucleinopathy, i.e. BMPR2, BMP2, BMP5, TGFβ1 and the downstream signals of Smad1/5/8 and Smad2. Additionally, there is a time-dependent change in the expression of GFAP, Iba1, inflammasome and the complement system, with most gene expression decreased at 8 months, while increased at 12-13 months after PFF injection. Moreover, amount of AMPA1/pSer845 of glutamate receptor increased after post-PFF injection at both time intervals. Collectively, our findings present a model of Parkinson's disease dementia (PDD) in which α-Syn aggregation begins in the substantia nigra (SN) and subsequently leads to changes in the glutamatergic system, potentially contributing to the development of dementia. Consistently, hippocampal synucleinopathy successfully replicates the cognitive impairments associated with dementia, with notable change observed in the BMP/TGFβ pathways, immune complement and glutamate systems.

Disclosures: Y. Huang: None. **Y. Lee:** None. **H. Li:** None. **J. Chen:** None.

Poster

PSTR403. Alpha-synuclein: Mechanisms and Transmission

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR403.25/U7

Topic: C.03. Parkinson's Disease

Support: Grants-in-Aid for Scientific Research from the Japan Society for Promotion of Science 21K14999

Title: The expression of E50K optineurin affects the lysosome-related organelles in murine retinal cells

Authors: *W. OTSU[1], M. OBAYASHI[1], H. TSUSAKI[1], M. SHIMAZAWA[1,2];
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Abstract: Glaucoma is a group of eye disorders characterized by the progressive degeneration of retinal ganglion cells and their axons, leading to permanent vision loss or blindness. One of the genes linked to familial normal-tension glaucoma (NTG) is *OPTN*, which encodes optineurin (OPTN), a multifunctional adaptor protein involved in various intracellular vesicular trafficking pathways. The E50K mutation in the *OPTN* gene is the most prevalent one in patients with NTG. It has been reported that OPTN has an important role in selective autophagy, especially mitophagy, and E50K/OPTN impacts Rab8-mediated vesicular transport and autophagy-mediated degradation. However, the molecular mechanisms underlying the retinal cell death induced by glaucoma-associated *OPTN* mutations are not fully understood. Here we investigated the effect of the E50K/OPTN expression on the endolysosomal system in a murine immortalized retinal cell line, 661W, and in the mouse retina. To monitor the intracellular distribution of OPTN, we generated the construct expressing tandem fluorescent-tagged OPTN (OPTN-pHluorin-mCherry). Expressed wild-type OPTN was predominantly seen as both pHluorin- and mCherry-positive (yellow) puncta throughout the cell, indicating their distributions at the neutral environment of the cytosol. Wild-type OPTN with only mCherry fluorescence (red) was also found in some vesicular structures, suggesting it can exist in acidic compartments such as lysosomes or autolysosomes. On the other hand, E50K/OPTN was localized in red or yellow granular structures rather than the cytosolic pattern. The expression of E50K/OPTN also altered the morphology of Rab8-positive organelles to swollen vacuoles, which tend to form clustering at the perinuclear region. Immunoblotting revealed that lysosome-related proteins such as Transcription Factor EB and Lamp1 decreased in the presence of E50K/OPTN expression but not of wild-type. For *in vivo* experiments, embryonic retinal transfection was performed in Slc:ICR mice (Japan SLC, Hamamatsu) at gestational day 14.5 as reported previously (Otsu *et al.*, 2019, *J. Neurosci.*). Transfected eyes were harvested at postnatal 21 days, and subjected to immunostaining. Wild-type OPTN appeared as small puncta at both cell body and axon in retinal ganglion cells, whereas E50K/OPTN were detected as enlarged granules. These abnormal structures containing E50K/OPTN were frequently found at the axon in the prelaminar region of the optic nerve head. In conclusion, the exogenous expression of E50K/OPTN results in the suppression of the lysosomal gene expression and the morphological changes of the endolysosomal organelles in retinal cells.

Disclosures: **W. Otsu:** None. **M. Obayashi:** None. **H. Tsusaki:** A. Employment/Salary (full or part-time):; Shin Nippon Biomedical Laboratories, Ltd.. **M. Shimazawa:** None.

Poster

PSTR403. Alpha-synuclein: Mechanisms and Transmission

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR403.26/U8

Topic: C.03. Parkinson's Disease

Support: Sea Act Co. Ltd.

Title: Protective effect of active components of microalgae against photoreceptor damage induced by blue LED light and endoplasmic reticulum stress.

Authors: *M. OBAYASHI[1], K. YAMAZAKI[2], W. OTSU[1], S. NAKAMURA[2], H. ISHIKAWA[3], Y. SAKATA[3], M. TSUBOI[3], H. TSUSAKI[1,4], M. SHIMAZAWA[1,2]; [1]Biomed. Res. Laboratory, Gifu Pharmaceut. Univ., Gifu, Japan; [2]Mol. Pharmacology, Dept. of Biofunctional Evaluation, Gifu Pharmaceut. Univ., Gifu, Japan; [3]Sea Act Co. Ltd., Tokyo, Japan; [4]Shin Nippon Biomed. Laboratories, Ltd., Kagoshima, Japan

Abstract: In recent years, with remarkable advances in science and technology, we have been surrounded by many electronic devices in our daily lives, such as smartphones and tablets equipped with light-emitting diode (LED) displays. Although the widespread use of electronic devices has made it easy for us to access information worldwide, light emitted by these devices is a concern for vision health because too much exposure to light is known as a risk factor leading to retinal disorders, including age-related macular degeneration. It has been reported that endoplasmic reticulum (ER) stress in light-exposed retinas is linked to photoreceptor cell death. However, it has yet to fully understand the mechanism underlying the stress response to light and its link to the pathophysiology of retinal diseases. We have reported that exposure to blue LED light induces excessive production of reactive oxygen species (Kuse *et al*., 2014. *Scientific Reports*) and unfolded protein response, significantly the increase of Activating transcription factor 4 (ATF4) expression (Ooe *et al*., 2017. *Molecular Vision*). This study aims to search for compounds that exhibit protective effects against photodamage. Here, we examine the effect of PENTADECYL, a bioactive product obtained from *Aurantiochytrium limacinum*, on either chemical-induced or blue LED light-induced ER stress. PENTADECYL is a triglyceride mixture composed of odd-chain saturated fatty acids, including pentadecanoic acid. We used a murine photoreceptor cell line, 661W, as an *in-vitro* model. PENTADECYL at the concentration of 0.1-10 µg/mL suppressed either thapsigargin- or tunicamycin-induced cell death in a concentration-dependent manner. PENTADECYL showed a protective effect against blue LED light-induced cell death at the concentration of 10 µg/mL. In contrast, PENTADECYL did not inhibit cell death caused by hydrogen peroxide treatment. Quantitative RT-PCR analysis revealed that the treatment of PENTADECYL at 10 µg/mL significantly attenuated the thapsigargin-induced gene expression of unfolded protein response target genes, including *Atf4* and *Bip*. Consistently, immunoblotting showed that PENTADECYL suppressed the increased ATF4 protein level induced by either thapsigargin or blue LED light exposure. In conclusion, PENTADECYL is a novel compound having a protective effect against light-induced ER stress in photoreceptors.

Disclosures: **M. Obayashi:** None. **K. Yamazaki:** None. **W. Otsu:** None. **S. Nakamura:** None. **H. Ishikawa:** A. Employment/Salary (full or part-time):; Sea Act Co. Ltd. **Y. Sakata:** A. Employment/Salary (full or part-time):; Sea Act Co. Ltd. **M. Tsuboi:** A. Employment/Salary

(full or part-time):; Sea Act Co. Ltd. **H. Tsusaki:** A. Employment/Salary (full or part-time):; Shin Nippon Biomedical Laboratories, Ltd. **M. Shimazawa:** 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.; Sea Act Co. Ltd..

Poster

PSTR403. Alpha-synuclein: Mechanisms and Transmission

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR403.27/U9

Topic: C.03. Parkinson's Disease

Title: Recombinant progranulin protects dopaminergic neurons in vivo and decreases alpha-synuclein via promoting autolysosome degradation in vitro.

Authors: *H. FUJIMORI[1], T. OHBA[1], S. NAKAMURA[1], M. SHIMAZAWA[1,2], H. HARA[1,2]; [1]Mol. Pharmachology, Dept. of Biofunctional Evaluation, [2]Lab. of Collaborative Res. for Innovative Drug Discovery, Gifu Pharmaceut. Univ., Gifu, Japan

Abstract: [Purpose] Progranulin (PGRN) is a secreted protein that is linked to inflammation and lysosomal function. PGRN haploinsufficiency inhibits proteolysis and causes frontotemporal dementia through the accumulation of a denatured protein, TAR DNA-binding Protein of 43kDa. Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of nigrostriatal dopaminergic neurons due to the accumulation of alpha-synuclein (α-Syn), an also denatured protein, and subsequent motor dysfunction. α-Syn is degraded by the autophagy lysosomal Pathway, but the relationship between PGRN and PD pathogenesis is unknown. Therefore, we evaluated the effects of recombinant PGRN on dopaminergic neurons *in vivo* and on α-Syn degradation, including the autophagy-lysosome pathway, *in vitro*.
[Methods and Results] PGRN protein expression in the striatum was increase in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD model mice both after 1 and 3 days. Intracerebroventricular (i.c.v.) administration of PGRN ameliorated the decrease in expression of tyrosine hydroxylase, a dopaminergic neuron marker, in MPTP-treated mice. In addition, PGRN tended to improve the MPTP-induced decrease of TH-positive cells by immunofluorescence in substantia nigra. Furthermore, i.c.v. administration of PGRN ameliorated 6-hydroxydopamine-increased rotation locomotor by rotation test. In SH-SY5Y human neuroblastoma cells, 1-methyl-4-phenylpyridinium ion (MPP^+), an active metabolite of MPTP, increased α-Syn expression. In contrast, PGRN ameliorated MPP^+-induced increase in α-Syn expression. Although PGRN suppressed the increase of the levels of autophagy-related proteins sequestosome-1 (p62) and MAP1LC3 (LC3)-II by MPP^+ treatment, PGRN did not influence the phosphorylation of 5' adenosine monophosphate-activated protein kinase and mechanistic target of rapamycin, which are also proteins that regulate autophagy. Immunostaining analysis showed that PGRN ameliorated MPP^+-induced increase of LC3 puncta, an indicator of autophagosome, and co-localization of LC3 and α-Syn. The DALGreen assay showed that PGRN ameliorated the

MPP^+-induced decreasing trend of autolysosomes.
[Conclusion] These results suggest that PGRN has a protective effect against dopaminergic neuron injury, and decreases α-Syn expression via activation of lysosome function in autophagy degradation. Further studies on the functions of PGRN may lead to a definitive treatment for PD that targets α-Syn.

Disclosures: H. Fujimori: None. **T. Ohba:** None. **S. Nakamura:** None. **M. Shimazawa:** None. **H. Hara:** None.

Poster

PSTR403. Alpha-synuclein: Mechanisms and Transmission

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR403.28/U10

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: Ministry of Science and Technology, Taiwan: MOST-110-2311-B-002-001 -
National Taiwan University: NTU-112L104306
Ministry of Science and Technology, Taiwan: : MOST-109-2314-B-002 -120 -MY3

Title: Investigating Cholesterol Balance in Multiple System Atrophy (MSA) Patients Using Plasma MicroRNAs and Extracellular Vesicle Proteins

Authors: *H.-H. LIN-WANG[1], J.-W. HUANG[2], C.-C. LU[1], P.-J. KUNG[4], M.-C. KUO[6,7], Y.-T. TSAI[1], K.-H. PHOA[2], Y.-T. CHU[4,6], G. CHUNGUNCO[4], K. UEDA[8], T. OCHIYA[9], R.-M. WU[6], S.-P. LIN[1,5,3];
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Abstract: Multiple System Atrophy (MSA) is a unique subtype of Parkinson's Disease (PD), characterized by distinct biochemical indicators and medication responses compared to typical PD. To facilitate a differential diagnosis and promote targeted treatments, it is crucial to identify reliable biomarkers for MSA. In this study, we sourced blood plasma from MSA patients, contrasting miRNA and extracellular vesicle (EV) protein profiles with various Parkinsonian patient groups and healthy controls (HC). Using the Biomedical Oriented Logistic Dantzig (BOLD) selector, we narrowed down to 1-5 key markers from approximately 2700 microRNAs

and 4700 potential EV proteins. The identities of EV protein candidates revealed important insights into cholesterol homeostasis. Notably, the EV protein candidate Lecithin-Cholesterol Acyltransferase (LCAT) is necessary for converting the neurotoxic cholesterol metabolite, 24-hydroxylcholesterol (24OH-C), into 24-hydroxylcholesterol esters (24OH-CE). We discovered significantly lower quantities of plasma EV-derived LCAT in MSA patients than HCs or other PD patients, indicating aberrant accumulation of toxic cholesterol metabolite. On top of that, we found that the inflammation and oxidative stress pathways linked to MSA-related EV protein candidate Kallistatin (SERPINA4), which acts as an anti-inflammatory protein. MSA patients exhibited less Kallistatin in their plasma EV compared to HCs or PD patients, potentially leading to increased inflammation. Furthermore, analysis of MSA-associated plasma miRNAs from an independent patient cohort highlighted a significant correlation between fatty acid biosynthesis and the abovementioned pathways. Integrating putative targets from 22 MSA-associated miRNAs highlighted fatty acid biosynthesis, which may be involved in cholesterol homeostasis, inflammation, and oxidative stress. We propose an integrated regulatory network on cholesterol homeostasis, comprising miRNA target genes and EV proteins, potentially providing new diagnostic and therapeutic targets. Furthermore, the level of 24OH-CE in plasma may serve as another layer of novel biomarkers of MSA progression and contribute to the understanding of the systemic pathophysiology of MSA.

Disclosures: **H. Lin-Wang:** None. **J. Huang:** None. **C. Lu:** None. **P. Kung:** None. **M. Kuo:** None. **Y. Tsai:** None. **K. Phoa:** None. **Y. Chu:** None. **G. Chungunco:** None. **K. Ueda:** None. **T. Ochiya:** None. **R. Wu:** None. **S. Lin:** None.

Poster

PSTR404. Neuroprotective Mechanisms in Parkinson Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR404.01/V1

Topic: C.03. Parkinson’s Disease

Support: Michael J Fox Foundation (Project Grant No. 2013-8499)

Title: Dynamics of pathogenic alterations after injection of patient-derived α-synuclein extracts in non-human primates

Authors: R. KINET[1], M. BOURDENX[1], S. DOVERO[1], M.-L. AROTCARENA[1], S. CAMUS[1], G. PORRAS[1], M.-L. THIOLAT[1], I. TRIGO-DAMAS[2], C. ESTRADA[3], N. GARCIA-CARRILLO[4], ***V. PLANCHE**[1], M. TRINIDAD HERRERO[3], M. VILA[5], J. OBESO[2], E. BEZARD[1], B. DEHAY[1];
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Abstract: Parkinson’s disease (PD), Dementia with Lewy Bodies (DLB), and Multiple System Atrophy (MSA), all gathered in the synucleinopathies family disease, share the aggregation of α-synuclein (α-syn) in intracellular inclusions in neurons and/or glial cells as a pathological hallmark. Aggregated forms of α-syn accumulated in neurons are called Lewy Bodies (LB). They are found in PD and DLB and are associated with a dopaminergic neuronal loss in the substantia nigra. We have previously shown that injection of patient-derived LB and noLB fractions in the striatum of non-human primates (NHP) induces nigral and striatal degeneration of dopaminergic neurons as well as α-synuclein pathology two years after administration.This study examines the effect of PD-patients derived fraction containing LB or noLB following striatal injections in baboon monkeys from different ages over time, including various pre-determined time points (6, 12, or 24 months after injection) (n=37). One hundred eighty variables were examined, covering behavioural, histological, western blot, scan sampling, or dot blot results. Extensive analysis showed increased significantly different variables between experimental and control groups over time. To understand pathogenic alterations mechanisms, we performed a proteomic analysis of the putamen and the entorhinal cortex, two brain regions strongly impacted at 24 months of this large cohort of NHP.Overall, we observed that experimental groups injected with LB- and noLB-enriched fractions followed different neuropathological pathways over time in response to the injection and that a significant level of shared variables between LB and noLB experimental groups diverged from the control group.

Disclosures: R. Kinet: None. **M. Bourdenx:** None. **S. Dovero:** None. **M. Arotcarena:** None. **S. Camus:** None. **G. Porras:** None. **M. Thiolat:** None. **I. Trigo-Damas:** None. **C. Estrada:** None. **N. Garcia-Carrillo:** None. **V. Planche:** None. **M. Trinidad Herrero:** None. **M. Vila:** None. **J. Obeso:** None. **E. Bezard:** None. **B. Dehay:** None.

Poster

PSTR404. Neuroprotective Mechanisms in Parkinson Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR404.02/V2

Topic: C.03. Parkinson’s Disease

Support: MJFF Project Grant No. 2019- 008814

Title: Harnessing chaperone-mediated autophagy through viral- based LAMP2A overexpression in non-human primates as a Treatment of Parkinson's Disease

Authors: M.-L. AROTCARENA[1], M. FOUKA[2], L. STEFANIS[2], E. BEZARD[1], M. XILOURI[2], ***B. DEHAY**[1];
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Abstract: Lysosomal impairment is strongly implicated in Parkinson’s disease (PD). Chaperone-Mediated Autophagy (CMA) is a major lysosomal pathway responsible for alpha-synuclein

(aSyn) clearance but, at the same time, can be a direct target of aSyn-related neurotoxic effects. The rate of CMA depends mainly on the levels of LAMP2A (lysosomal transmembrane protein 2A) and the presence within the lysosomal lumen of the lys-HSC70 chaperone. This project aimed to investigate whether the CMA lysosomal pathway induction may benefit the highest-order mammalian synucleinopathy model, the non-human primate, by targeting CMA's rate-limiting step, the LAMP2A receptor. To this end, we performed bilateral injections of the AAV2/9-LAMP2A-HA vector (or the control Stuffer vector) in the SNpc of 14 male rhesus macaque monkeys (*Macaca mulatta*) together with unilateral intrastriatal injections of low doses of aSyn-containing Lewy body (LB) extracts purified from the SNpc of PD brains (or extracts from non-PD brains as control). Animals were terminated 15 months later, and brains were harvested. Extensive histochemical and biochemical analyses were performed to evaluate cerebral pathological markers known to be affected in PD. We characterized the pattern of dopaminergic loss in the striatum and the substantia nigra, the regional distribution of aSyn immunoreactivity in several brain structures, as well as its pathological status (i.e., S129 phosphorylation) and the occurrence of lysosomal dysfunction. Overall, our data so far show that viral-mediated LAMP2A overexpression protects dopaminergic neurons from the cell loss induced by the injection of PD brain extracts. Interestingly, LAMP2A-injected animals displayed significantly improved performance in the behavioral tests related to pre-frontal cortex-dependent cognitive function, suggesting that our gene therapy approach induces a beneficial cognitive effect. Lastly, LAMP2A overexpression decreases extracellular aSyn levels in the monkey biological fluids.In conclusion, this study demonstrates that viral-based overexpression of LAMP2A attenuates the dopaminergic neurodegeneration in a non-human primate model of PD. These results support the idea that enhancement of CMA through LAMP2A overexpression or other means, possibly pharmacological, might open new therapeutic opportunities for slowing down the degenerative process in patients with PD and related synucleinopathies.

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Poster

PSTR404. Neuroprotective Mechanisms in Parkinson Disease: Animal Models

Location: WCC Halls A-C

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Program #/Poster #: PSTR404.03/V3

Topic: C.03. Parkinson's Disease

Support: NIH Grant R35ES030523

Title: A partial Drp1 knockout improves autophagy flux independent of mitochondrial function

Authors: *R. Z. FAN[1], C. SPORTELLI[2], Y. LAI[2], S. S. SALEHE[2], J. R. PINNELL[2], J. R. RICHARDSON[2], S. LUO[3], K. TIEU[2];
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Abstract: Dynamin-related protein 1 (Drp1) is a member of the dynamin GTPase superfamily typically known for its role in mitochondrial fission. A partial inhibition of this protein has been reported to be protective in experimental models of neurodegenerative diseases. The protective mechanism has been attributed primarily to improved mitochondrial function. Drp1 inhibition has also been demonstrated to reduce protein aggregation in experimental models of Parkinson's disease, Alzheimer's disease, and Hungtington's disease, indicating the potential involvement of protein removal pathways such as autophagy. However, it is not feasible to untangle with certainty whether Drp1 inhibition reduces protein aggregation via mitochondria, autophagy, or a combination of both in those models since these two pathways bidirectionally regulate each other Herein, we provide evidence showing that a partial Drp1-knockout improves autophagy flux independent of mitochondria using low doses of manganese (Mn) as a model. Mn causes parkinsonian-like symptoms in humans and it has been proposed as a risk factor for PD.. In this study, first, we characterized in cell models that at low non-toxic concentrations (up to 125μM in both N27 rat dopaminergic neuronal cells and Hela autophagy reporter cells), Mn impaired autophagy flux but not mitochondrial function or morphology. Furthermore, chronic low dose of Mn treatment in mice through drinking water impaired autophagy pathways but not OXPHOS in the ventral mid brain based on RNAseq and KEGG pathway analysis. Imaging using autophagy reporter mice as well as Laser-capture microdissection-assisted cell-type specific immunoblotting revealed nigral dopaminergic neurons were more sensitive than their neighbouring GABAergic counterparts. Second, in cells with a partial Drp1-knockdown and $Drp1^{+/-}$ mice, autophagy impairment induced by Mn was significantly attenuated. This study demonstrates that autophagy is a more vulnerable target than mitochondria to Mn toxicity. Furthermore, improving autophagy flux is a separate mechanism conferred by Drp1 inhibition independent of mitochondrial fission. In summary, the present study provides two major novel mechanisms relevant to neurological disorders. The combined protective mechanisms of improving autophagy flux and mitochondrial function conferred by Drp1 inhibition make this protein an even more attractive therapeutic target.

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Poster

PSTR404. Neuroprotective Mechanisms in Parkinson Disease: Animal Models

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Program #/Poster #: PSTR404.04/V4

Topic: C.03. Parkinson's Disease

Support: NIEHS Grant R35-ES030523

Title: Drp1 inhibition reduces neuroinflammation in vivo

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Abstract: Dynamin related protein 1 (Drp1) is a “master regulator” of mitochondrial fission. We previously reported that Drp1 inhibition attenuated mitochondrial dysfunction, oxidative stress, impaired autophagy flux, and release of exosomes that contain neurotoxic α-synuclein. Given the important role of neuroinflammation in Parkinson's disease (PD), herein we investigated the impact of Drp1 inhibition on neuroinflammation. First, as a model of neuroinflammation, we injected $Drp1^{+/-}$ mice and their wild-type (WT) littermates with lipopolysaccharides (LPS), collecting the ventral midbrains (VMB) 6h later. Nanostring neuroinflammation analysis showed that LPS increased levels of many pro-inflammatory genes in WT mice; however, significant protection was observed in $Drp1^{+/-}$ littermates. The most dramatic change was lipocalin 2 (*Lcn2)* gene, whose gene product activates NLRP3 inflammasome. These results were validated through qPCR and Meso Scale Discovery cytokine assay. Sholl analysis confirmed morphologically less microglial activation in $Drp1^{+/-}$ mice. To further investigate the role of Drp1 in microglia, we treated LPS in mice with inducible microglia-specific Drp1 deletion by crossing Drp1-LoxP with $Cx3Cr1\text{-}Cre^{ERT}$ mice, then captured individual microglia in the substantia nigra using laser microdissection, followed by qPCR analysis. Results revealed that microglia with $Drp1^{+/-}$ expressed less pro-inflammatory genes. Lastly, we also detected increased levels of *Lcn2* and other pro-inflammatory genes in transgenic α-synuclein mice but attenuated in those crossed with $Drp1^{+/-}$ mice. Together, our data indicate that a partial Drp1 knockout is sufficient to reduce neuroinflammation in multiple animal models, suggesting Drp1 is a promising therapeutic target for PD. The mechanism underlying the anti-inflammatory effects conferred by Drp1 inhibition is being investigated.

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Poster

PSTR404. Neuroprotective Mechanisms in Parkinson Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR404.05/V5

Topic: C.03. Parkinson’s Disease

Support: ERC #951294

Title: Intracerebrally-delivered AAV-synuclein (with or without fibroin) leaks to the mouse peripheral organs: in vivo and ex vivo monitoring by bioluminescence imaging

Authors: C. MAZZOCCO[1], C. GENEVOIS[2], E. DOUDNIKOFF[3], ***M. LANDRY**[4], N. DUTHEIL[1], T. LESTE-LASSERRE[6], E. BEZARD[5];
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Abstract: The popular viral vector-mediated delivery of wild-type or mutated (A53T) α-synuclein requires improving its induced lesion in mice as well as following up *in vivo* its expression. With these objectives in mind, we tested a bioluminescent expression reporter of α-synuclein embedded or not in a fibroin solution, the main protein of the silkworm's silk, classically used in optogenetic studies for increasing viral vector-mediated transfection.We developed an in vivo bioluminescent expression reporter of α-σψνυχλειν under the control of the synapsin promoter engineered into an AAV2/9. We used the bioluminescence enzyme Nanoluc, which is more efficient for deep-tissue imaging. We first verified the expression of the fused protein in vitro by bioluminescence imaging, then established the acquisition time for the most intense bioluminescence imaging signal and evaluated substrate concentrations to optimise the signal. Next, two groups of 15 male C57Bl6Jr mice were unilaterally injected with the fused protein Nanoluc_humanA53Tα-synuclein above the substantia nigra (SN) combined (or not) with fibroin, a silkworm protein, as a vehicle to stabilise its expression in space and time. We first validated the expression of the Nanoluc-humanA53Tα-synuclein fusion protein *in vitro* by optical imaging on transfected HEK293T cells and Western blotting using anti-Nanoluc and anti-human α-synuclein antibodies. The *in vivo* cerebral bioluminescence signal was more intense in the presence of fibroin. Using immunohistochemistry, we found that the humanA53Tα-synuclein staining was more restricted to the striatum and the SN with fibroin. Counting of tyrosine hydroxylase-labelled neurons revealed a greater induced-lesion when injected with fibroin. We, however, detected a bioluminescence signal in peripheral organs such as the prostate, the intestine and the liver, all more intense in the absence of fibroin. We suspected expression leakage and therefore verified by RT-qPCR the presence of viral RNA corresponding to the injected AAV in the positive organs, confirming this leakage despite using a specific neuronal promoter. Our data have two main consequences: (i) Mixing AAV with fibroin ensures accurate control of transgene expression while diminishing the peripheral leakage. (ii) In vivo Nanoluc_humanA53Tα-synuclein protein expression tracking by bioluminescence imaging would enable proper animal selection in preclinical testing of therapeutic strategies, first by allowing post-surgery animal selection and (ii) by possibly providing an in vivo monitored end-point of success (or failure) of the tested therapy.

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Poster

PSTR404. Neuroprotective Mechanisms in Parkinson Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR404.06/V6

Topic: C.03. Parkinson's Disease

Support: ERC #951294

Title: Disentangling the respective role of aggregopathy versus neurodegeneration on extracellular space nanometric diffusional parameters in synucleinopathy

Authors: J. ESTAUN-PANZANO[1], S. NANDI[2], Q. GRESIL[2], E. DOUDNIKOFF[3], C. MAZZOCCO[4], ***W. MEISSNER**[5], B. DEHAY[6], M.-H. CANRON[4], M.-L. AROTCARENA[7], L. COGNET[2], E. BEZARD[6];
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Abstract: Synucleinopathies represent a group of neurodegenerative disorders characterised by the accumulation of aggregated α-synuclein inside cells. The pathogenic mechanisms underlying synucleinopathies are believed toinvolve the spread and propagation of α-synuclein aggregates throughout the central nervous system and at least partially through the extracellular space (ECS). The ECS and its proteic scaffold, the extracellular matrix, are emerging as a critical component for communication and regulation in health and disease. Single particle tracking of near-infrared emitting Single Wall Carbon Nanotubes (SWCNTs) has become a powerful tool to characterise some of the fundamental ECS properties at the nanoscale. The recording of long trajectories in the so-called transparency window of tissue permits the reconstruction of ECS maps with nanometric resolution and extracts instantaneous diffusion coefficients and estimation of channel width. Using SWCNTs single-particle tracking, we previously unravelled a significant increase in Substantia Nigra pars compacta extracellular diffusion values in the context of α-synuclein pathology accompanied by nigral neuron degeneration. To disentangle the respective roles of neurodegeneration and aggregopathy in leading these dramatic changes, we here investigated ECS instantaneous diffusion coefficients and estimation of channel width in the substantia nigra and the striatum of mice featuring neurodegeneration (our classic PD patient-derived Lewy bodies extract) versus ones exhibit a synuclein aggregopathy without degeneration (strain 1B of synuclein pre-formed fibrils (PFF)). Our results show that: (i) SN and striatum present different diffusional regimes; (ii) neurodegeneration models present increased diffusion regimes in the SN but also in the striatum, (iii) the sole presence of secondary aggregates (without neurodegeneration) in the striatum in an aggregopathy model also causes an increased diffusion regime in the striatum. The spread of the synucleinopathy can therefore affect ECS diffusional parameters, thereby likely further contributing to its propagation. We hypothesise that these aggregates can affect the surrounding microenvironment, triggering inflammatory responses, which would widen the ECS. Understanding the distinct effects of these synucleinopathy models on the ECS is crucial for unravelling the mechanisms underlying synuclein propagation, neurodegeneration, and associated clinical manifestations. Elucidating the interplay between aggregated α-synuclein and the ECS may unveil potential therapeutic targets to modulate synucleinopathy progression.

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Poster

PSTR404. Neuroprotective Mechanisms in Parkinson Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR404.07/V7

Topic: C.03. Parkinson's Disease

Support: Coave Therapeutics

Title: Human TFEB overexpression prevents mutant human A53T-α-synuclein toxicity in a prevention design applied to a rat model of Parkinson's disease

Authors: M.-L. AROTCARENA[1], **R. KINET**[2], G. DABEE[3], *E. BEZARD[3], B. DEHAY[3]; [1]Neurodegeneratives Dis. Inst., Bordeaux Cedex, France; [2]Inst. of Neurodegenerative diseases, Bordeaux, France; [3]Inst. of Neurodegenerative Dis., Bordeaux, France

Abstract: The synucleinopathies Parkinson's disease (PD) and Multiple system atrophy (MSA) — characterised by neurodegeneration and α-synuclein intracytoplasmic inclusions into, respectively, neurons and oligodendrocytes — are associated with impairment of the autophagy-lysosomal pathways (ALP). Increased expression of the master regulator of ALP, transcription factor EB (TFEB), is hypothesised to promote the clearance of WT α-synuclein and survival of dopaminergic neurons.Here, we explore the efficacy of targeted human TFEB overexpression in substantia nigra neurons to reduce the pathological burden of α-synuclein in a viral-based rat model of nigral human A53T α-synuclein overexpression. To assess whether the timing of therapeutic intervention application vis-à-vis the pathological trigger matters, we evaluated the effects of AAV-hTFEB in three experimental designs: (i) in a therapeutic setting (i.e., striatal AAV-hTFEB injection at one mo p.i. after nigral AAV-Synuclein injection) and two prevention settings (ii) concomitant injections of nigral AAV-hTFEB and AAV-Synuclein; and (iii) concomitant injections of nigral AAV-Synuclein and intrastriatal AAV-hTFEB. All groups were followed behaviourally. The brains were harvested four months after AAV-Synuclein injection. Extensive histochemical analyses were performed to evaluate cerebral pathological markers known to be affected in PD. We characterised the human TFEB expression, the pattern of dopaminergic loss in the striatum and the substantia nigra, the regional distribution of α-synuclein immunoreactivity in several brain structures, as well as its pathological status (i.e., S129 phosphorylation). We observed that human TFEB was correctly expressed in the rat substantia nigra and the striatum. Human TFEB nigral expression was sufficient to prevent nigrostriatal neurodegeneration in this PD rat model both at the cell body (substantia nigra) and terminal (striatum) levels when injected only at the nigra level. This beneficial effect was associated with a decreased accumulation of α-synuclein into the substantia nigra and a strong clearance of phosphorylated (S129) α-synuclein.Our study confirms the disease-modifying potential of human TFEB by extending the demonstration to an AAV-A53T synuclein rat model and, for the first time, validating the human version of TFEB transgene, paving the way for gene therapy of PD.

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Poster

PSTR404. Neuroprotective Mechanisms in Parkinson Disease: Animal Models

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Program #/Poster #: PSTR404.08/V8

Topic: C.03. Parkinson's Disease

Support: NIH Grant NS127391
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Parkinson's Cell Therapy Research Func
SERB Grant EMR/2014/001127

Title: An optimized Nurr1 agonist provides disease-modifying effects in Parkinson's disease models

Authors: *W. KIM[1,2], M. TRIPATHI[3], C. KIM[1,2], S. VARDHINENI[3], Y. CHA[1,2], S. K. KANDI[3], M. FEITOSA[1,2], R. KHOLIYA[3], E. SAH[1,2], A. THAKUR[3], Y. KIM[1,2], S. KO[1,2], S. MANOHAR[3], Y.-B. KONG[1,2], K. BHATIA[1,2], G. SINDHU[3], Y.-S. KIM[4], B. M. COHEN[1], D. S. RAWAT[3], K.-S. KIM[1,2];
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Abstract: The nuclear receptor, Nurr1, is critical for both the development and maintenance of midbrain dopamine neurons (mDANs), representing a promising molecular target for Parkinson's disease (PD). We previously identified three Nurr1 agonists (amodiaquine, chloroquine and glafenine) that share an identical chemical scaffold, 4-amino-7-chloroquinoline (4A7C), suggesting a structure-activity relationship. Herein we report a systematic medicinal chemistry search in which over 570 4A7C-derivatives were generated and characterized. Multiple compounds enhance Nurr1's transcriptional activity, leading to identification of an optimized, brain-penetrant agonist, 4A7C-301, that exhibits robust neuroprotective effects in vitro. In addition, 4A7C-301 protects mDANs in the MPTP-induced male mouse model of PD and improves both motor and non-motor olfactory deficits without dyskinesia-like behaviors. Furthermore, 4A7C-301 significantly ameliorates neuropathological abnormalities and improves motor and olfactory dysfunctions in AAV2-mediated α-synuclein-overexpressing male mouse models. These disease-modifying properties of 4A7C-301 may warrant clinical evaluation of this or analogous compounds for the treatment of patients with PD.

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Poster

PSTR404. Neuroprotective Mechanisms in Parkinson Disease: Animal Models

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Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR404.09/V9

Topic: C.03. Parkinson's Disease

Support: NIH Grant 1ZIAES103310

Title: High metabolic rate reveals selective vulnerability of SNc dopamine neurons in Parkinson's Disease

Authors: *C. MENG[1], S. ELIASEN[2], A. PAPANERI[2], G. CUI[2];
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Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disease featuring progressive loss of dopamine (DA) neurons in substantia nigra pars compacta (SNc). However, the mechanism underlying the selective vulnerability of SNc-DA neuron in PD is still unknown. Computational studies proposed that SNc-DA neurons are more vulnerable than other neurons during aging because of their high bioenergetic demand due to the longer and more branched axons paired with non-stopped pacemaker firing. Here, we explored whether SNc-DA neurons have higher basal metabolic rate than other neuronal types, including glutamatergic-, GABAergic-, cholinergic- and norepinephrinergic neurons, and DA neurons in the ventrotegmental area using genetically encoded fluorescent ATP/ADP ratiometric sensor percevalHR in freely moving mice. We show that SNc-DA neuron has the lowest ATP/ADP ratio, indicating the highest ATP utilization and metabolic rate in the brain. We further confirm that the axonal terminals of SNc-DA neurons show higher ATP demands on energy supplies than somata, which may trigger the early axonal degeneration when energy demand exceeds the supply in the early stages of PD. These findings provide definitive evidence that SNc-DA neurons are more metabolically demanding than other neuronal types, which may contribute to the their selective vulnerability in PD.

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Poster

PSTR404. Neuroprotective Mechanisms in Parkinson Disease: Animal Models

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Program #/Poster #: PSTR404.10/V10

Topic: C.03. Parkinson's Disease

Title: Lysosomal mechanisms of Oxidation resistance 1 (OXR1)-mediated neuroprotection in preclinical models of Parkinson's disease.

Authors: *N. AMMAL KAIDERY[1,2,3], M. AHUJA[2,7,3], A. SOKRATIAN[8], P. L. OLIVER[9,10], D. WINTER[11], S. M. SHARMA[4], A. B. WEST[8], B. THOMAS[2,5,3,6];
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Abstract: Parkinson's disease (PD) is a neurodegenerative movement disorder characterized by the loss of nigral dopaminergic neurons and the presence of alpha-synuclein positive fibrillar cytoplasmic inclusions known as Lewy bodies. Oxidative stress is a prominent etiological feature in the loss of dopaminergic neurons in PD, although the pathways that govern defense against reactive oxygen species in neurodegeneration remain unclear. Oxidation resistance 1 (*OXR1*) was first identified in a screen for human genes that could rescue the DNA oxidation repair-defective phenotype of a spontaneous *E. coli* mutant. Increasing evidence suggests that OXR1 has a regulatory role in controlling several stress response genes associated with survival during oxidative stress. Here we show the upregulation of OXR1 in cellular and mouse models of PD and suggest that OXR1 is a novel early marker of stress in neurons and potentially a new neuroprotective factor. Oxr1 overexpression in the mouse prion promoter-driven (PRP)-OXR1 transgenic mice attenuated 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) as well as mouse alpha-synuclein preformed fibril-mediated loss of nigrostriatal dopaminergic neurons and reduced expression of markers for oxidative stress and neuroinflammation. Co-immunoprecipitation studies show that OXR1 interacts with the vacuolar-type H+-ATPase (v-ATPase), a proton pump critical for lysosomal acidification. Neuronal cells lacking OXR1 exhibited an increase in lysosomal pH, reduced lysosomal proteolytic activity, and exacerbated neurodegeneration in preclinical models of PD. We employed innovative systems biology approaches to compare similarities in affected pathways between single-nuclei transcriptomic data from human PD patients and proteomic data from preclinical models of alpha-synucleinopathy overexpressing OXR1. Our analysis revealed that lysosomal pathways are involved in neuronal survival due to the overexpression of OXR1 in PD preclinical models. These results suggest that OXR1 plays an integral role in lysosomal mechanisms of neuroprotection and that upregulation of OXR1 is a promising therapeutic approach for PD and synucleinopathies.

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Poster

PSTR404. Neuroprotective Mechanisms in Parkinson Disease: Animal Models

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Topic: C.03. Parkinson's Disease

Support: Parkinson Canada
Canada Research Chair
Canadian Institutes of Health Research
Canada Foundation for Innovation

Title: Optogenetic stimulation of astroglia prevents dopamine neuron degeneration

Authors: *J. L. MCNEILL[1], I. TRUJILLO-PISANTY[2], S. SIMARD[1], C. A. RUDYK[1], C. GROULX[1], K. FARMER[1], S. P. HAYLEY[1], G. COPPOLA[3], N. SALMASO[1,4];
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Abstract: Astrocytes are an abundant and heterogenous glial cell type that are intimately involved in several critical functions including the modulation of ion and neurotransmitter homeostasis, the regulation of oxidative stress and the production of key energy substrates for neurons. They are also essential in injury and disease response through morphological, physiological and functional changes, in a process known as reactive astrogliosis. Given this, it is unsurprising that astrocyte dysfunction is observed in Parkinson's disease (PD). For example, mutations in genes associated with PD have been linked to impaired mitochondrial function in astrocytes[1], and the overexpression of α-synuclein specifically in astrocytes is sufficient to produce earlier and more severe motor deficits.[2] Furthermore, the selective targeting and stimulation of astroglia using optogenetics has been shown to stimulate the release of FGF2 and increase functional repair in vitro and in vivo following administration of MPTP and 6-hydroxydopamine (6-OHDA)[3].This suggests a neuroprotective effect of astrocytes in PD. In this study, we further investigate the therapeutic potential of astroglia using optogenetics to stimulate astroglial cells in the substantia nigra (SNc) of rats injected with 6-OHDA. Briefly, rats were injected unilaterally with 6-OHDA into the dorsal striatum followed the next day by optogenetic stimulation of astroglia within the SNc. The rats were then tested on a battery of behavioral tests either 8- or 21-days post 6-OHDA administration. While there was no significant effect of the lesion 8 days post 6-OHDA administration, there were, as expected, significant motor deficits on the rotarod and apomorphine-induced rotations test 21 days post lesion. Interestingly, these deficits were attenuated with stimulation of SNc astroglia. In addition, a significant decrease in tyrosine hydroxylase (TH) positive neurons was noted in lesioned animals that was also attenuated with optogenetic stimulation of SNc astroglia. Taken together, this suggests an astroglial-induced neuroprotective effect at both the cellular and behavioral level. RNA-sequencing analysis of the SNc revealed differentially expressed genes that are implicated in microglial activation, immune pathways, and cytokine signaling. Altogether, results from this sequencing analysis further supports the notion that multi-cellular cross-talk is integral to neurodegeneration and neuroprotective processes.

Disclosures: J.L. McNeill: None. **I. Trujillo-Pisanty:** None. **S. Simard:** None. **C.A. Rudyk:** None. **C. Groulx:** None. **K. Farmer:** None. **S.P. Hayley:** None. **G. Coppola:** None. **N. Salmaso:** None.

Poster

PSTR404. Neuroprotective Mechanisms in Parkinson Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR404.12/V13

Topic: C.03. Parkinson's Disease

Title: Exercise-induced angiogenesis and midbrain neuroprotection in a mouse model of experimental Parkinson's Disease.

Authors: *T. N. RODRIGUEZ, R. J. SMEYNE, M. SMEYNE;
Dept. of Neurosci., Farber Inst. of Neurosci. Thomas Jefferson Univ., Philadelphia, PA

Abstract: Parkinson's disease (PD) is a debilitating neurological disorder that affects 2% of the population aged 50 and older. Characteristic PD pathology includes loss of dopaminergic (DA) neurons in the Substantia Nigra pars compacta (SNpc). Loss of 60-70% of these neurons in the SNpc manifests in motor symptoms: tremor, bradykinesia, and gait disturbances. Currently, PD symptomology can be addressed with drugs/surgery, but these treatments fail to slow the progression of the disease. Epidemiological studies offer an intervention that appears to slow physical motor symptom progression, may protect DA neurons from further degeneration and lowers the risk of developing the disease: exercise. Aerobic exercise has been shown to stop progression of motor symptoms in humans, as well as protect against neuronal pathology in mice. However, the mechanisms underlying exercise-induced neuroprotection from PD symptoms remain largely unknown. Previous work in our lab has shown that administration of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes a 40% loss of DA neurons in the SNpc of standard-house mice, compared to a 5% loss in exercised mice. We demonstrated that one mechanism underlying this neuroprotection is exercise's ability to cause cellular hypoxia in the SNpc. Cellular hypoxia induces transcription of target genes that include those that increase angiogenesis. Early studies demonstrated that DA neurons in the SNpc have intimate spatial relationships with capillaries. In PD patients, normal contacts between nigral neurons and capillaries are lost at early disease stages, accompanied by abnormal capillary morphology. Exercise has profound angiogenic capabilities in the muscular and cardiovascular systems, but exercise-induced microvascular changes have yet to be characterized in the SNpc in the context of neuroprotection. In this study, we perfused mice with a tomato lectin conjugate and co-labeled DA neurons to model microvasculature in the SNpc. Using Imaris Filament Tracer, we are able to reconstruct a model of the microvasculature and characterize angiogenesis in the SNpc in response to exercise. The goal of this study is to assess the correlation between exercise-induced angiogenesis and SNpc DA neuron survival when MPTP is administered. Future studies to determine biochemical and epigenetic changes to the NVU architecture in response to neuroprotective amounts of exercise and blockade of those pathways to assess exercise-induced neuroprotection are planned.

Disclosures: T.N. Rodriguez: None. **R.J. Smeyne:** None. **M. Smeyne:** None.

Poster

PSTR404. Neuroprotective Mechanisms in Parkinson Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR404.13/V14

Topic: C.03. Parkinson's Disease

Title: Differential Effective of Exercise on Young and Old Rats in Protecting Against 6OHDA Toxicity

Authors: *M. HANNA[1], V. FERNANDEZ[2], A. TALAUGON[1];
[2]Biol., [1]Vanguard Univ., Costa Mesa, CA

Abstract: Parkinson's disease (PD) is a neurodegenerative movement disorder characterized by the degeneration of the dopaminergic neurons of the nigrostriatal pathway. Patients with PD typically experience motor symptoms, including tremors, rigidity, slow movement, and postural instability. Lewy Body pathology from misfolded α-synuclein aggregates is another major hallmark of the disease. In animal models of PD, increased oxidative stress, reactive oxygen species, inflammatory cytokines, and microglial activation have been reported to play a significant role in mediating PD pathology. Previous studies mostly look at young rats in examining whether exercise can act as a protective factor against 6-OHDA toxicity. This study examined the effects of four weeks of spaced, high-intensity exercise in protecting against the dopaminergic neurotoxin 6-hydroxydopamine in 2-month and 12-month-old rats. After a unilateral injection of 6-OHDA, behavioral tests were assessed within one week by examining apomorphine-induced rotation, asymmetric paw use, and stride length. Apomorphine-induced rotation is used to evaluate the extent of impairment caused by 6-OHDA and directly correlates to the degree of dopaminergic loss. Results showed that in 6-OHDA injected rats, exercise training reduced apomorphine-induced contralateral rotation compared to the non-exercised 6-OHDA group. Young, exercised rats showed less apomorphine-induced rotation compared to old rats. Exercised rats also showed less asymmetric paw use compared to non-exercised rats. Old, exercised rats had higher asymmetric paw use than young, exercised rats. The data suggest that high-intensity, short-term exercise can act as a neuroprotectant factor by reducing the extent of dopaminergic neurodegeneration. The data also indicate that old rats are less susceptible to the protective effects of exercise.

Disclosures: M. Hanna: None. **V. Fernandez:** None. **A. Talaugon:** None.

Poster

PSTR404. Neuroprotective Mechanisms in Parkinson Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR404.14/V15

Topic: C.03. Parkinson's Disease

Support: Center for Development and Behavioral Neuroscience
Arnold and Mabel Beckman Foundation

Title: Optimizing levodopa-treatment through treadmill controlled exercise in a hemiparkinsonian rat model

Authors: *S. VENKATESH[1], H. HOLDEN[2], N. KINZONZI[3], A. CENTNER[4], C. R. BISHOP[4]; [2]Binghamton Univ., [1]Binghamton Univ., Vestal, NY; [4]Binghamton Univ., [3]Binghamton Univ., Binghamton, NY

Abstract: Parkinson's disease (PD) is a progressive neurodegenerative motor disorder characterized by a degeneration of dopamine (DA) neurons within the substantia nigra pars compacta (SNpc). DA replacement therapy using levodopa (L-DOPA)improves motor symptoms, however chronic use results in L-DOPA-induced dyskinesia (LID) characterized by abnormal involuntary movements (AIMs). In recent clinical and experimental studies, exercise has demonstrated potential disease-modifying effects within PD while also reducing LID liability. To better characterize the benefits of exercise, the aim of this study was to evaluate the effect of treadmill exercise on motor performance, L-DOPA's pro-motor efficacy and the development of LID.To do this, male and female Sprague-Dawley rats were rendered hemiparkinsonian by a unilateral injection of 6-hydroxydopamine (6-OHDA) into the left medial forebrain bundle. Following lesion, animals were counterbalanced into equally lesioned sedentary and exercise groups using the forepaw adjusting steps (FAS) test and amphetamine-induced rotations. Thereafter, a 4-week testing period was implemented, where exercised animals underwent 35 mins of treadmill-controlled exercise every weekday and received a performance score between 1-4. One hour post exercise, all animals were administered 4 mg/kg of L-DOPA. To observe LID development across both treatment groups, AIMs were observed on days 1, 7, 14, and 21, rated on a scale of 1-4 based on their presence of axial, limb and orolingual (ALO) dyskinesia behaviors. Additionally, rotarod testing was conducted a day prior to or following AIMs testing to evaluate latency to fall as an additional measure of motor performance. After the 4-week testing period, FAS and rotarod were conducted 60 min after L-DOPA administration to assess differences in L-DOPA efficacy between sedentary and exercise groups. Results demonstrated that all lesioned rats displayed a clear reduction in motor performance on FAS, however no exercise-related improvements were observed. Interestingly, consistent treadmill exercise appeared to attenuate and delay the onset of LID development. These findings suggest chronic exercise can maintain the efficacy of L-DOPA while reducing side effects like LIDin PD.

Disclosures: S. Venkatesh: None. **H. Holden:** None. **N. Kinzonzi:** None. **A. Centner:** None. **C.R. Bishop:** None.

Poster

PSTR404. Neuroprotective Mechanisms in Parkinson Disease: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR404.15/V16

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

Support: This technology was supported in part by an award from the Kentucky Cabinet for Economic Development, under Grant Agreement CED 2021-002-008
NIH Grant NS107148

Title: A Non-Invasive Method for Quantitative Tremor Assay in Mice Using Piezoelectric Sensors

Authors: *D. HUFFMAN[1], R. BERNAT[1], S. SUNDERAM[2], K. DONOHUE[1], B. F. O'HARA[3,1];
[1]Signal Solutions, LLC, Lexington, KY; [2]F Joseph Halcomb III MD Dept of Biomed. Engin., [3]Dept of Biol., Univ. of Kentucky, Lexington, KY

Abstract: Essential Tremor (ET) is the most prevalent movement disorder, affecting an estimated 25 million individuals worldwide. Involuntary shaking (tremor) of the body is a hallmark symptom of ET, and significantly impacts quality of life. Thus, assessing and quantifying tremor and its response to therapeutic intervention is a key component of ET research. However, while means of reliably inducing tremor in preclinical models are well established, existing methods for assessing tremor and its response to intervention are limited in either their throughput or specificity, and better research tools are needed. Previously, we have shown that the *PiezoSleep* system can resolve various rodent behaviors, such as sleep and wake, breathing, and seizures. Here, we investigate the application of the system in resolving movements specific to tremor to serve as the basis for a high-throughput tremor assay in mice. Nine female C57BL/6 mice (3 months aged) were transferred to *PiezoSleep* cages where motion data was continuously recorded throughout two experiments: one in which animals received saline and one in which animals received harmaline (15 mg/kg) (both subcutaneously administered). During experiments, animals were visually monitored to confirm presence of tremor, and a continuous video record was collected. Following experiments, motion signals (sampled at 120 Hz) corresponding to the 30-minute pre- and 240-minute post-injection periods were loaded into MATLAB™, and processed to visualize signals and quantify the relative contribution of tremor-band frequencies (11-15 Hz) to total signal power (Tremor Band Contribution; TBC). Of the nine mice treated with harmaline, eight developed visible motor tremors that resulted in clear tremor-band motion signals. Tremor signals were successfully resolved by the TBC measure both in terms of tracking discrete tremor events as well as the course of tremor progression throughout the experiment. Following harmaline, TBC significantly deviated from levels observed following saline, with no significant difference observed during the pre-treatment period. This rise and fall of TBC throughout the recording also followed observed tremor dynamics. These results suggest the *PiezoSleep* system shows promise in its utility as a non-invasive, high-throughput alternative for tremor research.

Disclosures: **D. Huffman:** A. Employment/Salary (full or part-time):; Signal Solutions, LLC. **R. Bernat:** A. Employment/Salary (full or part-time):; Signal Solutions, LLC. **S. Sunderam:**

None. **K. Donohue:** A. Employment/Salary (full or part-time):; Signal Solutions, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Signal Solutions, LLC. **B.F. O'Hara:** A. Employment/Salary (full or part-time):; Signal Solutions, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Signal Solutions, LLC.

Poster

PSTR405. Cellular and Molecular Mechanisms of Neuromuscular Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR405.01/V17

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Maria Joana and Family philanthropic gift

Title: Biochemical and cellular effects of atp13a2 mutations linked to hereditary spastic paraplegia

Authors: *C. MASSARI, D. MOORE;
Van Andel Res. Inst., Grand rapids, MI

Abstract: ATP13A2 is a transmembrane P5-type ATPase that can function to export polyamines from the lysosomal lumen to the cytosol. Mutations in the *ATP13A2* gene have been reported to cause a range of autosomal recessive, familial neurodegenerative diseases, such as Kufor-Rakeb syndrome, juvenile-onset Parkinson's disease, neuronal ceroid lipofuscinosis, and hereditary spastic paraplegia (HSP). HSP comprises a range of heterogeneous inherited neurodegenerative and neurodevelopmental disorders characterized by weakening of the lower limbs. However, familial HSP-linked mutations in ATP13A2 have not yet been biologically characterized, and there is a lack of knowledge regarding their pathogenic effects. Here, we developed a series of expression plasmids that comprise full-length human ATP13A2, either wild-type (WT) or 9 familial mutations linked to HSP (Q122*, R444*, Q486RFS*26, T512I, R704T, R740*, L820NFS*30, P851RFS*26 and Q1135*), in order to evaluate how these mutations might lead to phenotypes in HSP. We initially find that a number of HSP-linked mutations reduce the steady-state levels of ATP13A2 protein in human cell lines, suggesting that they may impair protein stability. Pulse-chase assays using the protein synthesis inhibitor, cycloheximide, confirm that most HSP mutations accelerate the turnover of ATP13A2 protein compared to the WT protein. Consistent with this effect, HSP-linked ATP13A2 mutants exhibit enhanced degradation by the proteasome and lysosome compared to WT protein. We also find that HSP-linked mutations can induce the mislocalization of ATP13A2 away from LAMP1-positive lysosomes and towards the protein disulfide isomerase-positive endoplasmic reticulum in human cells, consistent with a loss of proper protein folding and stability. In primary cortical neurons we find that HSP-linked mutations also disrupt the normal lysosomal localization of ATP13A2. We have recently developed new plasmids expressing ATP13A2 fused to APEX2 for proximity-based

labeling to allow the identification of novel ATP13A2-interacting proteins in live cells, where we will further compare the effects of WT and HSP-linked mutations. Our data support the idea that HSP-linked mutations in *ATP13A2* exert their pathogenic effects through a loss-of-function mechanism. The study of HSP-linked *ATP13A2* mutations will enable a better understanding of HSP pathobiology and related neurodegenerative diseases.

Disclosures: C. Massari: None. **D. Moore:** None.

Poster

PSTR405. Cellular and Molecular Mechanisms of Neuromuscular Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR405.02/V18

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: A conditional mouse model of GEMIN5 neurodevelopmental disorder with motor dysfunction and cerebellar atrophy

Authors: *C. H. NELSON, IV, S. KOUR, T. R. FORTUNA, S. AGNIHOTRI, U. B. PANDEY;
Univ. of Pittsburgh, Pittsburgh, PA

Abstract: GEMIN5 is a multidomain RNA binding protein with varying functions. As a member of the SMN complex GEMIN5 is involved in snRNP biogenesis while also has RNA regulatory functions as an independent protein. Recent studies have identified GEMIN5 as the cause of a novel neurodevelopmental disorder characterized by cerebellar atrophy, global developmental delay, and motor dysfunction (NEDCAM). Patients display low levels of GEMIN5 protein expression and possess variants in a homozygous or compound heterozygous manner, suggesting a LOF modality. Previous model organisms have included *Drosophila*, Zebrafish, and KO mice; however, larval/embryonic lethality has been observed in all models, hindering investigation. The cre-loxP system is a well characterized framework for the creation of conditional mouse models with temporospatial control. Here, we report the creation of a conditional mouse model for the study of *Gemin5* using Cre-ERT2 linked to a human ubiquitin C promoter and a uniquely engineered floxed *Gemin5* transgene. Animals homozygous for this transgene, both with (hom-cre) and without UBC-Cre-ERT2 (hom-ctrl) expression were used for experimentalpurposes.3.5-week-old hom-cre animals (n=7) injected with a high dose of tamoxifen showed rapid deterioration, significant short-term differences in mass, and some alterations in gait when compared to hom-ctrl mice (n=7). To increase survival time in the hopes of observing a phenotype closer to NEDCAM, tamoxifen dosage and injection timepoint were lowered. 3-week-old hom-cre animals (n=6) injected with 50 mg/kg bodyweight of tamoxifen showed slower deterioration while still showing bodyweight differences when compared to control (n=14). Significant alterations in Hom-cre (n=12) gait and movement were also observed in gait analysis and open field testing when compared with control (n=12). Importantly, hom-cre mice (n=3) also showed significant differences in cerebellar volume based on MRI when compared with

control(n=3), a hallmark of NEDCAM. Together, we report a conditional mouse model displaying motor dysfunction as well as cerebellar atrophy for the study of NEDCAM. While further testing and characterization is required to maximize utility, the temporospatial control offered by the cre-loxP system allows fora wide range of future studies, including GEMIN5 mutant expression *in utero*. Further, the model will allow for testing of genetic influencers as well as potential treatment options.

Disclosures: **C.H. Nelson:** A. Employment/Salary (full or part-time):; University of Pittsburgh. **S. kour:** A. Employment/Salary (full or part-time):; University of Pittsburgh. **T.R. Fortuna:** A. Employment/Salary (full or part-time):; University of Pittsburgh. **S. Agnihotri:** A. Employment/Salary (full or part-time):; University of Pittsburgh. **U.B. Pandey:** A. Employment/Salary (full or part-time):; University of Pittsburgh.

Poster

PSTR405. Cellular and Molecular Mechanisms of Neuromuscular Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR405.03/V19

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant R21NS123539-01

Title: Lis1 plays a crucial role in projection neurons of the adult mice

Authors: *S. MATOO, A. M. VENTRONE, J. OTTERSON, D. S. SMITH;
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Abstract: Mutations in LIS1 (lissencephaly-1) cause lissencephaly, a severe neurodevelopmental disorder. These genetic mutations disrupt neuronal migration and cortical organization during embryonic development, leading to significant neurological impairments. However, our understanding of LIS1's functions after brain development is limited. LIS1 regulates cytoplasmic dynein, an essential motor protein involved in cellular transport, cell division, and organelle positioning. Current models indicate that LIS1 directly interacts with dynein to enhance motor activity. Our study focused on Lis1's post-developmental role due to its elevated levels in the adult mouse nervous system. Severe phenotypes were documented using a tamoxifen-inducible LIS1 knockout mouse model, where CreER expression was controlled by an actin promoter (Act-LIS1iKO). Homozygous iKO mice displayed early-onset neurological phenotypes and death shortly after tamoxifen administration. These findings strongly support LIS1's role in adult mice, but the underlying cellular defects causing the severe phenotypes remain unknown. During the occurrence of severe phenotypes, the Cre reporter exhibited mosaic recombination, primarily in subcortical and peripheral regions. Furthermore, recombination was observed in various cell types, including glial and neuronal cells. Considering the important role of LIS1 in regulating dynein-dependent axonal transport, we hypothesized that LIS1 depletion in neurons was the primary underlying cause of the observed Act-LIS1iKO phenotypes. We have

now selectively depleted LIS1 exclusively in projection neurons of adult mice using the Thy1 promoter to drive the expression of a $CreER^{T2}$ fusion protein. Projection neurons are distinguished by their long axons, which place significant demands on microtubule-based intracellular transport. The Thy1 promoter also drives YFP expression in these mice, enabling visualization of the specific cells expressing the recombinase. Adult Thy1-LIS1iKO mice received a tamoxifen treatment regimen of 0.05 mg/g body weight per day for five consecutive days. Homozygous iKO mice exhibited neurological phenotypes, including severe shivering and leg clasping behaviors, followed by seizures that ultimately resulted in their death within 10 days after the initial injection. A reduced tamoxifen dosage led to a moderate phenotype suggesting that the lower dose targets fewer neurons. Control mice had no observable phenotypes. Lis1 expression notably decreased in YFP-positive cells, highlighting its crucial role in adult projection neurons. Future studies will investigate the mechanisms behind these phenotypes.

Disclosures: S. Matoo: None. **A.M. Ventrone:** None. **J. Otterson:** None. **D.S. Smith:** None.

Poster

PSTR405. Cellular and Molecular Mechanisms of Neuromuscular Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR405.04/V20

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: R21NS119671 (R.M., X.Z.)
R21NS124936 (R.M.)
ABCD Charitable Trust (R.M.)
Karen Toffler Charitable Trust (X.Z.)

Title: Behavioral and electrophysiological deficits in corticostriatal pathways of mice lacking junctophilin-3, the gene mutated in Huntington’s Disease-like 2

Authors: *H. JAARO-PELED[1], X. ZHAN[2,1], R. L. MARGOLIS[1];
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Abstract: Huntington’s Disease like 2 (HDL2), a neurodegenerative disorder clinically and pathologically nearly indistinguishable from Huntington’s disease, is caused by expansion of a CTG/CAG repeat on chromosome 16q24. In the sense strand, the repeat falls in a variably spliced exon of *junctophilin3 (JPH3)*. JPH3 contributes to the formation of the Cav1-RyR2-KCa3 complex between the plasma membrane and the endoplasmic reticulum in neurons, critical for slow afterhyperpolarization and hence neuronal excitability. JPH3 is lower in HDL2 postmortem brains, and mice lacking JPH3 develop motor abnormalities, suggesting that JPH3 loss-of-function contributes to HDL2 pathogenesis. Here we aimed to examine the role of JPH3 in corticostriatal pathways by testing the effect of loss of *JPH3* expression on relevant mouse behaviors and on electrophysiological properties of striatal medium spiny neurons (MSNs) in

brain slices.
The development and initial motor analysis of *JPH3* KO mice (on C57BL/6J background) have been previously described (Nishi et al, 2002; Seixas et al, 2012). We tested young adult *JPH3* KO mice (wildtype, heterozygous and homozygous littermates, N>=9) of both sexes in 3 behavioral paradigms representing 3 different aspects of corticostriatal circuitry: novel object recognition - recognition memory; prepulse inhibition (PPI) - sensorimotor gating; sucrose preference - responsiveness to a natural reward. *JPH3* KO mice were not impaired in novel object recognition or sucrose preference, but did show a lower startle response and a lower PPI. Slices were prepared from adult KO mice and controls using standard procedures. MSNs were identified, and examined using current or voltage clamps. In homozygous or heterozygous *JPH3* KO mice, rheobase currents were smaller than in wildtypes, whereas there was no significant difference in depolarization-induced spiking rates. Ca-dependent afterhyperpolarization currents were significantly reduced in *JPH3* KO mice.
Taken together, the attenuated startle response and PPI in *JPH*3 KO mice provide evidence that JPH3 leads to abnormal sensorimotor gaiting in addition to previously detected motor deficits, consistent with the sensorimotor deficits detected in many neuropsychiatric disorders, including HD. Even heterozygous loss of *JPH3* expression appears to have prominent effects on afterhyperpolarization in MSNs, suggesting a potential contributing factor to deficits in corticostriatal circuitry. Our data support the hypothesis that loss of *JPH3* expression leads to abnormal MSN function and contributes to HDL2 pathogenesis via disruptions of corticostriatal circuity.

Disclosures: H. Jaaro-Peled: None. **X. Zhan:** None. **R.L. Margolis:** None.

Poster

PSTR405. Cellular and Molecular Mechanisms of Neuromuscular Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR405.05/V21

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH NS100783
ABCD Charitable Trust

Title: Significantly less somatic expansion in HDL2 than in HD human blood and brain revealed by DNA fragment analysis and Nanopore sequencing

Authors: *Z. ZHA[1], H. FENG[1], S. ABDOLLAHI[1], V. C. WHEELER[2], C. ROSS[1], P. LI[1], S. DOLL[1], D. MOHR[1], A. SCOTT[1], H. JAARO-PELED[1], R. L. MARGOLIS[1];
[1]Johns Hopkins Univ., Baltimore, MD; [2]Harvard Med. Sch., Boston, MA

Abstract: Huntington’s Disease Like-2 (HDL2) is an autosomal dominant, late-onset neurodegenerative disease that is clinically and pathologically nearly indistinguishable from Huntington’s Disease (HD). HDL2 is caused by an expanded CTG/CAG repeat (> 40 triplets) on

chr:16q24; the repeat falls in exon 2A of the variably-spliced junctophilin-3 (JPH3) gene on the sense strand, and in a cryptic transcript on the antisense strand. Given the accumulated evidence that somatic expansion (i.e., the progressive increase in triplet count with age in somatic tissue) is a key feature of HD pathogenesis, we explored whether somatic expansion is similarly present in HDL2.
We extracted genomic DNA from HD and HDL2 blood and brain samples from age and repeat length-matched patients. DNA was amplified using FAM-labelled HD and HDL2 primers. After fragment measurement and visualization by GeneMapper, the expansion index (EI; Lee JM et al, 2010) was calculated to quantify the degree of expansion, normalizing by the initial repeat length and controlling for background signal. In HD (N = 5), EI = ~1.0 in cerebellum and = ~ 3 in frontal cortex, consistent with previous findings. In HDL2 (N =4), cerebellum EI = ~1.0. However, EI in frontal cortex was < 1.0. Replication, starting with new DNA extraction from the same brains and followed by amplification using two HDL2 PCR primer pairs, yielded the same finding.
In parallel, DNA was amplified using nested PCR and sequenced (Nanopore). We developed an "Island-Building" algorithm to analyze error/mutation-adjusted repeat length counts from each tissue sample, and again found lower EI in HDL2 than in HD in most brain regions. EI ranged from ~3.0 to 6.0 in amygdala, caudate, superior frontal gyrus, and thalamus from HD brains, but was ~< 2.0 in these regions from HDL2 brains (N = 2-3). Additionally, analysis of DNA-derived from blood (N: HD = 19, HDL2 = 21) using the same approach yielded a strong correlation between EI and initial repeat length in both HD and HDL2 (R_0=0.80, 0.56), but a 3.5x steeper slope in HD, indicating substantially faster growth of EI per increment in baseline repeat length in HD than in HDL2.
In summary, using both DNA fragment analysis and Nanopore sequencing, we found that somatic expansion is markedly less prominent in HDL2 than in HD, despite the remarkably similar phenotypes of the two disorders, including age of onset for a given repeat length. This suggests that somatic expansion may not be strictly necessary for the pathogenesis of CAG/CTG repeat expansion disorders. Determining the difference in DNA-repair pathway response to the HD and the HDL2 repeat expansion may shed light on the mechanism of somatic expansion of both disorders.

Disclosures: Z. Zha: None. **H. Feng:** None. **S. Abdollahi:** None. **V.C. Wheeler:** None. **C. Ross:** None. **P. Li:** None. **S. Doll:** None. **D. Mohr:** None. **A. Scott:** None. **H. Jaaro-Peled:** None. **R.L. Margolis:** None.

Poster

PSTR405. Cellular and Molecular Mechanisms of Neuromuscular Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR405.06/V22

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Transgenic mouse model of Parkinsonism Dystonia type2 and Structural 3D modeling of VMAT2 mutations

Authors: *R. A. ALKHATER[1], K. CARDONA-LONDOÑO[2], H. FIUMELLI[3], S. AROLD[5], P. J. MAGISTRETTI[4];
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Abstract: Transgenic mouse model of Parkinsonism Dystonia type2 and Structural 3D modeling of VMAT2 mutations
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Genomic medicine provides potential for novel diagnostic targets and therapeutic solutions. As mutations in human genes are identified and associated with Neurodevelopmental disease, transgenic animal models offer an opportunity to invasively study the mutant gene and its role in the pathophysiology of the disease. We have previously reported the first mutation in type 2 vesicular monoamine transporter (VMAT2), causing an infantile Parkinsonism phenotype Parkinsonism-dystonia-2 (PKDYS2) (OMIM 618049)[1]. Over the past decade, several additional mutations have been identified in VMAT2, giving rise to a broad phenotype spectrum and variable responses to treatment. Here, we characterize the disease's first viable transgenic mouse model and also analyze the molecular impact of the VMAT2 mutations in silico to understand the pathophysiology better and potentially improve treatment approaches. We based our analysis on the high-confidence three-dimensional structure of the VMAT2 protein produced by the AI-based Alphafold2 algorithm. The model was manually inspected, and mutations were evaluated using the Pymol program.Additionally, residue conservation was assessed with Consurf, and pathogenicity was predicted with Polyphen-2, Mutpred2, and Snpeff. We correlated the VMAT2 structural changes due to mutations with phenotypes. We found that there is a clear genotype-phenotype correlation, and this aids in guiding clinical decisions, enhancing patient outcomes, gaining an understanding of pathophysiology, and developing personalized treatment protocols. This work that includes animal model generation, behavioral characterization, and protein structure-phenotype analysis, advances our understanding of the disease and provides insights for creating personalized management protocols for this, and possibly other, neurodevelopmental disorders affecting neurotransmission.
References:
Rilstone JJ, Alkhater, R. A., & Minassian, B. A. Brain dopamine-serotonin vesicular transportdisease and its treatment. The New England journal of medicine. 2013;368(6):543-550.

Disclosures: R.A. Alkhater: None. **K. Cardona-Londoño:** None. **H. Fiumelli:** None. **S. Arold:** None. **P.J. Magistretti:** None.

Poster

PSTR405. Cellular and Molecular Mechanisms of Neuromuscular Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR405.07/V23

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: U54NS115052

Title: Self-limiting gene addition in a mouse model of hereditary spastic paraplegia due to a defect in the small subunit of serine palmitoyl transferase complex

Authors: *Y. GONG[1], A. QIAN[1], G. KENNETH[2], M. SELIG[1], C. MAGUIRE[1], T. DUNN[2], F. EICHLER[1];
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Abstract: Sphingolipids (SLs) are lipid molecules that play essential structural and signaling functions in eukaryotes and have been linked to many neurological diseases. Serine palmitoyl transferase complex (SPT) mediates the first and rate-limiting step in the de novo sphingolipid biosynthetic pathway. The larger subunits SPTLC1 and SPTLC2/SPTLC3 together form the catalytic core with a smaller third subunit either SSSPTA or SSSPTB propelling the catalytic efficiency and providing substrate specificity for the fatty acyl-CoA substrates. Recently, the SPTSSA^{T51I} mutation was discovered in two girls with stagnant neurodevelopment, and both showed substantially elevated levels of several SLs. To further investigate the pathophysiology of this disease-causing mutation and explore therapeutic approaches, we developed a mouse model T51I knock-in mouse using targeted introduction of the T51I allele into exon 2 of the *SPTSSA* gene by CRISPR technology in fertilized eggs and further assessed the sphingolipid biochemistry and behavior in these mice. Surprisingly, supplementation with 10% L-serine for 2-3 months increased SL levels, caused significant myelin deficits and worsened the hind limb clasping behavior. This suggests a critical threshold in lipid chemistry impacting myelin organization and functionality and thereby affecting motor behavior. *In vitro* data in patient fibroblasts demonstrated successful transduction with adeno-associated virus (AAV) vector encoding wildtype SPTSSA (AAV-SPTSSA) and improvement in lipid chemistry. This has provided the justification for ongoing *in vivo* studies of AAV-SPTSSA gene therapy in Sptssa^{T51I} heterozygous mice. In conclusion, our preliminary studies in mouse suggest the functional importance of the small subunit variant in Sptssa^{T51I} heterozygous mice by demonstrating the association of hind limb clasping with SL elevations. Our studies demonstrate the feasibility of adding a single unit of a multisubunit enzyme complex to confer functional benefit. AAV-mediated delivery in human fibroblasts suggests that addition of SPTSSA can combine with other native subunits and impart self-limiting specific catalytic efficiency and restore the normal homeostasis of SPT.

Disclosures: Y. Gong: None. **A. Qian:** None. **G. Kenneth:** None. **M. Selig:** None. **C. Maguire:** None. **T. Dunn:** None. **F. Eichler:** None.

Poster

PSTR405. Cellular and Molecular Mechanisms of Neuromuscular Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR405.08/V24

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH/NINDS R01 NS108114

Title: Mechanisms underlying the role of the deubiquitinase USP7 in the CAG triplet repeat disorder spinal and bulbar muscular atrophy

Authors: *M. SENGUPTA[1], E. H. NOLEN[1], A. DIBSIE[1], Y. LIU[1], S. M. SHEKARABI[1], A. PLUCIENNIK[1], S. V. TODI[2,3], D. E. MERRY[1];
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Abstract: Spinal and bulbar muscular atrophy (SBMA) is a progressive neurodegenerative disease affecting males with ≥38 glutamine-encoding CAG repeats in androgen receptor (AR) gene. Androgen-bound polyglutamine-expanded AR (**AR^{exp}**) misfolds, aggregates, causes lower motor neuron (MN) and muscle dysfunction, and, ultimately, cell death. Patients experience MN loss from brainstem and spinal cord, muscle weakness and atrophy, resulting in impaired mobility, dysphagia, and dysarthria. There is no effective treatment. E3 ubiquitin ligases add ubiquitin to proteins, tagging them for degradation, while deubiquitinases like **USP7** remove ubiquitin, altering the proteins' cellular fates. We previously showed that USP7 plays a role in SBMA as its knockdown reduced AR^{exp} aggregation and toxicity in PC12 cell, MN, *Drosophila*, and mouse models. USP7 deubiquitinates AR^{exp} at 8 lysine (8K) residues, and a lysine-to-arginine (KR) mutation blocking ubiquitination at one lysine site (K17) increased AR^{exp} aggregation. This suggests that USP7-mediated deubiquitination at K17 may prevent AR^{exp} degradation and contribute to its aggregation and associated cell death. This raises two research questions:**(1) Does USP7 contribute to AR aggregation and cell death by deubiquitinating AR^{exp} at K17 alone, or at 8K, or by acting on (an)other substrate?** We created PC12 AR^{exp} cell lines with ubiquitination blocked at either K17 (AR^{exp} K17R) with or without USP7 knockdown, or at 8K (AR^{exp} 8KR), and *Drosophila* AR^{exp} K17R or 8KR strains. Preliminary experiments indicate that USP7 knockdown in PC12 AR^{exp} K17R cells does not reduce AR^{exp} aggregation, suggesting that USP7-mediated deubiquitination at K17 is sufficient to promote AR^{exp} aggregation. We will quantify AR^{exp} ubiquitination, turnover, aggregation, and cell death with and without USP7 knockdown or inhibition in these SBMA mutant lines.
(2) Which E3 ligase ubiquitinates AR^{exp} at USP7-regulated sites? A differential ubiquitinome study found that 4 of the 8K (including K17) are less ubiquitinated in PC12 AR^{exp} S16A (phosphorylation blocked at S16) cells, suggesting that S16 phosphorylation may regulate ubiquitination at these sites. A peptide-pulldown assay identified several E3 ligases differentially interacting with pS16 over S16A. This list was further narrowed down based on USP7 interaction. We will knock down each E3 ligase in PC12 cells to understand its role in AR^{exp} ubiquitination, aggregation, and turnover. In summary, the insights revealed by our studies will help us understand the role of USP7 in SBMA.

Disclosures: **M. Sengupta:** None. **E.H. Nolen:** None. **A. Dibsie:** None. **Y. Liu:** None. **S.M. Shekarabi:** None. **A. Pluciennik:** None. **S.V. Todi:** None. **D.E. Merry:** None.

Poster

PSTR405. Cellular and Molecular Mechanisms of Neuromuscular Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR405.09/V25

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH BRAIN Initiative Grant 5UH3NS095553

Title: Chronic at-home thalamocortical recordings during sleep in patients with Essential Tremor

Authors: *N. GEIGEL[1], J. WONG[2], K. D. FOOTE[3], J. HILLIARD[3], A. GUNDUZ[4]; [2]Neurol., [3]Neurosurg., [4]J. Crayton Pruitt Family Dept. of Biomed. Engin., [1]Univ. of Florida, Gainesville, FL

Abstract: Essential Tremor (ET) is one of the most common movement disorders in the world. It afflicts over 7 million people in the US (2.2% of the population). It is a progressive neurodegenerative disease characterized by a 4-12Hz shaking of the upper extremities. Deep brain stimulation (DBS) has emerged as an effective therapy for people suffering from medication refractory ET. Significant tremor suppression for patients can be achieved by targeting and stimulating the thalamic ventral intermediate nucleus (VIM). In clinical practice, DBS is delivered continuously, resulting in stimulation when not needed, particularly during sleep when tremor disappears. Although the feasibility of intention-based closed-loop algorithms for ET has been demonstrated, there has been a lack of research on their behavior during sleep. To this end, we are investigating the modulations in the thalamocortical network during sleep. We captured at home-recordings from depth electrodes in the VIM and from a cortical strip over the primary motor cortex. The subjects were instructed to record 30 minutes of awake data, keep recording through the night, and indicate when they woke up. We recorded local field potentials overnight with interchanging stimulation at 0mA and at the clinical amplitude. Multi-taper spectral analyses are used to extract spectral information across sleep and awake states. We aim to uncover how intention-based closed-loop algorithms respond to changes from wakefulness to sleep. Furthermore, we aim to develop closed-loop algorithms that terminate stimulation during sleep.

Disclosures: **N. Geigel:** None. **J. Wong:** None. **K.D. Foote:** None. **J. Hilliard:** None. **A. Gunduz:** None.

Poster

PSTR405. Cellular and Molecular Mechanisms of Neuromuscular Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR405.10/W1

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Coupling between thalamus and cortex is spectrally and spatially organized in patients with essential tremor

Authors: *A. STEINA, S. SURE, M. BUTZ, J. VESPER, A. SCHNITZLER, J. HIRSCHMANN;
Heinrich-Heine-Universität Düsseldorf, Duesseldorf, Germany

Abstract: Essential tremor (ET) is a common movement disorder that is characterized by postural and intention tremor in the absence of other neurological signs. In severe ET the ventral intermediate nucleus of the thalamus (VIM) is an effective target for deep brain stimulation. Although the VIM is of therapeutic importance, its functional connectivity has rarely been investigated in humans. In this study, our goal was to identify the cortical areas coupled to the VIM in patients with ET, while the patients were at rest. We combined magnetoencephalography with local field potential recordings from the VIM of 19 ET patients and from the subthalamic nucleus (STN) of 19 patients with Parkinson's disease. Whole-brain maps of VIM-cortex and STN-cortex coherence were constructed in several frequency bands (alpha, low-beta, high-beta) using a beamformer and compared using a cluster-based permutation test. In order to estimate the directionality of coupling between cortical areas and subcortical nuclei spectral Granger causality was calculated. The topographies of VIM-cortex and STN-cortex connectivity resembled each other overall, but differed in coupling strength. Both nuclei were coupled to temporal cortex, brainstem and cerebellum in the alpha band, to sensorimotor cortex, brainstem and cerebellum in the low-beta band, and to ipsilateral sensorimotor cortex in the high-beta band. Coherence to deeper areas comprising brainstem and cerebellum was stronger for the VIM in the low-beta band ($p = 0.017$, cluster-based permutation test), whereas high-beta coherence to sensorimotor cortex was stronger for the STN ($p = 0.014$, cluster-based permutation test). The VIM led the sensorimotor cortex in the alpha band, while the STN was driven by cortical activity in the high-beta band. Our findings provide evidence for a spectral and spatial organization of thalamo-cortical coupling. The overall similar topographies of VIM-cortex and STN-cortex connectivity suggest that functional connections are not necessarily unique to one subcortical nucleus but might reflect larger frequency-specific networks. The directionality of VIM-sensorimotor cortex coupling supports the idea of a thalamic pacemaker for alpha oscillations. Elevated high-beta connectivity might be an electrophysiological marker of the hyperdirect pathway.

Disclosures: A. Steina: None. **S. Sure:** None. **M. Butz:** None. **J. Vesper:** None. **A. Schnitzler:** None. **J. Hirschmann:** None.

Poster

PSTR405. Cellular and Molecular Mechanisms of Neuromuscular Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR405.11/W2

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: BPAN Foundation
Families of SCN2A

Title: In vitro model system development and therapeutic testing for Beta-propeller protein-associated neurodegeneration (BPAN)

Authors: *A. KALEEM[1], S. SINHA RAY[2], F. ROUSSEL[1], M. PARVATE[3], R. TALEKAR[3], X. ZHANG[3], A. SIERRA DELGADO[3], S. B. LIKHITE[4], K. C. MEYER[5];
[1]Ctr. for Gene Therapy, Abigail Wexner Res. Inst. at Nationwide Children's Hosp., Columbus, OH; [2]Ctr. for Gene Therapy, Res. Inst. At Nationwide Children's Hospit, Hilliard, OH; [3]Ctr. for Gene Therapy, [4]Ctr. for gene therapy, Nationwide Children's Hosp., Columbus, OH; [5]Ctr. for Gene Therapy, Res. Inst. Nationwide Childrens Hosp., Columbus, OH

Abstract: Beta-propeller protein-associated neurodegeneration (BPAN) is the most common Neurodegeneration with Brain Iron Accumulation (NBIA) disorders comprising 35 to 45% of NBIA population. BPAN is an X-linked dominant disorder caused by de novo mutations in WDR45 gene, affecting approx. 1 in 500,000 individuals at the rate of 20 females for 3 males. With a childhood onset, BPAN is characterized by intellectual disability, developmental delay, and speech delay. Other common symptoms include sleep disorders, seizures or behaviors on the autistic spectrum disorder. While neurons are heavily implicated in BPAN disease pathogenesis, WDR45 is expressed in both neurons & astrocytes. To date, very little is known about the disease modifying impact of astrocytes towards the disorder. Astrocytes regulate extracellular environment of neurons promoting survival and regulating synaptic transmission and plasticity. Thus, identifying the role of astrocytes in disease pathology is critical as enhanced understanding of contribution of non-neuronal cell types may offer invaluable insights of underlying pathophysiology of BPAN and provide novel avenues for therapeutic development. To achieve this, we developed a novel in-vitro disease model system by reprogramming BPAN patient skin fibroblasts, carrying different mutations, using direct conversion method to induced Neuronal Progenitor Cells (iNPCs) which were further differentiated into induced astrocytes (iAs). We also generated induced neurons (iNs) directly from BPAN fibroblasts. These cells were further subjected to a battery of physiological, molecular and cell biology assays to evaluate their contributions to BPAN pathology. Our studies demonstrate that both BPAN iAs and iNs show novel disease phenotypes in vitro. With reduced levels of WDR45 protein expression, BPAN iAs show a highly significant decrease in mitochondrial respiration suggesting aberrant mitochondrial activity while BPAN iNs show reduced conversion rate and morphological defects. Moreover, treatment of BPAN iAs and iNs with various therapeutic molecules including AAV9 gene therapy, demonstrates rescue of disease phenotypes in mutation specific manner. Our work provides a robust model system to study the underlying disease mechanisms of BPAN pathology in mutation specific and cell type specific way. It also provides an excellent platform for high-throughput screening of multiple therapeutics with the potential to stratify patients based on the treatment responsiveness which is extremely crucial for optimal treatment development for this dreadful disease.

Disclosures: **A. Kaleem:** None. **S. Sinha Ray:** None. **F. Roussel:** None. **M. Parvate:** None. **R. Talekar:** None. **X. Zhang:** None. **A. Sierra Delgado:** None. **S.B. Likhite:** None. **K.C. Meyer:** None.

Poster

PSTR405. Cellular and Molecular Mechanisms of Neuromuscular Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR405.12/W3

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Don't Forget Morgan Foundation
NIH Grant: P20GM103620
NSF USD N3: DGE-1633213

Title: Exploring mechanisms of neurodegeneration and neurodevelopmental delay in WDR45 associated neurological disease.

Authors: *B. L. MEYERINK[1,2], K. S. KARIA[1], A. EDWARDS[1], J. HOWARD[1], E. AASENG[1], J. M. WEIMER[1,3], L.-J. PILAZ[1,3];
[1]Sanford Res., Sioux Falls, SD; [2]Dept. of Basic Biomed. Sci., [3]Dept. of Pediatrics, Univ. of South Dakota - Sanford Sch. of Med., Vermillion, SD

Abstract: Beta-propeller protein-associated neurodegeneration (BPAN) is a devastating rare neurodegenerative disease caused by mutations in the gene *WDR45* located on the X chromosome. Currently, there is no cure or disease altering treatment for this disease. While previous work has established WDR45's molecular role in autophagosome formation in vitro, its function in the development and maintenance of the CNS is still unknown. To explore these questions, we used CRISPR based genetic editing to generate a model of BPAN which mimics a patient mutation in *Wdr45* and confirmed that it ablates protein expression. We performed a comprehensive examination of the early pathophysiology of this model and explored the molecular role of WDR45 to understand its contribution to neural development and maintenance of cellular health. In WDR45 mutant animals, we observed neuroinflammation at 3 months of age and motor impairment consistent with disease progression in patients. This model additionally shows altered mitochondria and autophagosome number in the neurites of cultured neurons suggesting degradation and metabolic pathways are impaired in this disease. To build on this, we isolated the synapses from the cortex of WDR45 mutant animals through centrifugal fractionation. We found an accumulation of proteins labeled for autophagy degradation and elevated ROS response proteins. These data are supported by experiments using BioID biotin ligation to label proximal and interacting proteins with WDR45 in neuroblastoma cells. This analysis suggests novel interactions between WDR45 and proteins associated with mitochondrial metabolism and response to reactive oxygen species. Further, we performed RNAseq in cortical tissue of these animals revealing perturbed expression of genes previously linked to symptoms of BPAN and neurological disorders more broadly. This study provides key insights into the

pathological progression of BPAN and sheds new light on the potential role for this protein in establishing and maintaining proper function in the brain.

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Poster

PSTR405. Cellular and Molecular Mechanisms of Neuromuscular Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR405.13/W4

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant NS102417

Title: Autoreactive antibodies from pediatric transverse myelitis patients induce stress in astrocytes

Authors: *C. SMITH, K. TSE, K. TELESFORD, W. ZHANG, B. GREENBERG, N. L. MONSON;
Neurol., UT Southwestern Med. Ctr., Dallas, TX

Abstract: B cells play a critical role in the progression of central nervous system autoimmune diseases such as Multiple Sclerosis (MS). Adult patients who present with transverse myelitis (TM) symptoms and later evolve to MS diagnosis, display an expansion of neuron-targeting plasmablasts (PBs), a subpopulation of antibody-secreting B cells. In this study, we examined the cellular and genetic immune profile of PBs in pediatric TM patients and found a decreased frequency of peripheral PBs compared to healthy controls. Also in contrast to adult TM patients, pediatric TM patients did not display an overuse of VH4 heavy chain genes in PBs. To examine PB autoreactivity, we cloned recombinant human antibodies (rhAbs) and performed immunostaining in human neuroblastoma and human astrocytic cultures, as well as spinal cord and brain from mice who were either healthy or displayed MS-like symptoms due to disease induction. We found that rhAbs derived from pediatric TM patients preferentially targeted astrocytes in mouse tissue and human cell culture, with increased reactivity in antibodies that had accumulated somatic hypermutation. These antibodies induced stress granules in human astrocytes compared to controls, demonstrating functional significance of autoreactivity in live human astrocyte cell cultures. These studies indicate that pediatric TM patients have a distinct autoimmune profile that separates them from adult TM patients and highlights the need for thorough investigation of inflammatory mechanisms that may lead to adult MS.

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Poster

PSTR405. Cellular and Molecular Mechanisms of Neuromuscular Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR405.14/W5

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: 1RF1AG069483-01A1

Title: Novel splicing of UBQLN2 and its potential pathological implication in amyotrophic lateral sclerosis and frontotemporal dementia

Authors: *A. S. H. KOO, R. S. TIBBETTS;
Human Oncology, Univ. of Wisconsin Madison, Madison, WI

Abstract: *Ubiquilin 2* (*UBQLN2*) is a mono-exonic gene located on chromosome X (coordinates: 56,563,627:56,567,868) that encodes a 624 amino acids protein belonging to the eukaryotic ubiquilin family of ubiquitin chaperones. All ubiquilins harbour N-terminal ubiquitin-like (UBL) and C-terminal ubiquitin-associated (UBA) domains that mediate the shuttling of ubiquitylated substrates to the proteasome for degradation. Mutations in the proline-rich-repeat unique to UBQLN2 cause X-linked dominant inheritance of amyotrophic lateral sclerosis and frontotemporal dementia (ALS/FTD) (Deng *et al.*, 2011). These mutations are associated with cytosolic aggregation of UBQLN2; however, how these mutations cause ALS/FTD is unknown. Our analysis of long-read sequencing data from Pacific Biosciences Iso-seq public datasets revealed a cryptic intron with two alternative 3' splice sites at the 5' end of the UBQLN2 coding sequence (CDS), which are not currently annotated in NCBI and Ensembl. The alternatively spliced UBQLN2 transcripts, dubbed UBQLN2$^{\Delta 193}$ and UBQLN2$^{\Delta 210}$, are predicted to encode truncated UBQLN2 proteins lacking the first 193 or 210 amino acids of the UBQLN2 CDS. Both truncated versions lack the UBL domain, which mediates proteasome targeting, and the first STI1-like domain that mediates chaperone binding. We verified the presence of UBQLN2$^{\Delta 210}$ transcript in human cell lines (HeLa and U-2 OS) and a commercial human brain RNA sample (Invitrogen AM7962) via Sanger sequencing and RT-qPCR. An initial screen of short-read RNA-sequencing data from TargetALS, which were done in Illumina sequencing platforms, detected the splice junctions of both UBQLN2$^{\Delta 193}$ and UBQLN2$^{\Delta 210}$ transcripts. However, these novel transcripts were not detected in C57BL/6J mouse brain tissues (cortex, cerebellum, and hippocampus) by PCR and RT-qPCR. Experiments to verify the expression and localisation of truncated UBQLN2 isoforms in human cell lines are ongoing. We postulate that these human-specific truncated UBQLN2 species possess altered biochemical and functional properties that may impact cellular proteostasis as well as pathologic protein aggregation and neurotoxicity in UBQLN2-associated ALS/FTD. Future studies on the function and splicing regulation of UBQLN2 isoforms will inform better diagnostic and therapeutic options for ALS/FTD patients.

Disclosures: A.S.H. Koo: None. **R.S. Tibbetts:** None.

Poster

PSTR405. Cellular and Molecular Mechanisms of Neuromuscular Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR405.15/W6

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Characterizing the Multifunctional Roles of Neurodegeneration-associated TDP43 in Regulating Neuronal DNA Mismatch Repair

Authors: *V. E. PROVASEK;
HMRI, Houston, TX

Abstract: The DNA mismatch repair (MMR) pathway is widely recognized for its role in preventing replication-associated mutations. MMR proteins are also expressed in nondividing neurons, yet their function remains unknown. TDP43 is an essential RNA/DNA binding protein whose nucleocytosolic mislocalization has been linked to neurodegeneration in frontotemporal dementia (FTD) and Amyotrophic Lateral Sclerosis (ALS). Most FTD/ALS cases develop sporadically, and ~95% of these show TDP43 pathology. Over the disease course, neurons accumulate DNA damage and exhibit diminished repair capacity. These observations suggest multiple DNA repair pathways (DRPs) are defective. To this end, we questioned which neuronal DRPs are affected by TDP43. Here, we report for the first time that TDP43 exerts a multifactorial role in the regulation of MMR in neurons. Initial RNA microarray analysis of cells treated with TDP43 siRNA showed dysregulation of several DRPs, including MLH1, MSH2, MSH3, MSH6. Subsequent immunoblot and qRT-PCR of differentiated SH-SY5Y cells confirmed these results. We also discovered that TDP43 modulates splicing of multiple MMR transcripts. Endpoint PCR analysis using primers covering intron-exon boundaries of these transcripts revealed specific accumulation of MLH1 and MSH6 pre-mRNA. Furthermore, we show that loss of TDP43 results in accelerated degradation of MSH3 and MSH6 transcripts. Importantly, we confirmed altered expression and splicing of MMR transcripts in a subset of ALS patient CNS tissues. Finally, we show that TDP43 may also affect MMR at the protein level, as proximity ligation and co-immunoprecipitation assays show TDP43 interaction with MSH3 increases following DNA alkylation treatment of neuronal cells. Taken together, these data reveal multiple novel means by which TDP43 pathology may contribute to neurodegeneration associated genomic instability.

Disclosures: V.E. Provasek: None.

Poster

PSTR405. Cellular and Molecular Mechanisms of Neuromuscular Disease

Location: WCC Halls A-C

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Program #/Poster #: PSTR405.16/W7

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01NS094564
NIH R21NS106307
The Hartwell Foundation
Cure SMA
Agape Foundation

Title: Mitigating aberrant Cdk5 activation alleviates mitochondrial defects and motor neuron disease symptoms in spinal muscular atrophy

Authors: N. MILLER[1], A. JI[1], Z. XU[2], ***Y. MA**[2];
[2]Lurie Children's Hosp. of Chicago, [1]Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: Spinal muscular atrophy (SMA), the top genetic cause of infant mortality, is characterized by motor neuron degeneration. Mechanisms underlying SMA pathogenesis remain largely unknown. Here we report that the activity of cyclin-dependent kinase 5 (Cdk5) and the conversion of its activating subunit p35 to the more potent activator p25 are significantly upregulated in mouse models and human induced pluripotent stem cell (iPSC) models of SMA. The increase of Cdk5 activity occurs before the onset of SMA phenotypes, suggesting it may be an initiator of the disease. Importantly, aberrant Cdk5 activation causes mitochondrial defects and motor neuron degeneration, as the genetic knockout of *p35* in a SMA mouse model rescues mitochondrial transport and fragmentation defects, and alleviates SMA phenotypes including motor neuron hyperexcitability, loss of excitatory synapses, neuromuscular junction (NMJ) denervation, and motor neuron degeneration. Inhibition of the Cdk5 signaling pathway reduces the degeneration of motor neurons derived from SMA mice and human SMA iPSCs, suggesting a potential therapeutic strategy. Our findings highlight a positive feedback loop in SMA formed by motor neuron hyperexcitability-induced high bioenergetic demand and increased intracellular Ca^{2+}, which leads to Ca^{2+}/calpain-dependent conversion of p35 to p25 and aberrant Cdk5 activation, and subsequent Cdk5 hyperphosphorylation-mediated mitochondrial defects in energy production and Ca^{2+} buffering. Activation of this loop in SMA exacerbates energy shortage and Ca^{2+} homeostasis disruption, turning motor neuron hyperexcitability into excitotoxicity and degeneration. Altogether, our studies reveal a critical role for the aberrant activation of Cdk5 in SMA pathogenesis and suggest a potential avenue for therapeutic intervention.

Disclosures: **N. Miller:** None. **A. Ji:** None. **Z. Xu:** None. **Y. Ma:** None.

Poster

PSTR405. Cellular and Molecular Mechanisms of Neuromuscular Disease

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Program #/Poster #: PSTR405.17/W8

Topic:

Support: Philanthropy
Farber Family

Title: Cystamine as a potential therapeutic agent to restore altered heat shock response in the fibroblasts from a neuropathy patient with DNAJB2 c.823+6C>T mutation

Authors: *R. PRADHAN, H. S. ILIEVA;
Farber Inst. of Neurosci. Thomas Jefferson Univ., Philadelphia, PA

Abstract: Neuropathy is a debilitating disorder characterized by peripheral nerve dysfunction and damage. Homozygous mutations in DNAJB2 have previously been shown to cause early neuropathy. A heterozygous DNAJB2 c.823+6C>T mutation was found in a patient with adult-onset severe, sensory-motor polyneuropathy and is predicted to affect both isoforms of the protein. DNAJB2 (HSP40) belongs to a class of Heat Shock Proteins (HSPs) that play a crucial role in cellular protection and stress, including heat stress. HSP40 traffics unfolded proteins to another heat shock protein, HSP70, and activates its ATPase activity to result in a correctly folded protein. In our study, we investigated the effect of heat stress on the heat shock response of fibroblasts obtained from a neuropathy patient with a heterozygous DNAJB2 mutation. In order to elicit a stress response, these cells were subjected to one hour of heat shock, and the expression levels of HSP40 and HSP70 were analyzed after 0, 3, 8, and 24 hours by Western Blot and Immunocytochemistry. Additionally, we evaluated the therapeutic efficacy of Cystamine, a transglutaminase inhibitor, which has been shown to modulate DNAJB2 levels in cellular and animal model of Huntington's disease.While the baseline levels were similar to controls, we found a decreased response to heat shock in the fibroblasts with the DNAJB2 mutation compared to healthy controls, as evidenced by reduced HSP40 and HSP70 levels. Experiments are ongoing to assess if Cystamine can improve/correct expression compared to untreated cells.
In summary, this study demonstrates a diminished heat shock response in the fibroblasts carrying the c.823+6C> DNAJB2 mutation, indicating a loss-of-function pathogenic mechanism. Moreover, Cystamine treatment is evaluated as a potential therapeutic agent to correct DNAJB2 levels. Additional experiments to elucidate Cystamine mechanism of action are planned.

Disclosures: **R. Pradhan:** A. Employment/Salary (full or part-time):; Thomas Jefferson University. **H.S. Ilieva:** A. Employment/Salary (full or part-time):; Thomas Jefferson University.

Poster

PSTR406. Stroke Recovery: Pharmacological Approaches to Therapy

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR406.01/W9

Topic: C.09.Stroke

Support: by Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

Title: Targeting ferroptosis by clinically approved and brain penetrant HIF Prolyl hydroxylase inhibitors improves recovery in diverse neurological disease models in mice

Authors: *S. KARUPPAGOUNDER, C. CORONA, I. G. CHAMBERS, Y. CHEN, R. R. RATAN;
Burke Neurolog. Inst., White Plains, NY

Abstract: Ferroptosis is a non-apoptotic form of cell death which is dependent on iron. The prevailing view is that iron's role in ferroptosis emerges from its direct ability to drive lipid peroxidation. Here we provide evidence that target-selective iron chelation can also block ferroptosis. Specifically, we show that roxadustat, a clinically approved inhibitor of the iron-dependent, HIF prolyl hydroxylases (HIF PHDs), and adaptaquin, a brain-penetrant HIF PHD inhibitor, abrogated erastin and RSL3-induced ferroptotic death in mouse primary cortical neuronal cultures and a HT1080 human fibrosarcoma cell line. Molecular deletion of all three HIF PHD isoforms in each cell type also reduced ferroptosis. The protective effects of HIF PHD inhibition were correlated with suppression of ferroptosis-induced ATF4 activation but not a reduction in non-chelatable iron levels or inhibition of lipid peroxidation. A dose of adaptaquin that inhibited the HIF PHDs in the brain and eye vivo were validated using HIF-reporter mice and in vivo bioluminescence imaging. At this concentration (30 mg/kg), we found that adaptaquin could reduce cell death and improve functional recovery after ischemic stroke, traumatic brain injury and in a genetic model of retinal degeneration. Together, these findings demonstrate that iron chelators can participate in ferroptosis via target-selective inhibition of the iron-dependent HIF prolyl hydroxylases. Target selective iron chelation offers the opportunity to interdict ferroptosis and neuropathological events in vivo without negatively affecting the physiology of iron in the nervous system.

Disclosures: S. Karuppagounder: None. **C. Corona:** None. **I.G. Chambers:** None. **Y. Chen:** None. **R.R. Ratan:** None.

Poster

PSTR406. Stroke Recovery: Pharmacological Approaches to Therapy

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR406.02/W10

Topic: C.09.Stroke

Support: NIH Bench-to-Bedside Research Grant
NIDA Intramural Research Training Award

Title: Measuring serum proteins for potential biomarkers of endoplasmic reticulum stress following acute ischemic stroke in mice

Authors: *L. K. GRASSO[1], J. J. HINKLE[1], K. A. TRYCHTA[1], Y. LI[2], M. TANG[2], M. W. HALTERMAN[2], B. K. HARVEY[1];
[1]Natl. Inst. on Drug Abuse, Baltimore, MD; [2]Univ. Med. Center, Stony Brook, Stony Brook, NY

Abstract: Acute ischemic stroke (AIS) has been shown to induce endoplasmic reticulum (ER) stress. Our lab has identified a form of ER stress called exodosis whereby ER resident proteins exit the cell via the secretory pathway. In vitro experiments demonstrate that hypoxia and hypoglycemia can induce exodosis. The current exploratory study examines ER exodosis in vivo using a mouse model of AIS to identify ER-resident proteins that may be affected by stroke. Blood serum was collected from male mice twenty-four or seventy-two hours after middle cerebral artery occlusion (MCAO) or sham surgery. Serum cytokine levels were analyzed via an Ella multiplex immunoassay, which measures multiple chemokines and inflammatory markers within one sample. Serum IL-6 and TNF-a were increased twenty-four hours post-MCAO compared to sham and seventy-two hours. Mesencephalic astrocyte-derived neurotrophic factor (MANF), a marker of ER exodosis, was measured via ELISA to correlate MANF serum levels with the timing and severity of AIS. No significant difference in MANF levels was detected across groups; however, MANF levels trended higher in twenty-four-hour MCAO serum than all other groups. To screen for additional serum markers of ER exodosis in AIS, western blots were exposed to a polyclonal antibody that reacts with proteins containing an endoplasmic reticulum retention sequence (ERS). Increased immunoreactivity with this antibody suggests an increase in exodosis. No significant trend was seen for any of the ERS proteins detected by the antibody. A higher *n* value may reveal more trends in this data set. Additional timepoints, including baseline serum, may aid in characterizing ERS secretion in AIS. Identifying a panel of ERS proteins that react in measurable ways to AIS would allow us to establish a new timeline of post-stroke cellular mechanisms. With this knowledge, we may be able to therapeutically target ER exodosis and attenuate post-stroke symptomology in those who have experienced AIS.

Disclosures: L.K. Grasso: None. **J.J. Hinkle:** None. **K.A. Trychta:** None. **Y. Li:** None. **M. Tang:** None. **M.W. Halterman:** None. **B.K. Harvey:** None.

Poster

PSTR406. Stroke Recovery: Pharmacological Approaches to Therapy

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR406.03/W11

Topic: C.09.Stroke

Support: Canadian Institutes of Health Research grant 451168
Canadian Institutes of Health Research Canada Graduate Scholarship - Doctoral

Title: A Meta-Analysis of Glibenclamide after Experimental Intracerebral Hemorrhage (ICH)

Authors: *T. KUNG, C. M. WILKINSON, L. J. LIDDLE, F. COLBOURNE;
Univ. of Alberta, Edmonton, AB, Canada

Abstract: Edema has occasionally been shown to worsen behavioural outcomes and often contributes to mortality after intracerebral hemorrhage (ICH), a devastating subtype of stroke.

Glibenclamide (GLC) is a sulfonylurea 1- transient receptor potential melastatin 4 (Sur1-Trpm4) channel blocker, and attenuates edema in ischemic stroke models, suggesting possible benefits in ICH. As clinical interest in GLC is high with multiple clinical trials in ischemia currently underway, this meta-analysis was performed to synthesize all animal studies investigating a post-ICH administration of GLC vs. no-treatment controls on behavioural outcomes, edema, hematoma volume, and injury volume after ICH. Six studies were included in our meta-analysis, two null (i.e., GLC did not affect any endpoints) and four positive (i.e., GLC improved one or more endpoints). GLC significantly improved behaviour (standardized mean difference (SMD) = −0.63, [−1.16, −0.09], n = 70-74) and edema (SMD = −0.91, [−1.64, −0.18], n = 70), but did not affect hematoma volume (SMD = 0.0788, [−0.5631, 0.7207], n = 18-20), or injury volume (SMD = 0.2892, [−0.4950, 1.0734], n = 24). Although it appears that significant benefit may be realized with GLC after ICH, limitations suggest these results should be interpreted with caution. Publication bias was significant in edema (p = 0.0001) and indicated missing negative data, and the same trended towards significance in behaviour (p = 0.0766). Experimental quality was generally low, and risk of bias was generally high among studies. Experiments were often low-powered; future researchers should employ sample sizes of 33 to detect our observed effect in edema, and 41 to detect our observed effect in behaviour. Lastly, many important experimentally rigorous recommendations to improve translatability of pre-clinical findings (e.g., use of female, aged, and co-morbid animals) have not yet been completed. Thus, missing negative data, generally low study quality and high risk of bias show that additional high-powered confirmatory animal work is needed prior to future clinical GLC work in ICH.

Disclosures: **T. Kung:** None. **C.M. Wilkinson:** None. **L.J. Liddle:** None. **F. Colbourne:** None.

Poster

PSTR406. Stroke Recovery: Pharmacological Approaches to Therapy

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR406.04/W12

Topic: C.09.Stroke

Support: R01NS094535

Title: The Crucial Function of DNA Damage Response-Induced Senescence-Like Phenotype in Experimental Brain Hemorrhage: A Focus on Hemin Degradation and Cell Survival

Authors: *V. H. MALOJI RAO[1], J. MITRA[1], M. KODAVATI[1], V. VASQUEZ[1], S. MITRA[1], J. M. TOUR[3], G. W. BRITZ[2], T. A. KENT[4], M. L. HEGDE[1];
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Abstract: In brain hemorrhage, toxic hemin, a degradation product of hemoglobin, accumulates, potentially leading to cell death. The cells respond by expressing Heme Oxygenase-1 (HO-1), which breaks down hemin into biliverdin and free iron (Fe2+). Our recent studies highlighted that hemin triggers DNA double-strand breaks (DSBs) and a cellular senescence-like phenotype. The causal relationships between these observations remain unknown. In this study, we illuminate how DNA damage response (DDR) signaling directly mediates the induction of this senescence-like phenotype and HO-1 expression. We studied both cultured neurons and endothelial cells exposed to hemin, as well as in brain cells near the site of hemorrhage. Senescence induction may enable cells to survive and avoid ferroptosis, while simultaneously degrading hemin through HO-1. In culture and in tissue from individuals with intracerebral hemorrhage, we observed a co-localization of senescence-associated β-galactosidase expression and HO-1 positivity. DDR signaling inhibitors reduced both senescence markers and HO-1 expression. Interfering with senescence directly using p21 inhibitors during hemin exposure or pre-emptive HO-1 overexpression resulted in apoptosis and ferroptosis, suggesting that addressing only one aspect of hemin-induced toxicity was insufficient to prevent cell death. Therefore, we designed a multifunctional nanoparticle that incorporates deferoxamine (DEF), an iron chelator, with a pleiotropic nanozyme of oxidized, medical-grade coconut shell activated charcoal (OAC) and poly(ethylene)glycol (PEG). This nanoparticle efficiently mitigated hemin-induced senescence while also preventing iron-induced cell death. These findings emphasize the crucial function of DDR-induced senescence-like phenotype and HO-1 expression in the context of hemin degradation and cell survival in experimental brain hemorrhage, suggesting potential new therapeutic strategies for treating brain hemorrhage.

Disclosures: V.H. Maloji Rao: None. **J. Mitra:** None. **M. Kodavati:** None. **V. Vasquez:** None. **S. Mitra:** None. **J.M. Tour:** None. **G.W. Britz:** None. **T.A. Kent:** None. **M.L. Hegde:** None.

Poster

PSTR406. Stroke Recovery: Pharmacological Approaches to Therapy

Location: WCC Halls A-C

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Program #/Poster #: PSTR406.05/W13

Topic: C.09.Stroke

Support: supported by a Boston Children's Health Physicians' Neonatal Division pilot grant for stem cell research (GV and EFL),
Seed grant funding, Touro Universities system (GV & WM)

Title: Combined sulforaphane and deferoxamine improves hippocampal recovery after neonatal intraventricular hemorrhage

Authors: *G. VINUKONDA[1,4], M. ZIGELSTEIN[5], V. CASTRO DIAZ[5], F. HU[2], M. S. WOLIN[6], E. F. LA GAMMA[7,3];
[1]Pediatrics-Newborn Med. & Cell biology anatomy, [2]Pediatrics-Newborn Med., [3]Pediatrics,

Biochem. and Mol. biology, New York Med. Col., Valhalla, NY; [4]Newborn Med., Boston Childrens Hlth. Physicians (BCHP), Valhalla, NY; [5]Newborn Med., Maria Fareri Children's Hospital, Westchester Med. Ctr., Valhalla, NY; [6]Physiol., New York Meical Col., Valhalla, NY; [7]Dir Regional Neonatal Ctr., Maria Fareri Children's Hosp., Valhalla, NY

Abstract: Germinal matrix hemorrhage-intraventricular hemorrhage (IVH) is a common complication of extremely premature newborns and results in reduced neurogenesis, cortical atrophy and subsequent cerebral palsy, learning and memory disabilities. Significantly, the dentate gyrus (DG) of the hippocampus produces neuronal precursor cells throughout life. Despite this, survivors of Grade III-IV hemorrhage show smaller hippocampal volumes on MRI consistently with impaired hippocampal function and related cognitive scores (Strahle et al 2019). We hypothesize that following IVH, RBC lysis-released toxic mediators (hemin, free hemoglobin, Fe^{+++} accumulation) will disturb postnatal hippocampal formation of CA3, CA2, CA1 and dentate gyrus (DG) structures. To address this, we are evaluating the combined treatment with deferoxamine (DFN, Fe^{+++} chelator) and sulforaphane (SFN) an activator of the Nrf2 transcription factor, which enhances many antioxidant enzymes attenuating free radical toxicity and proinflammatory responses leading to preferential macrophage polarization (M1 vs M2). Using a preterm rabbit pup model of glycerol-induced IVH (Georgiadis et al, 2008 and Vinukonda et al, 2019), after DFN+SFN (SFN 25mg/Kg, 2 doses, DFN 50mg/Kg, twice a day starting from day 1 of IVH), we identified a reduced size of the parenchymal hematoma *qualitatively* on hippocampus sections using H&E staining control vs IVH alone on postnatal d3 (equivalent to term). Ongoing experiments will *quantify* free Hb and Fe^{+++} accumulation/clearance after combined treatment. $NeuN^{+}$ (mature neuron specific antibody) immunofluorescence staining showed a 5-6-fold increase in total cell density in the dentate gyrus (QuPath-software program) after IVH. Due to region-specific functions within the hippocampus, we also examined inner-pyramidal (DG_{inner}) and outer-pyramidal (DG_{outer}) blades of the dentate gyrus. We observed that after IVH, $NeuN^{+}$ neurons were densely distributed in both DG_{inner} and DG_{outer} blades; whereas in healthy controls, only the DG_{outer} blade was densely populated. DFN+SFN treatment after IVH resulted in ~40% reduction in overall and densely populated $NeuN^{+}$ in the DG_{outer} blade. We observed a two-fold increase in Iba-1^{+} microglia in IVH pups vs control that was reduced to near normal on day 3 after SFN-DFN treatment. These data suggest beneficial effects of SFN-DFN treatment at early IVH that may later impact long term memory or neurobehavioral function.

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Poster

PSTR406. Stroke Recovery: Pharmacological Approaches to Therapy

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR406.06/W14

Topic: C.09.Stroke

Title: Modulation of microglial polarization in hematoma clearance in hemorrhagic stroke by immunoproteasome inhibition

Authors: *W. HU[1], *W. HU[2], C. LEE[3,5], H. YU[4], H. LIU[4], C. LIN[1], C. PANG[3,4], H. LIEW[1,3,4]; [1]PhD Program in Pharmacol. and Toxicology, Tzu-Chi university, Hualien, Taiwan; [2]Tzu-Chi Univ., Hualien, Taiwan; [3]Neuro-medical Scientific Ctr., [4]Dept. of Med. Res., Tzu-Chi general hospital, Hualien, Taiwan; [5]Dept. of Neurosurg., Tzu Chi general hospital, Hualien, Taiwan

Abstract: Objective: Our objective was to investigate the impact of proteasome over-activation induced by intracerebral hemorrhage (ICH) on endoplasmic reticulum (ER) stress, protein homeostasis disruption, and neuroinflammation. We explored the role of proteasome inhibition in reducing proteasome activation, alleviating ER stress, and mitigating neuroinflammation. Additionally, we studied the diverse phenotypes of microglial activation, namely M1 polarization (inflammatory activation) and M2 polarization (anti-inflammatory/phagocytic activation), which are influenced by different signals received by microglial receptors. We aimed to elucidate the expression pattern of proteasome and their involvement in hematoma clearance. **Materials & Methods:** We examined the expression of proteasomes and neuroinflammation in rats with ICH using RT-qPCR and Western blotting, respectively. Immunofluorescent staining was employed to identify the cell types expressing specific proteasome subtypes. In an in vitro erythrophagocytosis assay, microglia were incubated with CFDA-labeled red blood cells (RBCs), and some groups were co-treated with ONX-0914 (a specific inhibitor of immunoproteasomes at a concentration of 100 nM). Phagocytic ability was measured by using water to induce cell swelling and dissolve the fluorescent dye. Microglial polarization levels were determined by flow cytometry, and the expression of inflammatory and anti-inflammatory genes was assessed using qPCR. **Results:** Immunoproteasomes were found in the vicinity of the hemorrhagic area and primarily co-localized with microglia expressing the clearance marker CD163. The *in vitro* erythrocyte phagocytosis assay revealed that co-treatment with ONX-0914 enhanced phagocytic ability and promoted microglial polarization toward an anti-inflammatory phenotype. **Conclusion:** Inhibiting immunoproteasomes exerts neuroprotective effects by facilitating hematoma clearance and suppressing inflammation.

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Poster

PSTR406. Stroke Recovery: Pharmacological Approaches to Therapy

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR406.07/W15

Topic: C.09.Stroke

Support: SIP 20221448
SIP 20210292
CONACyT 556028

Title: Evaluation of the antioxidant and antiapoptotic effect of a combination of cannabidiol and dapsone against in vitro brain ischemia and reperfusion in sh-sy5y cells.

Authors: *M. ISLAS[1], A. DIAZ-RUIZ[2], C. RIOS[3], R. PEREZ-PASTEN-BORJA[1];
[1]Mol. toxicology, Natl. Polytechnic Inst., Mexico city, Mexico; [2]Neurochemistry, Natl.Aut.Univ. of Mexico, INNN, Mexico City, Mexico; [3]Natl. Inst. Neurology, Neurosurg, Natl. Inst. Neurology, Neurosurg, Mexico City, Mexico

Abstract: EVALUATION OF THE ANTIOXIDANT AND ANTIAPOPTOTIC EFFECT OF ACOMBINATION OF CANNABIDIOL AND DAPSONE AGAINST IN VITRO BRAINISCHEMIA AND REPERFUSION IN SH-SY5Y CELLS.Marcela Islas-Cortez[1,2], Araceli Diaz-Ruiz[2], Camilo Rios[3], Ricardo Perez-Pastén-Borja[11]Laboratorio de Toxicología Molecular y Celular, Escuela Nacional de Ciencias Biológicas-CampusZacatenco, Instituto Politécnico Nacional, Ciudad de México 07738, Mexico[2]Departamento de Neuroquímica Instituto Nacional de Neurología y Neurocirugía Manuel VelascoSuárez, Ciudad de México, México.[3]Dirección de Investigación, Instituto Nacional de Rehabilitación Luis Guillermo Ibarra Ibarra, Ciudadde México, México | Laboratorio de Neurofarmacología Molecular, Universidad AutónomaMetropolitana Xochimilco, Ciudad de México, México.**Introduction:** Brain ischemia is the result of interruption of blood flow of a cerebral artery.This event is characterized by oxygen and glucose deprivation, which produces neuronaldeath. The**objective** of the present study was evaluated the effect of treatment with Cannabidiol (CBD)and Dapsone (DDS) administered alone or in combination in vitro model of ischemic cerebral with reperfusion in SH-SY5Y cells by means of an isobolographic study. **Experimental procedures:** This study was carried out in SH-SY5Y cells to evaluate the interaction of the combination of CBD and DDS for which concentration-response curves of DDS and CBD were performed. Where LC50 values were established separately for each drug. Concentration-response curves of the drugs were made on the model of brain ischemia through oxygen and glucose deprivation (OGD) to obtain the EC50. Once the EC50 of the DDS-CBD mixture was obtained, the interaction index was calculated and the isobologram was performed. Once the type of interaction between the drugs was determined, the mixture was evaluated on cell viability, cytotoxicity, caspase3 enzymatic activity, and determination of the production of reactive oxygen species. **Results:** The isobolographic results indicated a synergistic effect, this effect is of great interest since it is possible to increase the cell viability of the SH-SY5Y line after undergoing conditions of OGD followed by 24 h. of reperfusion. The results of the group treated with DDS-CBD showed a significant decrease in lactate dehydrogenase (LDH) release, ROS and caspase 3 enzyme activity, as well as an increase in cell viability and reduced glutathione. **In conclusion**, the results suggest that the combination of CBD and DDS could be a suitable alternative to achieve a good antioxidant antiapoptotic effect, safely maintaining cell viability.

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Poster

PSTR406. Stroke Recovery: Pharmacological Approaches to Therapy

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR406.08/W16

Topic: C.09.Stroke

Support: The Mathers Foundation (MF2204-0255)
Neurology Department, BIDMC
FAPESP 22/12780-0

Title: A neuroprotective state of suspended animation

Authors: *L. ANGENENDT DA COSTA[1], A. SHEHADAH[2], R. SOHUR[2], C. B. SAPER[3], N. L. MACHADO[4];
[1]Univ. of São Paulo, Ribeirao Preto, Brazil; [2]Beth Israel Deaconess Med. Center, Harvard Med. Sch., Boston, MA; [3]Harvard Med. Sch. Dept. of Neurol., Harvard Med. Sch. Dept. of Neurol., Boston, MA; [4]Beth Israel Deaconess Med. Ctr. - Harvard Med., Beth Israel Deaconess Med. Ctr. - Harvard Med., Boston, MA

Abstract: Suspended animation is a state in which life processes in a body are temporarily slowed down but can be restarted without damage. Some animals enter such a state naturally in response to a chronic low environmental temperature or times of food scarcity. These animals lower their body temperature and enter torpor or hibernation states, a deep hypothermic and hypometabolic condition that conserves energy. We have recently demonstrated that median preoptic neurons expressing the prostaglandin EP3 receptor (MnPOEP3R) are necessary and sufficient to cause torpor, and activating them promotes deep hypothermia - an artificially induced state of suspended animation without injury to the animals.Here we characterized the wake-sleep behavior of mice during a state of suspended animation and tested the neuroprotective benefits of deep hypothermia after ischemic stroke. We recorded the patterns of the electroencephalogram (EEG) and electromyogram (EMG) of mice during hypothermia or baseline (normothermic) conditions. We found that during deep hypothermia, there is a substantial reduction of the EEG power, an increase in time spent in NREM sleep, and a suppression of REM sleep. We also tested the ability of mice to be aroused from the deep hypothermic state by gently handling them and showed that these mice are responsive to external stimuli.Then, in a separate set of experiments, we used a permanent middle cerebral artery occlusion (pMCAO) or promoted a transient MCAO for 4 hours, followed by reperfusion (tMCAO). Thirty minutes from the MCAO onset, we promoted deep hypothermia by activating MnPOEP3R neurons. Body weight, survival rate, and neurological severity score were evaluated 24-72 hours following a stroke. Our deep hypothermic model caused core temperature to drop (23.84 ± 0.7 ℃) at room temperature for up to 72 h. Hypothermic mice had no evidence of weight loss during the first 48 hours of hypothermia, while normothermic mice showed 15% and 20% in body weight loss 48 hours from the onset of the permanent or transient stroke, respectively. Hypothermia also drastically reduced stroke severity, increasing survival by 30% (pMCAO) or 70% (tMCAO) after 72 hours. The hypothermia length also correlated with better neurological outcomes after tMCAO.Our results suggest that suspended animation is neuroprotective after ischemic stroke, and its therapeutic benefits are likely to be translatable from mice to humans.

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Poster

PSTR406. Stroke Recovery: Pharmacological Approaches to Therapy

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Program #/Poster #: PSTR406.09/W17

Topic: C.09.Stroke

Support: NIH Grant R01NS107853

Title: Inhibition of histone deacetylase 3 improves functional outcomes post-intracerebral hemorrhage via reduction of neuroinflammation.

Authors: ***N. J. WATSON**, F. BONSACK, S. SUKUMARI RAMESH;
Augusta Univ., Augusta, GA

Abstract: Secondary brain injury is a leading cause of neurological deficits after intracerebral hemorrhage (ICH), a severe stroke subtype. Stimulation of the immune system at the site of a brain hemorrhage, characterized by microglial activation, leads to neuroinflammation and secondary brain damage. We previously reported that genetic knockdown of HDAC3 in a macrophage cell line significantly attenuated hemin-induced release of TNF-α and IL-6 compared to a control, while genetic knockdown of both HDAC1 and HDAC2 significantly augmented hemin-induced release of TNF-α from cells without an effect on IL-6. To extend this observation further, we have now generated microglia-specific HDAC3 conditional knockout using Cre-Lox technology. Our ongoing preliminary studies indicate improved neurobehavioral outcomes in HDAC3 flox/flox: Cx3cr1 CreER post-ICH compared to experimental control in both males and females. Further, analysis of levels of pro- and anti-inflammatory cytokines in ipsilateral brain regions post-ICH via rt-qPCR have demonstrated a highly significant reduction of pro-inflammatory cytokines, a corresponding increase in anti-inflammatory cytokines, a reduction of activated microglia in both male and female subjects, and an increase in M2 microglial polarization. Additionally, use of a HDAC3 inhibitor, RGFP966, in mice has demonstrated an improvement in functional recovery in treated mice compared to control animals. RGFP966 treated mice also show a reduction in pro-inflammatory cytokines, indicating HDAC3i as a potential novel therapy for ICH.

Disclosures: **N.J. Watson:** None. **F. Bonsack:** None. **S. Sukumari Ramesh:** None.

Poster

PSTR406. Stroke Recovery: Pharmacological Approaches to Therapy

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR406.10/W18

Topic: C.09.Stroke

Support: Calico Labs

Title: Inhibiting Integrated Stress Response in a Stroke Model of Vascular Dementia

Authors: *M. HOVANESYAN[1], S. AZARAPETIAN[1], W. DAI[1], M. ZARAFSHAN[1], I. LORENZO LLORENTE[1], S. CARMICHAEL[2];
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Abstract: White matter stroke (WMS) is a debilitating disorder, characterized by the formation of ischemic lesions along the white matter tracts in the brain. The accumulation of these white matter lesions leads to vascular dementia, which is the second leading cause of dementia, and accelerates the pathology of Alzheimer's Disease. As of today, there are no therapy options available to halt the progression of this debilitating disease. Evidence from genetic diseases that affect the cerebral white matter, a support cell (or glial cell), termed the astrocyte, senses the injury and undergoes a prolonged stress response. This stress response, termed the integrated stress response (ISR), contributes to progressive damage in the white matter. We targeted the ISR response by using an inhibitor, 2BAct, in a mouse model of vascular dementia. We initially performed white matter strokes in C57BL/6J mouse models, and measured the behavioral recovery of these cohorts through drug administration. We conducted tests of motor control (cylinder and grid-walking) at baseline, 1-week, 1-month, 2-month, and 4-month timepoints to assess recovery after injury in the white matter. Our data indicates that there is an initial recovery in grid-walking at the 1-month timepoint, and a recovery in cylinder tests at the 2-month timepoint for cohorts that received a WMS + 2BAct administration. This data suggests that the ISR is an important target to promote recovery in a model of vascular dementia. Supported by a grant from Calico Labs

Disclosures: M. Hovanesyan: None. **S. Azarapetian:** None. **W. Dai:** None. **M. Zarafshan:** None. **I. Lorenzo Llorente:** None. **S. Carmichael:** None.

Poster

PSTR406. Stroke Recovery: Pharmacological Approaches to Therapy

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Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR406.11/Web Only

Topic: C.09.Stroke

Support: NSTC 110-2320-B-001-002-025-MY3

Title: Roles of equilibrative nucleoside transporter-1 in modulating energy homeostasis and meningeal lymphatics in mice with permanent ischemic stroke

Authors: *C.-J. HO[1], K.-C. WU[1], J. LIN[1], W.-R. CHEN[1], C.-F. CHANG[1], Y. CHERN[2], C.-J. LIN[1];
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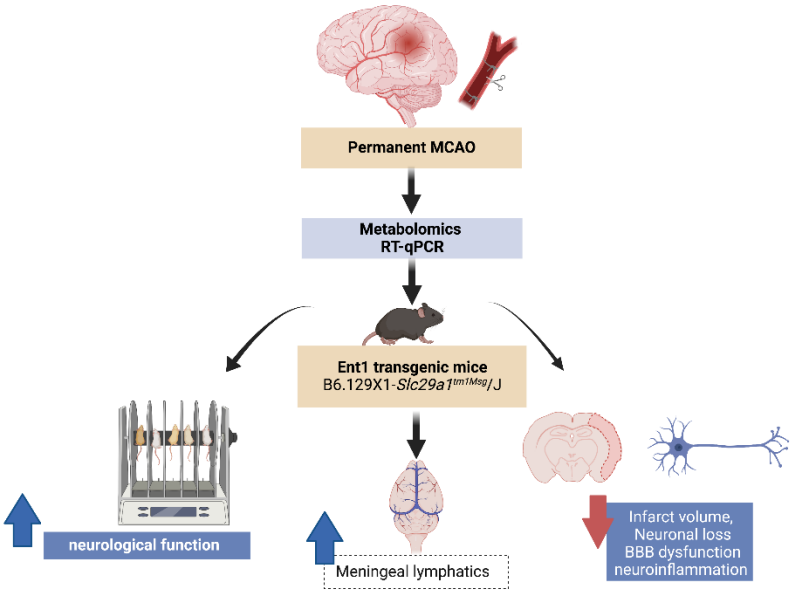
Abstract: Main purpose
During ischemic events, adenosine serves as a neuromodulator with a 100-fold upregulated level in the brain extracellular fluids. The equilibrative nucleoside transport 1 (Ent1) distributed in the neuron and glial cells is responsible for cellular uptake and the homeostasis of adenosine in the brain. Meanwhile, meningeal lymphatics have been found in transporting metabolites, wastes, and immune cells in the brain and therefore play a crucial role in neurological diseases. The objective of the present study was to explore the roles of Ent1 in the modulation of energy homeostasis and meningeal lymphatics in ischemic stroke.
Methods
$Ent1^{-/-}$ ($B6.129X1\text{-}Slc29a1^{tm1Msg/J}$) mice and its littermate controls were originally purchased from Jackson Laboratory. Mice (8-12 weeks old), both male and female mice, were subjected to permanent middle cerebral artery occlusion (pMCAO) to induce permanent ischemic stroke. The mRNA expression of adenosine-related enzymes was measured by RT-qPCR. The metabolomic profiles of adenine nucleotides were conducted by UPLC-QTOF/MS analysis. The infarct volume was measured on D1 post injury. Behavioral tests, astrogliosis, microgliosis, BBB integrity, and lymphagiogenesis were examined on D14 post injury.
Results
Metabolomic results showed that intracellular adenine, adenosine, and AMP were significantly decreased in mice with pMCAO. Meanwhile, the mRNA level of Ent1 was increased. $Ent1^{-/-}$ mice reduced the infarct volume on D1 with improved motor and recognition function until D14. In terms of meningeal lymphatics, $Ent1^{-/-}$ mice exhibited enhanced lymphagiogenesis in both uninjured and ischemic injury conditions. $Ent1^{-/-}$ mice also preserved neuron density and BBB integrity in the penumbra of the cortex, along with reduced neuroinflammation in mice with MCAO.
Conclusion
Ent1 deletion attenuated the injury in mice with permanent ischemic stroke. These findings indicate the important role of Ent1 in the pathogenesis and treatment of ischemic stroke.



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Poster

PSTR406. Stroke Recovery: Pharmacological Approaches to Therapy

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR406.12/Web Only

Topic: C.09.Stroke

Support: CONACyT A1-S-21433

Title: Effect of S allylcysteine on neurotrophin expression after ischemia/reperfusion injury in female rats

Authors: *S. BAUTISTA PÉREZ[1], C. A. SILVA-ISLAS[2], J. PEDRAZA-CHAVERRÍ[3], M. OROZCO-IBARRA[5], D. BARRERA-OVIEDO[4], P. MALDONADO-JIMENEZ[6], O. APARICIO-TREJO[7];
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Medicine, Natl. Autonomous Univ. of Mexico, Mexico, Mexico; [7]Natl. Inst. of Cardiol., Mexico, Mexico

Abstract: Stroke is a leading cause of death and mortality, despite that it affects mainly the elderly in a homogeneous way, there are differences in terms of epidemiology, recovery, motor and cognitive impairments, and the response and effectiveness of the treatments between men and women. Initially, the research for treatments was focused on limiting the damage; however, regeneration therapies have attracted attention. Regeneration is a process naturally activated after cerebral ischemia damage, in part in response to the neurotrophins. One of its main advantages is that these therapies are not limited by time. However, is an insufficient process due the presence of proinflammatory molecules. In relation to treatments, the S-allylcysteine (SAC), has been used by its antioxidant and antiinflammatory properties, showing to have protective effect against acute ischemia/reperfusion injury in male young animals. However, the protective mechanism is not fully understood and its effects on regenerations have not been studied yet. Thus, the aim of this work was to evaluate the effect of SAC on motor defict and the neurotrophins levels in young female rats after 1 h of ischemia and 15 days of reperfusion. The ischemia was induced by the middle cerebral artery occlusion. Daily doses of SAC (100 mg/kg) were administered via intraperitoneal. The motor deficits were analyzed, and the neurotrophins vascular endothelial growth factor (VEGF), neuronal growth factor (NGF) and brain derived neurotrophic factor (BDNF) were quantified by immunohistochemistry. Animals treated with SAC showed fewer motor deficits and higher levels of NGF in cortex and striatum, VEGF in striatum and BDF in subventricular zone. This project was partially supported by CONACyT A1-S-21433 to PDM.

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Poster

PSTR406. Stroke Recovery: Pharmacological Approaches to Therapy

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR406.13/W19

Topic: C.09.Stroke

Support: NIH Grant R01NS112642 to ZAS

Title: Cofilin knockdown with siRNA prevents microglial activation, neuroinflammation, and cofilin-actin rods/aggregates after intracerebral hemorrhage in young and aged mice

Authors: *D. ALMARGHALANI[1], G. BAHADAR[2], F. SHEHJAR[2], W. ANTONISAMY[2], Z. A. SHAH[3];
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Abstract: Studies from our lab have shown that cofilin-actin rods/aggregates are localized within the microglia and around the hematoma of the human autopsy brain and young mouse brain after intracerebral hemorrhage (ICH). Considering age as a risk factor for ICH and associated with higher mortality rates than young, this study focused on the role of cofilin in aged mice, and the results were compared to young mice. Furthermore, cofilin siRNA gene therapy in mice subjected to inter-striatal collagenase injection-induced experimental ICH was tested. The outcomes were assessed 15 days after ICH. The cortex and hippocampus of the mouse brain were analyzed for cofilin-actin rods/aggregates, microglial activation, and neuroinflammation and serum was collected to evaluate proinflammatory cytokines (IL-6 and IL-1β). Cofilin-actin rods/aggregates were observed in the cortex and hippocampus of aged males and females but not in young-adult males and females. The length and intensity of cofilin-actin rods/aggregates increased in adult and aged males and females around the hemorrhagic area. Activated microglia increased in both young and aged males and females but were prominent in aging male and female mice. However, cofilin gene therapy with siRNA mitigated cofilin-actin rods/aggregates and microglial activation in young adults and aged male and female mice. There were no changes in IL-1β in the cortex and hippocampus of young males and females. An increased expression of IL-1β was observed in the hippocampus of aged males and females, but cofilin siRNA treatment significantly reduced IL-1β in old females but not males. The serum IL-6 levels were significantly increased in aged males and females compared to young-adult males and females and attenuated by cofilin siRNA treatment. The serum IL-1β levels were diminished in the cofilin siRNA treatment of young and aged males and females compared to ICH groups, but the results were insignificant. These results suggest that reducing cofilin-actin rods/aggregates with siRNA gene therapy could potentially treat ICH-related pathology in aged and young mice.

Disclosures: D. Almarghalani: None. **G. Bahadar:** None. **F. Shehjar:** None. **W. Antonisamy:** None. **Z.A. Shah:** None.

Poster

PSTR406. Stroke Recovery: Pharmacological Approaches to Therapy

Location: WCC Halls A-C

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Program #/Poster #: PSTR406.14/W20

Topic: C.09.Stroke

Title: 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3) blockade is neuroprotective in an acute mouse model of ischemic stroke

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Abstract: Stroke is the second leading cause of death and the leading cause of adult disability worldwide. Ischemic stroke, characterized by the blockage of a blood vessel, is the most

prevalent type. A major hallmark of stroke is the dysregulation of normal metabolic functions. In the ischemic environment, cells shift towards glycolysis, a metabolic pathway that is associated with a prolonged inflammatory response and delayed apoptotic cell death. One primary driver of glycolysis is 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3). This enzyme is upregulated after injury and synthesizes fructose-2,6-biphosphate, an allosteric activator of phosphofructokinase-1 which catalyzes the first-rate limiting step in glycolysis. In diseases involving glycolytic shift such as atherosclerosis, pulmonary hypertension, and endotoxemia, PFKFB3 inhibition has been shown to increase survival rate and reduce injury; however, little research has been conducted in stroke. We hypothesize that because aberrant glycolysis contributes to inflammation and cell death and PFKFB3 upregulates glycolysis after injury, inhibition of PFKFB3 is neuroprotective in stroke. We administered a 45-minute transient middle cerebral artery occlusion in male C57BL/6J mice followed by treatment with a highly selective PFKFB3 inhibitor, AZ67 (N=10) or vehicle (N=11). The vehicle or the inhibitor (30mg/kg) were administered starting at the onset of reperfusion via the jugular vein with three repeated retro-orbital doses over the course of 48h. Open field, vertical grid, weight test, and neural deficit scorings were conducted at 24 and 48h post stroke after which the mice were euthanized by perfusion with saline and stained with TTC to quantify infarct size. Our findings show a neuroprotective effect of PFKFB3 inhibition in ischemic stroke with improved functional and behavioral recovery and smaller infarct sizes. These results support the hypothesis that PFKFB3 inhibitors may be used as a novel pharmacological treatment for reducing delayed cell death after ischemic stroke. Future studies are underway to investigate the molecular mechanisms and cell types involved in the protection conferred by PFKFB3 blockade in the context of ischemic stroke.

Disclosures: R.E. Gunraj: None. **J. Larochelle:** None. **S. Stansbury:** None. **C. Yang:** None. **L. Liu:** None. **E. Candelario-Jalil:** None.

Poster

PSTR406. Stroke Recovery: Pharmacological Approaches to Therapy

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR406.15/W21

Topic: C.09.Stroke

Support: NIH Grant R01NS112642

Title: Iron overload and the protective effects of deferoxamine and a cofilin inhibitor

Authors: *F. SHEHJAR, R. MAHAJAN, W. ANTONISAMY, Z. A. SHAH;
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Abstract: Background: Iron overload plays a critical role in neurodegenerative disorders, particularly hemorrhagic brain injury, characterized by brain bleeding. Increased iron levels contribute to oxidative stress, inflammation, and neuronal damage. Cofilin-1, an actin-binding

protein, is implicated in neurodegeneration, including hemorrhagic brain injury, as it regulates actin dynamics essential for neuronal survival. Understanding the role of iron overload and cofilin-1 in hemorrhagic stroke is crucial for developing targeted therapies. **Methods:** To mimic iron overload, we investigated protein expression modulation in SH-SY5Y cells treated with ferric ammonium citrate (FAC). We examined the potential protective effects of deferoxamine (DFX) and a cofilin inhibitor (CI). Cells were treated with FAC (150uM), FAC+DFX (150uM+150uM), FAC+CI (150uM+5uM), and vehicle (control group). Western blot was used to assess protein levels associated with neurodegeneration. **Results:** FAC-treated cells exhibited significantly increased protein expression of BAX, caspase-1, BIP, eIF2-α, STAT-1, AIF, cleaved PARP, cleaved caspase-3, COX-2, ferritin, HO-1, NRF-2, and SOD-2 compared to the vehicle (control group), suggesting activation of apoptotic pathways, endoplasmic reticulum stress, and elevated oxidative stress. In contrast, the DFX and CI groups showed significantly decreased expression of these proteins compared to the FAC-treated group, indicating potential protective effects by attenuating pro-apoptotic, pro-inflammatory, and oxidative stress responses induced by iron overload. **Conclusions:** Our findings highlight the potential of DFX and CI as therapeutic strategies to mitigate iron-induced neurotoxicity in hemorrhagic brain injury. Targeting iron overload and cofilin-1 signaling pathways may offer novel approaches to preserve brain tissue and promote recovery in neurodegenerative diseases associated with iron dysregulation.

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Poster

PSTR406. Stroke Recovery: Pharmacological Approaches to Therapy

Location: WCC Halls A-C

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Program #/Poster #: PSTR406.16/W22

Topic: C.09.Stroke

Support: NRF-2019R1A5A2026045
NRF-2021R1F1A1061819
HR21C1003

Title: The NFkB-cIAP2 axis is essential and sufficient to protect oligodendrocyte from ischemic insult

Authors: *J. CHOI[1], H. KIM[2], S. KOH[1], B. KIM[2], B. KIM[1];
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Abstract: Myelinating glia, oligodendrocyte (OL), provides saltatory conduction and various support to the axon through axomyelinic synapse. White matter stroke (WMS) is an important clinical entity that comprises one-fourth of ischemic stroke and is the second most common cause of senile dementia but underestimated neurologic disorders. The main pathology of WMS

is ischemic OL loss. Thereafter, a therapeutic strategy for WMS should focus to reduce the ischemic OL death. Previously, our group showed Toll-like receptor 2 in OLs protects OLs from ischemic damage, promotes OPC migration to the WMS lesion through interaction with the one of the endogenous TLR2 ligands, the high-mobility group box 1 released from dying OL. However, there is a need to uncover more detailed intracellular survival pathways mediated from TLR2 in oligodendrocytes. We investigated primary OL culture from P1 pups and oxygen-glucose deprivation as an in vitro ischemic model. Firstly, we examined the phosphorylation or activation of canonical TLR2 pathway signaling proteins, such as AKT, ERK1/2, p38, CREB, or NF-kB in OL after Pam3CSK4, a TLR2 agonist. TLR2 activation didn't phosphorylate AKT but phosphorylate ERK1/2, p38, and CREB and activate NF-kB. Secondly, we used a chemical inhibitor or siRNA to each signal pathways proteins and found p38 and NF-kB were essential for TLR2-mediated OL protection from ischemic insults but not ERK1/2 and CREB. To validate our findings, we performed bulk RNA seq in OL with or without Pam3CSK4 or OGD. According to RNA seq data, NF-kB is located in the central position of TLR2-mediated OL protection. Also, we found that cIAP2 and Bcl2 have upregulated in TLR2-NFkB dependent manner. After the acquisition of RNA seq, we transfected OL with various constitutive active plasmids or siRNA to manipulate signaling pathways. cIAP2 rather than Bcl2 was increased after transfection in either p38 or NF-kB active state. Interestingly, p38 constitutive activation with NF-kB knockdown couldn't increase cIAP2 and protect OLs from OGD. On the contrary, NF-kB constitutive activation with p38 knockdown could increase cIAP2 and protect OLs from OGD. Additionally, cIAP2 overexpression with NF-kB knockdown also showed OLs protective effects but not vice versa. To confirm the protective effects of NF-kB, we injected AAV-mMBP-Flag-IKK2 into right internal capsule. Similarly with in vitro results, activation of NF-kB in OLs showed reduced WMS lesion volume and improved neurobehavioral deficit measured by adhesive removal test. These findings show TLR2 in OLs exerts a protective effect through NF-kB and cIAP2 and suggest a novel therapeutic target to overcome ischemic OL death and WMS.

Disclosures: **J. Choi:** None. **H. Kim:** None. **S. Koh:** None. **B. Kim:** None. **B. Kim:** None.

Poster

PSTR406. Stroke Recovery: Pharmacological Approaches to Therapy

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Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR406.17/W23

Topic: C.09.Stroke

Support: NIH Grant ZIA-AA000242

Title: A Mendelian Randomization Study of PCSK9 Inhibition and Stroke: Evidence for Benefits Beyond Low-Density Lipoprotein Cholesterol Lowering

Authors: *L. M. PARK[1], D. B. ROSOFF[1,3], L. A. MAVROMATIS[1], A. S. BELL[1], A. M. HAMANDI[1], J. JUNG[1], J. WAGNER[1], P. MUKHOPADHYAY[1], P. PACHER[1], L. F. VENDRUSCOLO[2], G. F. KOOB[2], F. W. LOHOFF[1];

[1]NIAAA, NIH, Bethesda, MD; [2]NIDA, NIH, Baltimore, MD; [3]Univ. of Oxford, Oxford, United Kingdom

Abstract: Background: Stroke is the second leading cause of death globally, and recent clinical trials have found that pharmacological proprotein convertase subtilisin kexin 9 (PCSK9) inhibition (PCSK9i) reduces stroke risk. However, the impact of long-term PCSK9i on stroke risk and the complex interplay of PCSK9 with various lipids remains unknown. Our goal was to leverage data on PCSK9 protein levels using drug-target Mendelian randomization (MR) and multivariable MR (MVMR) to investigate the impact of genetically-lowered PCSK9 levels on stroke risk. Methods: We created genetic instruments using *PCSK9* variants associated with plasma PCSK9 concentration ($N_{primary}$=35,559; $N_{replication}$=10,186); low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG; N=1,320,016); apolipoprotein B (ApoB; N=439,125); lipoprotein a (Lp[a]; N=361,194); stroke (N_{cases}=40,585); ischemic stroke (IS; N_{cases}=34,217); and stroke subtypes ($N_{cases}\leq$7,193). We performed drug-target MR investigating relationships between plasma PCSK9 and stroke phenotypes as well as MVMR assessing PCSK9 inhibition on stroke risk while controlling for five major lipid subfractions (LDL-C, HDL-C, TG, ApoB, and Lp[a]). Results: We identified a strong protective association between genetically-lowered PCSK9 protein levels and stroke (odds ratio (OR)=0·86, 95% CI [0·83-0·88], P=$1.74x10^{-28}$), ischemic stroke (OR=0·88, 95% CI [0·85-0·90], P=$1·70x10^{-18}$), cardioembolic stroke (OR=0·84, 95% CI [0·76-0·93], P=0·001), and large artery ischemic stroke (OR=0·74, 95% CI [0·68-0·80], P=$1·28x10^{-14}$). We replicated these findings with complementary MR methods and two genetic instruments from independent data. MVMR analysis suggested that genetically proxied circulating PCSK9 inhibition was protective both for ischemic stroke (OR=0·89, 95% CI [0·82-0·97], P=0·008) and cardioembolic stroke (OR=0·83, 95% CI [0·69-1·00], P=0·04) supplementary to its established effect on lowering lipid levels, while PCSK9 inhibition was protective against large artery atherosclerosis entirely through its lipid pathways. Conclusions: Our data provide new evidence that plasma PCSK9 protein levels inversely associate with stroke risk, resolving discrepancies between randomized clinical trials and previous genomic studies. MVMR suggested pleiotropic relationships of PCSK9 on stroke risk beyond its effects on lipid fractions, underlining that future studies are needed to investigate the protective effect of PCSK9i beyond LDL-C-related pathways. Broadly, our study should reassure clinicians that anti-PCSK9 therapies reduce stroke.

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Poster

PSTR406. Stroke Recovery: Pharmacological Approaches to Therapy

Location: WCC Halls A-C

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Topic: C.09.Stroke

Support: Swedish Reserack Council
Swedish Brain Foundation
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Title: Complement C3a treatment attenuates secondary neurodegeneration after stroke

Authors: *M. PEKNA[1], A. STOKOWSKA[1], M. ASWENDT[2], S. LOHMANN[1], D. ZUCHA[4], Y. LI[1], F. WIETERS[2], P. ABAFFY[4], M. KUBISTA[5], L. VALIHRACH[4], M. HOEHN[3], M. PEKNY[1];
[1]Univ. of Gothenburg, Gothenburg, Sweden; [2]Univ. Hosp. Cologne, Univ. of Cologne, Koeln, Germany; [3]Univ. of Cologne, Cologne, Germany; [4]Inst. of Biotech. AS CR, [5]Inst. of Biotech. AS CR, Prague 4, Czech Republic

Abstract: Secondary neurodegeneration in brain regions remote to the infarct impedes functional recovery after ischemic stroke. The complement system has been implicated as a driver of neuroinflammation, neuronal dysfunction and synapse loss associated with aging and Alzheimer's disease type neurodegeneration. We previously showed that signaling through the receptor for complement-derived peptide C3a (C3aR) stimulates peri-infarct neural plasticity and intranasal treatment with C3a facilitates recovery of sensorimotor function after ischemic stroke. Here, we investigated the role of the C3a-C3aR axis in secondary neurodegeneration following cortical ischemic stroke. We found that mice lacking C3aR had increased beta-amyloid accumulation and more prominent reactive microgliosis in the ipsilesional thalamus 21 days after stroke. Daily intranasal treatment of mice with C3a for 2 or 3 weeks starting 7 days after stroke induction reduced beta-amyloid accumulation and reactive gliosis, and increased density of synaptophysin-positive presynaptic terminals in the ipsilesional thalamus. Using in vivo magnetic resonance imaging, we assess stroke-induced long-term changes in the ipsilesional thalamus. We conclude that the C3a-C3aR axis is an important modifier of post-stroke secondary neurodegeneration. Intranasal treatment with C3a presents an attractive therapeutic strategy to inhibit secondary neurodegeneration after stroke.

Disclosures: **M. Pekna:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder. **A. Stokowska:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder. **M. Aswendt:** None. **S. Lohmann:** None. **D. Zucha:** None. **Y. Li:** None. **F. Wieters:** None. **P. Abaffy:** None. **M. Kubista:** None. **L. Valihrach:** None. **M. Hoehn:** None. **M. Pekny:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder.

Poster

PSTR406. Stroke Recovery: Pharmacological Approaches to Therapy

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

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Topic: C.09.Stroke

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Neuroscience Program, College of Medicine, Department of Chemistry and Biochemistry, John G. Kulhavi Professorship in Neuroscience, and E. Malcolm Field and Gary Leo Dunbar endowed Chair in Neuroscience at Central Michigan University
A generous gift from Joan Allinder.

Title: Progesterone Reduced Behavioral Deficits in MCAo Rat When Delivered Intraperitoneally Using PAMAM Dendrimers

Authors: *A. UPRETY, A. POUDEL, M. J. M. KING, B. SRINAGESHWAR, N. DAY, J. D. GROSS, R. C. SCHALAU, L. K. BOLEN, S. SCHWIND, B. M. GALVIN, S. R. SHUHAG, O. SMITH, A. SHARMA, D. SWANSON, G. L. DUNBAR, J. ROSSIGNOL;
Central Michigan Univ., Mount Pleasant, MI

Abstract: A stroke occurs when the blood supply to a specific area of the brain is interrupted, resulting in brain tissue damage, loss of brain function, and neuroinflammation. Ischemic stroke, the most common type, happens when a blood clot or narrowed artery blocks the flow of blood to the brain. Developing effective treatments for stroke is challenging due to the complex nature of events and circuitry within the central nervous system. Tissue plasminogen activator (tPA) is the first FDA-approved treatment for acute ischemic stroke, which dissolves blood clots that obstruct brain blood flow. For maximum effectiveness, tPA must be administered within 3-4 hours of ischemic stroke onset. Progesterone, a hormone naturally produced in the body, has neuroprotective and anti-inflammatory properties and might be a potential treatment for an ischemic stroke. Previous studies have indicated that progesterone may be a viable option for mitigating the effects of neuroinflammation and oxidative stress. In this study, we used G4 PAMAM dendrimers for the systemic delivery of progesterone in rat models with middle cerebral artery occlusion (MCAo). The rats received intraperitoneal (IP) injections of dendrimer-progesterone complex, dendrimer only, and HBSS every other day for 10 days, starting on the sixth day after surgery. The dosage was reduced to half of the previous dose for the ninth and tenth injections to prevent potential withdrawal effects. Behavioral assessments, including the ladder test, cylinder test, and modified Garcia scale for neuro-scoring, were conducted weekly to evaluate motor function. The study results indicated that the use of dendrimers to deliver progesterone as a treatment in the stroke rat model showed positive outcomes by improving motor function. Results of the ladder test showed reduced left paw slips in dendrimer-progesterone and dendrimer-only treated MCAo rats. The cylinder test showed improved left paw placement in dendrimer-only treated MCAo rats. The neuro-scoring test showed improvement in score in dendrimer progesterone-treated MCAo rats. These findings confirmed that dendrimers could cross the blood-brain barrier when injected intraperitoneally. These results further suggest that dendrimers have potential as a promising delivery method for drugs targeting the brain. In the future, we will perform immunohistochemistry to see GFAP, IBA-1, and Progesterone receptor A/B expression in brain sections.

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Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR407.01/W26

Topic: C.10. Brain Injury and Trauma

Support: Merit Review Award # 1 I01 RX003123-01A1, from the United States (U.S.) Department of Veterans Affairs Rehabilitation Research and Development Service (RR&D)
Merit Review Award # B3986-R/1 I01 RX003986-01A1, from the United States Department of Veterans Affairs Rehabilitation Research and Development Service (RR&D)

Title: Neuroplasticity of the central noradrenergic system following chronic mild-to-moderate closed-head traumatic brain injury in rats

Authors: *F. THOMPSON[1,2], S. TSUDA[1,3], G. M. DOOLEY[1,3], K. KLIPPEL[1,3], G. HWANG[1,3], J. HOU[1,3], P. BOSE[1,3,4];
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Abstract: Anxiety disorder, balance disability, and motor impairments are common and frequent co-morbidities following closed-head traumatic brain injury (cTBI) in humans, although the precise pathological mechanisms are not well understood. Our previous work showed that chronic mild-to-moderate cTBI resulted in significant injury to the major noradrenergic (NA) cell cluster (i.e., locus coeruleus, LC), whose NA neurons play critical roles in the regulation of emotion, balance, and motor functions. Therefore, it is likely that LC injury contributes to cTBI-induced morbidity. However, studies have not examined to what extent compensatory mechanisms for the LC injury might be mobilized by other NA-cell clusters. Therefore, the purpose of this study was to investigate cTBI-induced NA cell loss in the LC and potential changes in NA cell numbers in alternative NA cell clusters (A1 and A2 cell groups), respectively. It was hypothesized that cTBI-induced LC cell loss would be compensated by increased DβH cell expression in the A1/2 cell groups. Adult female rats were randomly assigned into the normal and cTBI groups (n = 5 per group), and a weight-drop model of mild-to-moderate cTBI was induced in the latter group. Eighteen weeks later, the animals were perfused (4% paraformaldehyde), the brains were further fixed and the brain stems cryosectioned in 40-µm thickness. Every other section was incubated with primary antibodies against neuronal nuclei (NeuN) and dopamine beta-hydroxylase (DβH). On the next day, the sections were incubated

with secondary antibodies conjugated to fluorescent dyes. Then, confocal pictures taken for quantitative analyses of NA cells, revealed cTBI-induced severe loss of NeuN/DβH-immunoreactive NA cells in the LC. However, the number of NA cells in subregions of the A1/2 cell groups was observed to be significantly increased following chronic mild-to-moderate cTBI, compared to normal animals ($p < 0.05$). These studies indicate that the total number of NA cells in the central nervous system (i.e., LC and A1/2 cell groups) was potentially conserved following TBI via enhanced NA expression properties of a subpopulation of the A1/2 cell groups (DβH-positive). Accordingly, the critical roles of NA cells in a variety of essential functions following a severe loss of NA cells in the LC might have been potentially compensated by the elevation of the NA expression in a subpopulation of A1/2 cells. However, our current and previous studies showing lingering significant disabilities, suggest an incomplete neurobehavioral compensation likely, in part, associated with the lingering dysregulation in multiple structures uniquely innervated by LC.

Disclosures: F. Thompson: None. **S. Tsuda:** None. **G.M. Dooley:** None. **K. klippel:** None. **G. Hwang:** None. **J. Hou:** None. **P. Bose:** None.

Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR407.02/W27

Topic: C.10. Brain Injury and Trauma

Support: Supported by SPiRE Award B4097-P/I21 RX004097, from the United States Department of Veterans Affairs Rehabilitation Research and Development Service (RR&D).
Merit Review Award # 1 I01 RX003123-01A1, from the United States (U.S.) Department of Veterans Affairs Rehabilitation Research and Development Service (RR&D)

Title: Therapeutic effects of electroacupuncture on TBI-induced facial and somatic allodynia/hyperalgesias in a rodent model

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Abstract: Traumatic brain injury (TBI)-induced pain, such as headache and hypersensitivity (allodynia) to mild tactile and thermal stimuli, are common comorbidities observed in TBI patients. Currently, no effective therapy is available. More worryingly, TBI induces high

vulnerability to addiction since the common use of addictive pain medication makes dependency and ultimately leads to addiction. Therefore, finding an alternative therapeutic approach to treat TBI-induced pain is urgent. In this study, we evaluated the therapeutic effects of electroacupuncture (EA) on TBI-induced pain in a rat model of TBI. Twenty-four Sprague Dawley rats received mild/moderate closed-head TBI and were randomly divided into TBI-Ctrl and TBI-EA groups (n=12/group). The TBI-EA group received a total of six sessions of EA treatments (30 min/session) starting immediately after TBI and then every other day for two weeks. Three pairs of acupuncture points: ST 36 (Zusanli), LI 4 (He Gu), and LIV 3 (Tai Chong), were used in this study. Operant orofacial allodynia testing (OPAD), lick-guard responses (thermal pain testing), and Magnetic Resonance Imaging (MRI) were conducted at pre-injury, and post-treatment (PO Tx) weeks 1 and 3. The animals were then euthanized with 4% PFA and the spinal cord and brain tissues were collected for immunohistochemistry (IHC) study. Our data to date showed that TBI significantly reduced the lick/facial contact ratio at 42 °C in OPAD testing compared to pre-injury, but the EA treatment significantly improved this ratio. In the lick-guard responses testing, both groups showed a significant reduction in hind paw lick latency compared to pre-injury; but the TBI-EA treated animals showed significant recovery compared to the TBI-Ctrl group. Magnetic resonance diffusion tensor imaging (DTI) detected the recovery of the fiber integrity of the trigeminothalamic pathway in the TBI-EA group. IHC studies showed TBI-induced significant upregulation of the pain signaling molecules [Substance P (SP), Calcitonin gene-related peptide (CGRP), and Piezo2], activated microglia marker (Iba-1), and the pro-inflammatory cytokine interleukin-1β (IL-1β) in spinal cord dorsal horn, spinal trigeminal nuclei, ventral posteromedial nucleus (VPM) of the thalamus, and sensorimotor cortex. Interestingly, the TBI-EA treatment significantly reduced the upregulation of these pain and inflammation molecules. Our data showed that the EA treatment significantly alleviated TBI-induced orofacial and somatic allodynia/hypersensitivity via the downregulation of pain and inflammatory signaling. Thus, EA could be an effective therapeutic approach for TBI-induced pain/headache.

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Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

Location: WCC Halls A-C

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Support: Merit Review Award # 1 I01 RX003123-01A1, from the United States (U.S.) Department of Veterans Affairs Rehabilitation Research and Development Service (RR&D)
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States (U.S.) Department of Veterans Affairs Rehabilitation Research and Development Service (RR&D)

Title: NaHBED iron chelator therapy improved hallmark traumatic brain injury (TBI) disabilities by reducing hemorrhagic iron toxicity and inflammation in a rodent model of acceleration/deceleration TBI

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Abstract: Acceleration/deceleration TBI causes microvessel shear injury, blood-brain barrier dysfunction, and hemorrhage. Iron deposited by diffuse bleeds fuels inflammation through reactive oxygen species, and other inflammatory pathways may further induce progressive disabilities. There is a growing concern that TBI may significantly elevate risk factors for long-term chronic inflammation-induced progressive diseases. Currently, effective therapies to address these issues are hindered by the insufficient neurobiological foundation to guide the refinement of therapy and prevention strategies for chronic diseases. The current studies tested the therapeutic impact of an iron chelator, NaHBED, on hallmark disabilities in a rodent model of acceleration-deceleration closed head TBI model. Mild/moderate TBI was produced using our previously reported protocol (450 g/1.25m). NaHBED or saline treatment was initiated at both acute and chronic time points (NaHBED, 50 mg/kg/day; saline, equal amount, SQ). Behavioral tests for motor, anxiety, and cognitive functions were conducted at multiple time points during 9 months of post-injury. Clinically relevant MRI (SWI/QSM, T2* map, and DTI), immunohistochemistry (IHC), and histology were performed to chart the time course for iron deposition and inflammation. Our data revealed long-term enduring disabilities in motor/vestibulomotor, anxiety, and cognitive behaviors following TBI, and significant reductions in these disabilities in the NaHBED-treated animals. IHC and histology studies of TBI tissues showed disruption of BBB, patterns of iron deposition, increased expressions of proinflammatory molecules in neuronal cells, and loss of regulatory noradrenergic and BDNF trophic supports in the specific regions essential for the motor, executive, cognitive, and anxiety behaviors. Tissue from the NaHBED-treated animals exhibited robust normalization of each of these markers. Collectively, these studies demonstrate that TBI-induced microhemorrhage contributes to the development and persistence of multiple chronic disabilities. This collective trauma portfolio of chronic disabilities and inflammation was attenuated by NaHBED iron chelator therapy. Taken together, NaHBED iron chelator therapy offers a potential mechanism-based therapy that provides a significant contribution to attenuate long-term TBI disabilities, contributes to trophic support for neuronal and vascular healing, and enhances neuroplasticity for adaptive compensation.

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Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

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Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR407.04/X1

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant NS110609
Research Advisory Committee, Children's Hospital of UPMC

Title: Neurobehavioral and physiological effects of traumatic brain injury in spontaneously hypertensive rats

Authors: M. BOZENKO[1], P. RENNERFELDT[1], E. MOSCHONAS[1], N. RACE[2], J. CHENG[1], A. KLINE[1], *C. BONDI[3];
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Abstract: Approximately 2.8 million people sustain a traumatic brain injury (TBI) yearly, with many experiencing long-term disabilities often exacerbated by pre-existing comorbidities. In the United States, an estimated 45-50% of adults suffer from hypertension, which may lead to blocked or damaged arteries, heart attacks, strokes, and premature death. There is a critical need to investigate TBI in hypertensive rats to better characterize neurological, physiological, and cognitive impairments, and to enhance clinical translatability of the TBI models. This study explores the effects of TBI on Spontaneously Hypertensive Rats (SHR) via a battery of behavioral assays, such as motor coordination/balance, hippocampal-dependent learning, sustained attention, and anxiety-like symptoms. Firstly, a novel pathophysiological study was conducted on SHR rats compared to normotensive Wistar Kyoto (WKY) rats. Adult male rats (17 weeks of age) were assigned to receive a controlled cortical impact (CCI; 2.8mm cortical deformation depth, 4 m/s) or a sham injury. Both sham and TBI rats underwent the Beam Walking Task (motor) as well as the Morris Water Maze (MWM; spatial learning). Open field testing (OFT) was performed to examine anxiety, while Shock Probe Defensive Burying Task (SPDB) inspected passive/active coping behavior. 3-Choice Serial Reaction Time Task (3-CSRT) was employed in a separate cohort of SHR rats to examine sustained attention and distractibility. Before surgery, rats underwent 3-CSRT training for 1-2 months to a 2 s cue in operant chambers. Starting on post-op day 14, rats underwent 10 days of 3-CSRT re-testing. Data were analyzed using ANOVAs followed by Newman Keuls post hoc tests. Adult male SHR TBI rats exhibit 10% higher heart rate and 30% higher mean arterial pressure than injured WKY counterparts. Moreover, injured SHR rats display impaired beam-walking capability, as well as reduced spatial learning compared to SHR shams ($p<0.05$). SHR TBI rats presented more immobility and anxiety-like behavior in comparison to SHR shams, seen as reduced center area exploration in OFT and less time approaching and burying the shock probe in SPDB ($p<0.05$). SHR TBI rats also displayed markedly reduced percent accuracy and increased omissions during 3-CSRT suggesting impairments in sustained attention ($p<0.05$). Results indicate that TBI in rats with a hypertensive phenotype renders neurobehavioral deficits across a variety of behavioral tasks. Understanding the impact that underlying conditions such as hypertension may have on TBI pre-clinically is critical to further developing clinically-relevant therapies

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Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

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Topic: C.10. Brain Injury and Trauma

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Research Advisory Committee, Children's Hospital of Pittsburgh (COB)
UPMC Children's Research Advisory Committee Dissertation Fellowship
Brain Injury Association of America Dissertation Grant (EHM)

Title: Combining a α7 nicotinic acetylcholine receptor allosteric modulator and environmental enrichment improves sustained attention and cholinergic neurotransmission after controlled cortical impact injury in male and female rats

Authors: *E. M. ANNAS[1], E. H. MOSCHONAS[1], P. L. RENNERFELDT[1], T. S. RANELLONE[2], M. A. BERTOCCHI[1], N. S. RACE[1], J. P. CHENG[3], A. E. KLINE[4], C. O. BONDI[1];
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Abstract: Introduction: Traumatic brain injury (TBI) is a leading cause of cognitive impairment. Pharmacological strategies that enhance acetylcholine (ACh) transmission may ameliorate cognitive deficits and the benefit may be greater when combined with a noninvasive rehabilitative strategy, mirroring clinical approaches. Hypothesis: Chronic NS-1738, a novel α7 nicotinic ACh receptor (α7-NAChR) positive allosteric modulator (PAM), will improve sustained attention post-TBI when provided alone and in combination with environmental enrichment (EE). Additionally, blocking α7-NAChRs with methylycaconitine (MLA) will negate the effects of NS-1738, confirming its mechanism of action. Methods: Adult male and female rats were trained in the 3-choice serial reaction time task (3-CSRT) prior to a right parietal controlled cortical impact injury (2.8 mm cortical deformation depth) or sham surgery. Rats were randomized to NS-1738 (5 mg/kg) or vehicle (saline; 1 mL/kg), as well as 6 h of daily EE (or standard housing) for 28d starting on post-injury day 1(PID 1). Male subgroups also received daily α7-NAChR blockade via MLA (3 mg/kg; i.p.). 3-CSRT retrials occurred on PID 14-24. Tissue from the medial prefrontal cortex (mPFC) was utilized for Western blot assessment of the cholinergic markers [acetylcholinesterase (AChE), choline acetyltransferase (ChAT), and α7-NAChR]. Microarray analysis examined inflammatory gene expression. Statistical analysis

utilized ANOVAs and Newman-Keuls post-hoc tests. Results: TBI rats of both sexes exhibited impaired sustained attention ($p<0.05$) and ChAT disruptions in the mPFC and basal forebrain. Sex differences were evident for therapeutic efficacy of treatment paradigms, such that attentional deficits in male rats improved by EE alone or in combination with chronic NS-1738 ($p<0.05$), whereas sustained attention was partially restored in females after NS-1738 treatment alone or when combined with EE ($p<0.05$). Moreover, NS-1738+EE rendered an additive effect on lowering omissions and improving inflammatory markers ($p<0.05$), including TREM-1 (triggering receptor expressed on myeloid cells-1) and IL-1 RA (interleukin-1 receptor antagonist). Moreover, a reinstatement of performance deficits was observed in the male TBI group following MLA. Taken together, the data support the hypothesis. Conclusions and Significance: The findings support the use of α7-NAChR PAM and/or EE treatment after preclinical TBI to benefit sustained attention and cholinergic neurotransmission

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Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR407.06/Web Only

Topic: C.10. Brain Injury and Trauma

Support: FIMSS-CIS IMSS R-2019-785-060
Ana Fernanda Salinas-García was supported by CONACYT (CVU 805864)
Jonathan Zamudio-Flores was supported by CONACYT (CVU 1187161)

Title: Early life stress negatively impacts cognitive performance and increases CA1 microglial activation after a mild traumatic brain injury in adult rats

Authors: *A. SALINAS GARCÍA[1,2], A. ROQUE[3], J. ZAMUDIO FLORES[2,3], E. MELENDEZ HERRERA[4], A. E. KLINE[5,6], N. LAJUD[3];
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Abstract: Early life stress (ELS) affects neurogenesis, cognitive performance, and increases neuroinflammation after a pediatric mild traumatic brain injury (mTBI). Previous studies have

shown that ELS has minimal effects in juveniles, but it shows an age-dependent effect in adults. Hence, we aimed to evaluate if ELS affects cognitive performance, hippocampal microglial activation, and neurogenesis after a mTBI in adult male rats. Maternal separation for 180 min per day (MS180) during the first 21 post-natal (P) days was used as an ELS model, while controls (CONT) remained undisturbed. At P110 the rats were subjected to a mild controlled cortical impact (2.6 mm) or sham injury. Cognition was evaluated in the Morris water maze (MWM) 14 days after injury and both hippocampal microglial activation and neurogenesis were quantified 24 h after the last day of behavioral testing. The results indicate that MS180 + mTBI, but not CONT + mTBI, rats show cognitive deficiencies in the MWM. mTBI equally increased hilus and cortical microglial activation; however, only MS180 + mTBI rats showed an increase in microglial activation in the CA1 hippocampus subfield. ELS and mTBI independently caused a decrease in hippocampal neurogenesis and this effect was not increased further in MS180 + mTBI rats. The findings demonstrate that ELS and mTBI synergistically affect cognitive performance and neuroinflammation, and suggest that ELS may cause an increase on TBI vulnerability that is only evident after mTBI.

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Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

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Topic: C.10. Brain Injury and Trauma

Support: Jonathan Zamudio-Flores was supported by CONACyT (CVU 1187161)
Ana Fernanda Salinas-García was supported by CONACyT (CVU 805864)

Title: Cohabitation in environmental enrichment conditions affects cognitive recovery and microglial activation in rats after pediatric TBI

Authors: *J. ZAMUDIO FLORES[1,2], A. SALINAS GARCÍA[1,2], E. MELÉNDEZ[3], A. E. KLINE[4,5], N. LAJUD[6];
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Abstract: Environmental enrichment (EE), a rodent model of neurorehabilitation promotes cognitive recovery after pediatric traumatic brain injury (TBI). It has been suggested that the gut

microbiota could be involved in TBI pathophysiology. Because rats are coprophagous, we proposed that microbiota transference due to cohabitation with non-injured controls during traditional EE exposure could be related to recovery. Male Sprague-Dawley rats received a controlled cortical impact (2.5 mm tissue deformation at 4 m/s) or sham surgery at PND 21 then randomly assigned to EE or standard (STD) housing conditions with different cohabitation settings and allowed to recover for 14 days. Cognitive performance was evaluated in the Morris water maze (MWM) on post-injury days 14-19. Microglial morphology was evaluated by immunostaining after the behavioral assessments. The TBI+STD rats showed cognitive impairments during the training phase of the MWM. Additionally, the EE groups showed cognitive recovery, but the TBI+EE rats cohabitating with sham controls did not perform as well as the TBI+EE alone group. TBI increased microglial activation in the ipsilateral cortex, the hippocampus CA1, and the hilus subregions. An increase in the percentage of active cells in both the ipsilateral CA1 and cortex was observed in the TBI+EE rats cohabitating with sham controls compared to the TBI+EE group with no sham cohabitants. There was no significant effect in the contralateral regions. These results suggest that there is an effect of cohabitation that could be related to microbiota transference in cognitive recovery after a pediatric TBI and may be explained by the role of microbiota in the immune response.

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Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

Location: WCC Halls A-C

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Program #/Poster #: PSTR407.08/Web Only

Topic: C.10. Brain Injury and Trauma

Title: Environmental enrichment improves cognitive performance in adult female rats experiencing early life stress and a mild traumatic brain injury

Authors: *M. A. ROQUE[1], S. GAONA[1], E. ARREOLA[1], A. E. KLINE[2,3], N. LAJUD[1]; [1]Ctr. de Investigación Biomédica de Michoacán, Morelia, Mexico; [2]Physical Med. & Rehabil., [3]Phys Med. & Rehab, Safar Ctr. Resuscitation Res., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Traumatic brain injury (TBI) induces cognitive and motor deficits and is more common in females with a history of early life stress (ELS). EE has been shown to reverse the effects of ELS on behavior and brain connectivity, however the effects on cognition in female rats with ELS after mild TBI (mTBI) have not been studied. Our aim was to evaluate the effects of EE on cognitive performance in female rats experiencing early stress after mTBI. Maternal separation for 180 min/day (MS180) from postnatal day 1 to 21 was used as the ELS model, while control (CONT) rats remained undisturbed. At P75 the rats were subjected to a mild controlled cortical impact (2.6 mm tissue depth) or sham surgery. Rats were randomly assigned to standard (STD) or EE living conditions. Cognition was evaluated in the Morris water maze

(MWM) beginning 14 days after injury. Our results show that EE improves the acquisition of spatial learning in the MS180+TBI+EE and CONT+TBI+EE groups compared to the CONT+TBI+STD group at PD89 ($p<0.05$, and $p<0.01$, respectively). We observed effects of mTBI in the CONT+TBI+STD and MS+TBI+STD groups compared to the CONT+SHAM+STD group ($p<0.05$, and $p<0.01$, respectively). These results suggest that EE can be a therapeutic strategy to reverse effects on cognitive process after ELS and mTBI in females.

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Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR407.09/X4

Topic: C.10. Brain Injury and Trauma

Title: Early life stress negatively impacts cognition, but not emotionality, after a mild traumatic brain injury in adult female rats.

Authors: *N. LAJUD[1], A. ALMONTE[1], E. VARGAS[1], A. SALINAS-GARCÍA[1], J. ZAMUDIO- FLORES[1], A. ROQUE[1], A. E. KLINE[2], E. MELÉNDEZ[3];
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Abstract: Early life stress negatively impacts cognition, but not emotionality, after a mild traumatic brain injury in adult female rats. Naima Lajud[1], Ana Karen Almonte[1], Emmanuel Vargas[1], Fernanda Salinas-García[1,2], Jonathan Zamudio-Flores[1,2], Angelica Roque[1], Anthony E. Kline[3,4], Esperanza Meléndez-Herrera[5]*[1]División de Neurociencias, Centro de Investigación Biomédica de Michoacán – Instituto Mexicano del Seguro Social, Morelia, Michoacán, México.[2]Maestría en Ciencias en Ecología Integrativa – Instituto de Investigaciones sobre los Recursos Naturales – (UMSNH) [3]Safar Center for Resuscitation Research, University of Pittsburgh, Pitt., PA[4]Physical Medicine & Rehabilitation, University of Pittsburgh, Pitt., PA[5]Instituto de Investigaciones sobre los Recursos Naturales- UMSNH*
Early life stress (ELS) has been shown to have detrimental effects on emotionality, cognitive performance, and neuroinflammation in male rats. However, the impact of ELS on female rats remains a topic of controversy. Previous findings have demonstrated that ELS increases vulnerability to mild traumatic brain injury (mTBI) in males. Therefore, the aim of our study was to assess cognition, emotionality, and neuroinflammation following mTBI in adult female rats. We employed maternal separation for 180 minutes per day (MS180) during the first 21 postnatal (P) days and controls (CONT) as an ELS model. At P75, the rats underwent a mild controlled cortical impact (2.6 mm) to induce mTBI, while the control group received a sham injury.

Cognition was assessed using the Morris water maze (MWM) and the object location test (OLT), and emotionality was evaluated utilizing the open field and forced swimming test. Additionally, microglial morphology was characterized through Iba1 immunostaining. Our results revealed that mTBI caused a mild impairment in spatial learning in the MWM. Notably, this effect was further exacerbated in the MS180 + mTBI group, indicating an enhanced vulnerability in rats that experienced early life stress. In the OLT, only the MS180 + mTBI rats exhibited an inability to discriminate between familiar and relocated objects. Importantly, neither depressive-like behavior nor anxiety-like behavior were affected by any of the treatments. Furthermore, mTBI induced comparable levels of hippocampal neuroinflammation in both the control and MS180 rats. In conclusion, our findings suggest that ELS exacerbates the cognitive deficits associated with mTBI in adult female rats. However, these effects were specific to spatial learning, as depressive-like behavior, anxiety-like behavior, and neuroinflammation were not significantly influenced by either ELS or mTBI.

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Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

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Program #/Poster #: PSTR407.10/X5

Topic: C.10. Brain Injury and Trauma

Support: MHC-2023-02-02-02
6S210201602S000100

Title: Administration of amlexanox reduces neuroinflammation and neuronal death after pilocarpine-induced seizure

Authors: *H. YANG[1], S. LEE[1], B. KANG[1], M. PARK[1], C. LEE[1], S. WOO[1], S. PARK[1], D. KIM[1], S. LEE[2], H. SONG[4], H. CHOI[3], S. SUH[1];
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Abstract: Epilepsy is a neurological disorder characterized by recurrent seizures resulting from abnormal electrical activity in the brain. In addition to genetic factors, specific traumatic events or brain injuries can also trigger epileptic seizures. Understanding the mechanisms underlying epilepsy is crucial for developing effective treatments.One important aspect of epilepsy is the role of lysosomal dysfunction in the pathogenesis of the disease. Lysosomes are cellular organelles responsible for the breakdown and recycling of various substances within cells. Impaired lysosomal function can lead to the accumulation of toxic materials, contributing to neuronal damage and cell death.In this study, we focused on the enzyme phosphodiesterase-4

(PDE4) and its involvement in lysosomal function and neuroinflammation. PDE4 regulates cyclic adenosine monophosphate (cAMP) levels by breaking it down into adenosine monophosphate (AMP). Increased PDE4 activity can result in reduced cAMP levels, affecting lysosomal function and promoting neuroinflammation.To investigate the potential therapeutic effects, we used amlexanox, a non-selective PDE4 inhibitor with known anti-inflammatory properties. We utilized a pilocarpine-induced epilepsy animal model and administered amlexanox following seizures. Brain tissue samples were collected at different time points for analysis.The results demonstrated that amlexanox effectively improved lysosomal function, reduced inflammation, attenuated hippocampal neuronal death, and improved cognitive impairment associated with epilepsy. These findings suggest that amlexanox may hold promise as a therapeutic agent for the treatment of epileptic brain disorders. Further research is needed to fully elucidate the underlying molecular mechanisms by which amlexanox exerts its neuroprotective effects. Understanding these mechanisms will help in optimizing treatment strategies and developing targeted therapies for epilepsy and other brain disorders associated with lysosomal dysfunction and neuroinflammation. **Keywords:** Epilepsy, cAMP, Phosphodiesterase4, Protein Kinase A, Lysosome, Autophagy, Neuro-inflammation

Disclosures: **H. Yang:** None. **S. Lee:** None. **B. Kang:** None. **M. Park:** None. **C. Lee:** None. **S. Woo:** None. **S. Park:** None. **D. Kim:** None. **S. Lee:** None. **H. Song:** None. **H. Choi:** None. **S. Suh:** None.

Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR407.11/X6

Topic: C.10. Brain Injury and Trauma

Support: NRF-2021R1A6A3A01087054
MHC-2023-02-02-02
6S210201602S000100

Title: An acid sphingomyelinase inhibitor, imipramine, enhances the survival of newborn neurons after pilocarpine-induced seizure

Authors: *S. LEE[1], B. KANG[1], M. PARK[1], H. YANG[1], C. LEE[1], S. WOO[1], S. PARK[1], D. KIM[1], S. LEE[2], H. SONG[3], H. CHOI[4], S. SUH[1];
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Abstract: Epilepsy is a progressive neurological disorder that can be triggered by factors such as traumatic brain injury (TBI) and abnormal neuronal activity in the brain. Acid sphingomyelinase (ASMase) is an enzyme involved in the breakdown of sphingomyelin, leading to the production

of ceramides. Excessive ceramide production has been implicated in various diseases, including cancer, cystic fibrosis, diabetes, Alzheimer's, and depression. Ceramide acts as a pro-apoptotic intracellular messenger and can promote the generation of reactive oxygen species (ROS), inflammation, and lysosomal damage. In this study, we aimed to investigate the role of ASMase and ceramide generation in epilepsy. We hypothesized that seizures induce ASMase activation, resulting in ceramide production, DNA damage, and neuronal apoptosis. To test this hypothesis, a pilocarpine-induced seizure model was used in rats. Imipramine, an inhibitor of ASMase, was administered continuously at a dose of 10 mg/kg via intraperitoneal injection for four weeks following the seizure induction. Histological analyses and cognitive function assessments were performed four weeks after the seizure. The results of the study revealed that post-seizure treatment with imipramine reduced markers of neuronal apoptosis and increased the number of newly generated neurons in the dentate gyrus of the hippocampus. Furthermore, imipramine treatment prevented seizure-induced cognitive impairment. These findings suggest that imipramine, through its inhibition of ASMase, may offer a promising therapeutic approach for enhancing the survival of newly generated neurons and improving cognitive function following seizures. By reducing ceramide production and apoptotic signaling, imipramine may mitigate neuronal cell death. Further research is needed to elucidate the precise molecular mechanisms underlying the effects of imipramine and to evaluate its potential clinical applications in the treatment of epilepsy-associated neuronal damage and cognitive dysfunction. **Keywords:** Epilepsy, Imipramine, Ceramide, Acid sphingomyelinase, Neuron death, Cell survival

Disclosures: **S. Lee:** None. **B. Kang:** None. **M. Park:** None. **H. Yang:** None. **C. Lee:** None. **S. Woo:** None. **S. Park:** None. **D. Kim:** None. **S. Lee:** None. **H. Song:** None. **H. Choi:** None. **S. Suh:** None.

Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

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Program #/Poster #: PSTR407.12/X7

Topic: C.10. Brain Injury and Trauma

Support: MHC-2023-02-02-02
6S210201602S000100

Title: L-theanine, an inhibitor of AMPA receptors, prevents traumatic brain injury-induced neuronal death

Authors: ***M. PARK**[1], S. LEE[1], B. KANG[1], C. LEE[1], H. YANG[1], S. WOO[1], S. PARK[1], D. KIM[1], H. CHOI[2], S.-K. LEE[3], S. SUH[4];
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Abstract: Traumatic brain injury (TBI) is a complex and debilitating condition characterized by brain damage and functional deficits. One of the key mechanisms contributing to TBI pathology is the excessive activation of glutamate receptors, particularly AMPA receptors. This overactivation leads to an influx of calcium and zinc ions into neurons, resulting in neuronal injury and oxidative stress. L-theanine, a bioactive compound found in tea leaves, has been shown to possess inhibitory effects on AMPA receptors, as well as antioxidant properties. In this study, we sought to investigate the potential neuroprotective effects of L-theanine in the context of TBI-induced hippocampal damage. Experimental rats were administered L-theanine at a dose of 200mg/kg immediately following the induction of TBI. After a 24-hour period, we performed histological analyses to assess various aspects of neuronal damage, including neuronal death, oxidative damage, microglial activation, and astrocyte activation, utilizing specific staining techniques. The findings of our study demonstrated that treatment with L-theanine effectively reduced neuronal death and mitigated cognitive impairments associated with TBI. These positive outcomes were attributed to the ability of L-theanine to suppress the excessive activation of AMPA receptors and enhance the production of glutathione, a critical antioxidant molecule. By modulating these pathways, L-theanine exhibited promising neuroprotective properties in the context of TBI-induced hippocampal damage. Further investigation is warranted to elucidate the underlying mechanisms through which L-theanine exerts its neuroprotective effects, as well as to explore its potential clinical applications in the management of TBI. The results of this study highlight L-theanine as a potential therapeutic intervention for mitigating neuronal damage and improving outcomes in individuals with TBI.

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Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

Location: WCC Halls A-C

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Program #/Poster #: PSTR407.13/X8

Topic: C.10. Brain Injury and Trauma

Support: MHC-2023-02-02-02
7S220401236S000100
6S210201602S000100

Title: Role of pyruvate kinase m2 (PKM2) on traumatic brain injury-induced neuronal death

Authors: *B. KANG[1], S. LEE[1], M. PARK[1], C. LEE[1], H. YANG[1], S. WOO[1], S. PARK[1], D. KIM[1], S. LEE[2], M. SOHN[4], S. SUH[3];
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Abstract: Traumatic brain injury (TBI) is a severe condition caused by physical force trauma to the head, resulting in primary brain edema, hemorrhage, and swelling, as well as secondary injuries involving oxidative damage, neuroinflammation, and mitochondrial dysfunction. Astrocytes, a type of brain cell, play a crucial role in the astrocyte-neuron lactate shuttle (ANLS), which transfers lactate as an energy source from astrocytes to neurons. This study aimed to investigate the role of the pyruvate kinase m2 (PKM2) gene in astrocytes and its impact on neuronal survival following TBI. We hypothesized that deleting the PKM2 gene in astrocytes would lead to increased neuronal death due to the lack of lactate supply through ANLS. Additionally, we hypothesized that administering lactate after TBI would mitigate neuronal death, improve cognitive impairment, and promote neurogenesis. To test these hypotheses, we used tamoxifen to specifically delete the PKM2 gene in astrocytes of Aldh1l1-CreERT2; PKM2f/f mice. After inducing TBI in these mice, we immediately administered sodium L-lactate and sacrificed the animals 24 hours later. The analysis included evaluating neuronal death, oxidative damage, microtubule disruption, and the activity of enzymes related to ANLS. The results of the study confirmed that deleting the PKM2 gene in astrocytes increased neuronal death. However, administering lactate after TBI reduced neuronal death, improved cognitive impairment, and promoted neurogenesis. These findings suggest that lactate administration could serve as a potential therapeutic intervention for the treatment and prevention of neurological damage following TBI.

Disclosures: B. Kang: None. **S. Lee:** None. **M. Park:** None. **C. Lee:** None. **H. Yang:** None. **S. Woo:** None. **S. Park:** None. **D. Kim:** None. **S. Lee:** None. **M. Sohn:** None. **S. Suh:** None.

Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR407.14/X9

Topic: C.10. Brain Injury and Trauma

Support: T.MRICD.2021.01- Treatment Interventions for Brain and Complex Injury, CCC-65

Title: Adjunct treatments positively impact mortality and recovery in a mouse traumatic brain injury and nerve agent exposure polytrauma model.

Authors: *J. LEIGHTON, E. JOHNSON, J. SAHARGUN, E. MILLER, J. JANSSEN, A. METHVIN;
Med. Toxicology, USAMRICD, Aberdeen Proving Ground, MD

Abstract: Polytrauma with traumatic brain injury (TBI) can complicate treatment with well-established pharmaceutical countermeasures in a military setting. Previously, our group established a TBI/nerve agent (NA) polytrauma mouse model that showed an increase in mortality, symptom incidence and severity, and impeded recovery compared to the individual

injuries even when standard countermeasures are utilized. This study used a TBI/NA polytrauma model with a unique human acetylcholinesterase knock-in/serum carboxylesterase knockout (C57BL/6-Ces1ctm1.1LocAChEtm1.1Loc/J; a.k.a KIKO) mouse strain to investigate the use of readily available pharmaceuticals as adjuncts to standard countermeasures to improve treatment of concurrent injuries. To produce polytrauma, male mice received a TBI and its countermeasures followed by NA exposure and its countermeasures. Mice were then administered either ketamine, scopolamine, hydromorphone, levetiracetam, diphenhydramine, promethazine, or hypertonic saline as an adjunct. Throughout and up to 72 hrs post-exposure, mice were monitored for physiological and behavioral changes, including but not limited to EEG signals, burrowing behavior, and nesting behavior. Results of this study indicate that several adjuncts have positive impacts on mortality following TBI/NA polytrauma. In addition, most adjuncts allowed for return to normal nesting and burrowing behavior, though changes in behavior associated with severe NA exposure symptoms were not entirely alleviated. These results provide plausible adjunct options to aid the treatment of TBI/NA polytrauma injuries alongside current countermeasures.

The views expressed in this abstract are those of the author(s) and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government. The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense, and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. These studies were funded by the Combat Casualty Care Research Program (CCCRP). J. Leighton, E. Miller, J. Janssen, & J. Sahargun were supported in whole or in part by an appointment to the Research Participation Program for the U.S. Army Medical Research and Development Command administered by the Oak Ridge Institute for Science Education (ORISE) through an agreement between the U.S. Department of Energy and U.S. Army Medical Research and Development Command.

Disclosures: **J. Leighton:** 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.; ORISE, Combat Casualty Care Research Program. **E. Johnson:** 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.; Combat Casualty Care Research Program. **J. Sahargun:** 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.; ORISE, Combat Casualty Care Research Program. **E. Miller:** 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.; ORISE, Combat Casualty Care Research Program. **J. Janssen:** 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.; ORISE, Combat Casualty Care Research Program. **A. Methvin:** 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.; Combat Casualty Care Research Program.

Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR407.15/X10

Topic: C.10. Brain Injury and Trauma

Support: T.MRICD.2021.01- Treatment Interventions for Brain and Complex Injury, CCC-65

Title: Methodology of a novel subcutaneous EEG implantation and open craniotomy surgery for a novel traumatic brain injury and chemical exposure polytrauma mouse model

Authors: *J. G. JANSSEN, J. A. LEIGHTON, J. C. SAHARGUN, E. Z. MILLER, A. METHVIN, E. JOHNSON;
Med. Toxicology, USAMRICD, Gunpowder, MD

Abstract: Pharmaceutical treatments for traumatic brain injury (TBI) and chemical exposure polytraumas are relatively unexplored. To this end, a novel polytrauma mouse model was developed to investigate treatments for concurrent TBI and chemical exposures. We created a surgical technique with two main objectives: inserting subcutaneous EEG implant leads into the skull to observe and record seizure activity and introducing an open craniotomy for post-operative TBI administration. Although wireless EEG transmitter and open craniotomy surgeries are common, this study developed a technique where both surgeries are done in tandem and kept viable for multiple weeks. We determined that a two-week healing period was required in this polytrauma model after shorter recovery windows triggered oversensitivity to chemical exposures, a potential impediment for seizure progression and survival. Methods detailing the placement of EEG burr holes, surgical implantation of a DSI ETA-F10 transponder with two leads secured to the skull with dental acrylic, and drilling of an open craniotomy for TBI in the skull are discussed. Post-operative care procedures, including pain management, fluid support, and recovery assessments, are also discussed. We used 5 physical and behavioral metrics as a measure of post-operative recovery and determined that pre- and post-operative weight metrics are useful for predicting a successful surgical outcome.
 The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011), and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. These studies were funded by the Combat Casualty Care Research Program (CCCRP). J. Janssen, J. Sahargun, J. Leighton and E. Miller were supported in whole or in part by an appointment to the Research Participation Program for the U.S. Army Medical Research and Materiel Command administered by the Oak Ridge Institute for Science Education (ORISE)

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Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR407.16/X11

Topic: C.10. Brain Injury and Trauma

Support: T.MRICD.2021.01- Treatment Interventions for Brain and Complex Injury, CCC-65

Title: Utilizing cardiopulmonary metrics to determine adjunct treatment efficacy in a traumatic brain injury and nerve agent exposure polytrauma mouse model

Authors: *J. SAHARGUN, J. LEIGHTON, E. MILLER, J. JANSSEN, A. METHVIN, E. JOHNSON;
Med. Toxicology Neurosci., USAMRICD, Gunpowder, MD

Abstract: Combat-related traumatic brain injury (TBI) may be accompanied by other injuries, such as nerve agent (NA) exposure, resulting in a polytrauma scenario. Pulse oximetry is a direct, combat medic-relevant diagnostic for assessing both TBI and NA exposure as each injury

initiates unique cardiopulmonary changes. Previously, our group created a TBI/NA polytrauma model using a transgenic mouse model with human acetylcholinesterase knock-in/serum carboxylesterase knockout (C57BL/6-Ces1ctm1.1LocAChEtm1.1Loc/J; a.k.a KIKO) that assessed cardiopulmonary outcomes following standard TBI/NA treatment strategies in a prolonged field care setting. This study expands on these findings by adding potential adjunct treatments that are readily available to medical providers to improve mortality and recovery. Heart rate, breath rate, and oxygen saturation (SPO_2) were recorded following polytrauma using a mouse pulse oximeter to assess the impact of adjunct treatments. Heart rate and SPO_2 changes were leading indicators of mortality, while breath rate increased in response to SPO_2 decline. In most outcomes, cardiopulmonary activity mostly recovered, but recovery was not observed in seizure outcome groups. When adjunct treatments were administered, polytrauma mortality rates decreased compared to controls, and cardiopulmonary metrics were consequently affected. This confirms the importance of pulse oximetry as a reliable diagnostic for combat medics based on the consistency of cardiopulmonary metrics at predicting mortality in a polytrauma model.
The views expressed in this poster are those of the author(s) and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government. The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense, and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. These studies were funded by the Combat Casualty Care Research Program (CCCRP). J. Sahargun, J. Janssen, J. Leighton and E. Miller were supported in whole or in part by an appointment to the Research Participation Program for the U.S. Army Medical Research and Materiel Command administered by the Oak Ridge Institute for Science Education (ORISE) through an agreement between the U.S. Department of Energy and U.S. Army Medical Research and Development Command.

Disclosures: **J. Sahargun:** A. Employment/Salary (full or part-time):; ORISE. 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.; Combat Casualty Care Research Program. **J. Leighton:** 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.; ORISE and Combat Casualty Care Research Program. **E. Miller:** 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.; ORISE and Combat Casualty Care Research Program. **J. Janssen:** 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.; ORISE and Combat Casualty Care Research Program. **A. Methvin:** 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.; Combat Casualty Care Research Program. **E. Johnson:** 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.; Combat Casualty Care Research Program.

Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

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Topic: C.10. Brain Injury and Trauma

Support: Combat Casualty Care Research Program (CCCRP) T.MRICD.2021.01
Treatment Interventions for Brain and Complex Injury, CCC-65

Title: Risk Benefit of Aggressive Intervention in a Nerve Agent and Traumatic Brain Injury Based Polytrauma: Electroencephalogram Analysis of Potential Adjunctive Treatments

Authors: *A. METHVIN[1], J. LEIGHTON[1], J. SAHARGUN[1], E. MILLER[2], J. JANSSEN[1], E. JOHNSON[1];
[1]USAMRICD, Gunpowder, MD; [2]USA Med. Res. Inst. of Chem. Def., Gunpowder, MD

Abstract: The effect of polytrauma on the brain and potential treatment interaction is a topic of increasing interest as the medical field continues to widen its lens for approaches to effective medical care. Current paradigms are established for treatment of nerve agent (NA) exposure and of traumatic brain injury (TBI) separately, though little had been done to assess a combined treatment regimen and potential adjuncts. We previously developed and characterized a polytrauma model in a human acetylcholinesterase knock-in/serum carboxylesterase knockout (C57BL/6-Ces1ctm1.1LocAChEtm1.1Loc/J; 15-20 wks) mouse strain which combines these injuries and allows monitoring of several physiological and behavioral responses after challenge. Electroencephalogram (EEG) analysis showed an exacerbated severity of seizure status following NA exposure in animals given a TBI, as shown by increased frequency and amplitude of spikes, longer duration of status epilepticus (SE), and prolonged bursting/refractory status in the 72 hours following the polytrauma. The synergistic nature of these injuries resulted in outcomes that, while compatible with all standard treatments, is not as responsive to the current standard of care as either individual injury. Potential adjuncts (ketamine, scopolamine, hydromorphone, levetiracetam, diphenhydramine, promethazine, hypertonic saline) are evaluated here for efficacy at reducing morbidity and mortality when given alongside current treatment. Results indicate that while most adjuncts were effective in improving seizure termination, a few also resulted in kindling and potentiation of near-seizure status in mice whose response to the polytrauma was not initially as severe. The views expressed in this poster are those of the author(s) and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government. The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. These studies were funded by the Combat Casualty Care Research Program (CCCRP). J. Sahargun, J. Leighton, J. Janssen, and E. Miller were supported in whole or in part by an appointment to the Research Participation Program for the U.S. Army Medical Research and

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Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

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Program #/Poster #: PSTR407.18/X13

Topic: C.10. Brain Injury and Trauma

Support: R01NS117598
Vaccine and Immunotherapy Center Education Fund
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Title: Immunomodulatory B Lymphocytes Promote a Neuroprotective Microenvironment After Traumatic Brain Injury Through Reciprocal Interaction with Infiltrating Peripheral Myeloid Cells

Authors: L. J. DWYER[1], S. MAHESHWARI[1], E. LEVY[1], M. C. POZNANSKY[1], M. J. WHALEN[1], ***R. F. SIRBULESCU**[2];
[1]Massachusetts Gen. Hosp., Boston, MA; [2]Neurol., Massachusetts Gen. Hospital, Harvard Med. Sch., Charlestown, MA

Abstract: Traumatic brain injury (TBI) remains a major cause of death and severe disability worldwide. Our work has demonstrated previously that exogenous mature naïve B220+/CD19+/IgM+/IgD+ B cells are potent modulators of inflammatory responses, and that therapeutic administration of B cells is associated with structural and functional neuroprotection after TBI. A single intraparenchymal injection of B cells at the time of injury significantly improved cognitive recovery after contusion TBI in multiple neurobehavioral paradigms, and reduced brain tissue loss by 40-60% as compared to injured groups treated with saline or equivalent numbers of splenic T cells. Naïve B cells placed in injured microenvironments become activated via Toll-like receptor (TLR) - MyD88-dependent signaling pathways and generate a variety of immunoregulatory cytokines. Here, we use a model of unilateral controlled cortical impact TBI in adult male mice to investigate cellular mechanisms of immunomodulation. Exogenous B cells show a complex time-dependent response in the injury microenvironment, including increased expression of IL-10, IL-35, and TGFβ, but also IL-2, IL-6, and TNFα. After 10 days *in situ*, B cell subsets expressing IL-10 or TGFβ dominate. In the presence of B cells, significantly more of the myeloid cells infiltrating at the injury site produced IL-10, TGFβ, and IL-35, and fewer produced TNFα, interferon-γ and IL-6 as compared to controls, up to 2 months post-TBI. B cell treatment significantly increased the proportion of

$CD206^+$ infiltrating monocytes/macrophages and reduced the relative proportion of activated microglia starting at 4 days and up to 2 months post-injury. Ablation of peripheral monocytes with clodronate liposomes showed that infiltrating peripheral monocytes/macrophages are required for inducing a regulatory phenotype in exogenous B cells. Reciprocally, B cells specifically reduced the expression of inflammatory cytokines in infiltrating $Ly6C^+$ monocytes/macrophages. We thus find evidence that infiltrating peripheral monocytes/macrophages are required for the regulatory activation of the transplanted B cells, and are in turn subject to the modulatory influence of the exogenous B cells, mediating their functional neuroprotective effects.

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Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

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Program #/Poster #: PSTR407.19/X14

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R01NS115815-03
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R21NS131689-01
Barrow Neurological Foundation
Chuck Noll Foundation for Brain Injury Research
Translational Neuroscience Trainee Fast Track grant 2021, from Barrow Neurological Institute
Translational Neuroscience Trainee Fast Track grant 2022, from Barrow Neurological Institute

Title: Glibenclamide Treatment Decreases Markers of Neurodegeneration and Increases Neurogenesis With Improved Cognitive Outcome Across Both Sexes After Murine Traumatic Brain Injury

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Abstract: Traumatic brain injury (TBI) is a major cause of mortality and morbidity worldwide and a significant risk factor for accelerated cognitive memory loss. Sexual dimorphism in cognitive dysfunction and recovery post-TBI are poorly understood. Treatment for secondary injury post-TBI is limited with no currently approved medications that improve functional outcome. We evaluated efficacy of Glibenclamide (GLI) in modulating TBI-induced cognitive dysfunction by sex. GLI is a promising drug that has shown promise in early clinical trials in stroke and contusional-TBI. Adult C57BL/6J mice were randomized into (n=6/group); Naive, TBI, TBI+Vehicle, TBI+GLI. CCI-TBI was induced :velocity=5.0m/s, depth=1.2mm, dwell time=50ms. GLI was injected post-TBI followed by 7D maintenance infusion. Cognitive function was analyzed by Morris water maze test (MWM). Immunofluorescence was performed at D21 post-TBI to study effects of GLI on neurogenesis and neurodegeneration post-TBI. We used markers of neurogenesis doublecortin (DCX), Sox2, Ki67, in the subgranular zone (SGZ) of dentate gyrus (DG) and subventricular zone (SVZ), neurodegeneration TDP43 and Tau. In females Sox2 and DCX expression increased post-TBI which was enhanced with GLI and expression of Ki67 in the SGZ and SVZ decreased post-TBI which increased with GLI ($p<0.001$). In males, similar expression pattern was observed for DCX and Sox2, Ki67 increased primarily in the contralateral SGZ. TDP43 and Tau expression was increased in both sexes in ipsilateral cortex, DG and thalamus which was restored after GLI treatment ($p<0.001$). Regional and sex based differences were observed in both neurogenesis and neurodegenerative markers after GLI treatment. Neurogenesis markers were increased with GLI in the SGZ in males and SVZ in females ($p<0.001$). In males, GLI had a more pronounced impact on decreasing TDP43 expression in the ipsilateral cortex versus females ($p<0.001$). Decreased expression of Tau after GLI was similar across sex in the ipsilateral cortex, and thalamus but more pronounced in DG in females ($p<0.001$). MWM results showed decrease in cognitive memory post-TBI which was restored with GLI treatment in both sexes ($p<0.001$), with more pronounced benefit in females. Our data suggest that GLI enhances markers of neurogenesis and decreases neurodegeneration in nuanced region and sex based manner. Nonetheless, based on current outcomes, GLI improves cognitive outcome in both sexes although there are sex-based differences in post-TBI deficits. Collectively, our data supports the therapeutic potential of GLI post-TBI and the critical need for rigorous and thoughtful study in large randomized human trials.

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Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR407.20/X15

Topic: C.10. Brain Injury and Trauma

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Title: Disodium Succinate as a Novel Therapeutic Agent for Secondary Injuries after Traumatic Brain Injury.

Authors: *C. A. HALL, K. BARANES, M. R. KOTTER, K. L. H. CARPENTER, P. J. A. HUTCHINSON;
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Abstract: Following traumatic brain injury, several downstream cascades are initiated, including deranged cerebral metabolism and inflammation, contributing towards secondary injury. It has been shown that unfavourable patient outcome correlates with a high brain extracellular lactate/pyruvate ratio (LPR). Initial in-vitro research using rat mixed glia showed that succinate, an intermediate of the tricarboxylic acid cycle, protected against rotenone-induced metabolic dysfunction. Rotenone acts as an inhibitor of complex I of the mitochondrial electron transport chain (ETC), with succinate interacting with complex II, thus bypassing the induced injury mechanism. We have subsequently studied the effect of rotenone-induced metabolic dysfunction in both human induced neurons (iNs) and human induced astrocytes (iAs), as a model for TBI secondary injuries, further investigating the possible neurotherapeutic effects on these cell types. Using the novel cellular reprogramming technique Optimised Inducible Overexpression, we produced iNs and iAs through transcription factor overexpression. These cultures were then treated with varying concentrations of rotenone and disodium succinate. At select time points

(12hrs-48hrs), the cellular metabolism and LPR were assessed using an ISCUSflex analyser, and the cell viability was determined using selected cellular stains (Hoechst 33342 and propidium iodide). Furthermore, extracellular acidification rate and oxygen consumption rate have also been recorded. Whilst the iNs responded to treatment of both rotenone and succinate, they did not do so consistently. iAs, however, proved to behave more consistently in the testing regime and produced results suggesting metabolic rescue, such that LPR was decreased, and cell viability increased following treatment, compared with non-succinate control cells. Co-culturing experiments have also been performed to investigate a mechanism whereby the astrocytes may provide a supportive or protective role to the neurons. Furthermore, TBI has been linked to later Alzheimer's disease (AD) development, therefore, using a patient-derived AD cell line, we are investigating the link between succinate treatment and AD development.

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Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR407.21/X16

Topic: C.10. Brain Injury and Trauma

Support: Shanghai basic research field Project (18JC1410100)
Shanghai Municipal Science and Technology Major Project (2018SHZDZX05)
The National Key Research and Development Program of China (2020YFA0112700)
National Natural Science Foundation of China (31871035)

Title: Integrated landscape of tectal glial heterogeneity and TBI responses across life stages in zebrafish

Authors: *H. QIN, J. HE;
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Abstract: Glial populations are highly diverse and exhibit complex traumatic brain injury (TBI) responses. However, the temporal heterogeneity of glial cells in the traumatic injured vertebrate brain remains elusive. Here we obtained single-cell transcriptomes of 99,456 cells enriched with three major glial types from injured and uninjured zebrafish optic tectum across post-embryonic developmental stages spanning from larval to adult. Across stages, we identified five microglia subtypes, each exhibiting specific stage distribution pattern. Notably, the injury evoked the stage-dependent emergence of three transient MG substates representing different injury responses. For oligodendrocyte lineages, we identified six clusters, which responded to the injury by triggering the embryonic developmental program at larval stages but by inducing multiple

transient oligodendrocyte-lineage substates beyond larval stages. For radial astrocytes (RA), we identified six subtypes with distinct spatial temporal characteristics. A temporal loss of Notch signaling accounted for the TBI-induced RA proliferation beyond the early larval stage. Thus, our study systematically uncovered the temporal heterogeneity of glial cells under physiological and TBI conditions across life stages and laid the framework of vertebrate glial responding to traumatic injuries.

Disclosures: **H. Qin:** None. **J. He:** None.

Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

Location: WCC Halls A-C

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Program #/Poster #: PSTR407.22/X17

Topic: C.10. Brain Injury and Trauma

Support: NIH 1R21NS097899

Title: Adenosine A1 receptor system is impaired in cortical organoids derived from patients with chronic mountain sickness

Authors: *H. YAO, J. WANG, H. ZHAO, G. G. HADDAD;
UCSD, La Jolla, CA

Abstract: Chronic mountain sickness (CMS) is manifested by neurological symptoms such as migraine headache, dizziness, and cognitive deficits. The underlying pathological mechanism has not been well understood. A recent functional MRI study has demonstrated an increased brain activity in CMS patients compared with their control counterparts, which is consistent with our previous findings showing the elevated neuronal network activity in CMS neurons under hypoxia (Yao, et al., SFN Abstract, 2018). Previous work has shown that hypoxia leads to increased adenosine secretion at synapses which can silence the synaptic transmission by preventing excessive glutamate release during hypoxia and hence protect the brain from hypoxia-induced excitotoxic damage. In this work, we examined if the adenosine A1 receptor system is altered in the CMS neuronal cultures. Skin biopsies were obtained from both CMS patients and healthy highlanders (non-CMS) who live in the Peruvian Andes (~14000 ft). Fibroblasts were grown and reprogramed into induced pluripotent stem cells (iPSCs) which then were differentiated into neurons in a 3D cortical organoid culturing system (human cortical organoid, hCO). Spontaneous activity was recorded extracellularly from hCOs using a multi-electrode array system (MEA, Axion Biosystems) and the firing rate (FR) was used to reflect the neural network activitiy. Our results show that pharmacological inhibition of adenosine A1 receptor with DPCPX increased FR in non-CMS neurons (increase by 146.0±10.5% of control, n=3, p=0.038) while its agonist CPA decreased FR (decrease by 78.2%±4.0% of control, p=0.030), suggesting the existence of adenosine A1 receptor-regulated presynaptic muting system in hCOs. Further, this CPA-induced decrease in FR in non-CMS disappeared in the hypoxia group (change

by 94.1±3.6% of control, p=0.363) although DPCPX still robustly enhanced FR under hypoxia (increase by 150.0±12.2% of control, p=0.046), probably due to the endogenous secretion of adenosine stimulated by hypoxia. In the CMS group however, both CPA and DPCPX did not affect the FR under either normoxia (CPA group: change by 108.5±19.5% of control, p>0.05; DPCPX group: change by 108.6±10.3% of control, p>0.05) or hypoxia (CPA group: change by 83.3±6.5% of control, p>0.05; DPCPX group: change by 83.3±9.2% of control, p>0.05). We conclude that adenosine A1 receptor may mediate presynaptic silencing under hypoxia in non-CMS organoids and this neuroprotection mechanism may be impaired in the CMS neurons which renders excessive network activity in the diseased neurons.

Disclosures: H. Yao: None. **J. Wang:** None. **H. Zhao:** None. **G.G. Haddad:** None.

Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

Location: WCC Halls A-C

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Program #/Poster #: PSTR407.23/X18

Topic: C.10. Brain Injury and Trauma

Support: NIH NS 111378
NIH NS 117148
NIH NS 116838

Title: The BDNF mimetic R-13 and exercise attenuate TBI pathogenesis

Authors: *Z. YING[1], P. THAPAK[1], G. SMITH[5], A. PAYDAR[6], N. G. HARRIS[2], F. GOMEZ-PINILLA[3,4];
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Abstract: Background: Traumatic brain injury (TBI) is a global neurological burden that leads to cognitive decline and escalates to psychiatric disorders. The R13 is a TrkB agonist molecule much smaller and more effective than BDNF with great therapeutic potential, which capacity has not been shown in the TBI pathology (Thapak et al, Biochim Biophys Acta Mol Basis Dis. (2023), 1869 (7), 166781).**Methods:** Sprague Dawley rats received moderate lateral fluid percussion injury (FPI). R13 (7.25 mg/kg, i.p) and vehicle were administered at 7 days post-FPI for 7 consecutive days to rats that either had access to voluntary running wheel or were sedentary. Memory and anxiety-like behaviors were assessed two-week post-TBI. Magnetic resonance imaging (MRI) was performed on post-TBI days 1 and 7 in rats receiving R13 or vehicle intervention. Protein levels were measured in the ipsilateral to the injury hippocampus using quantitative western blots.**Results:** Animals exposed to FPI showed a reduction in spatial memory and anxiety-like behavior at 2 weeks post-TBI which was counteracted by R13 and

exercise. Injured animals showed a reduction in the p-TrkB protein level in the hippocampus at 7 days post-TBI which was counteracted by R13 intervention. MRI scans revealed distinct connectivity variations between injured rats treated with R13 and the vehicle group, determined by analysing functional connectivity (FC) at 1 and 7 days post-injury. R13 treatment significantly increased FC throughout the cortex compared to the vehicle treatment.**Conclusion:** This study showed that delayed administration of R13 or exercise counteracted cognitive deficits post-TBI and increased FC. Overall, these findings delineate the neuroprotective potential of R13 against TBI.

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Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

Location: WCC Halls A-C

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Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R01NS076815
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Title: Degradation of endocannabinoid in astrocytes promotes traumatic brain injury

Authors: *M. HU[1], D. ZHU[1], J. ZHANG[1], F. GAO[1], J. HASHEM[1], C. CHEN[1], P. J. KINGSLEY[2], L. J. MARNETT[2], K. MACKIE[3];
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Abstract: Traumatic brain injury (TBI) is an important risk factor for development of Alzheimer's disease (AD) and dementia. Previous studies show that administration of the endocannabinoid 2-arachidonoylglycerol (2-AG) or inhibition of 2-AG degradation by pharmacological inactivation of monoacylglycerol lipase (MAGL), a key enzyme hydrolyzing 2-AG, attenuates TBI-induced neuropathology. However, the mechanism of the neuroprotective effects produced by inactivation of MAGL in TBI remains unclear. In particular, little is known about whether genetic inactivation of MAGL produces neuroprotection against TBI and whether the protective effects of 2-AG signaling are cell type-specific. In the present study, we provide evidence that genetic inactivation of MAGL reduces neuropathology and averts synaptic and cognitive declines in mice exposed to repetitive mild closed head injury. Importantly, our study reveals that these neuroprotective effects primarily result from inactivation of MAGL in astrocytes, rather than in neurons. Single-cell transcriptomic analysis shows that mice lacking MAGL in astrocytes exhibit great resilience to TBI-induced upregulation of genes involved in inflammation and downregulation of genes associated with anti-inflammatory responses or

associated with maintenance of brain homeostasis in astrocytes and microglia. The MAGL inactivation-produced neuroprotection is apparently mediated via CB1R as pharmacological inactivation of MAGL failed to produce neuroprotection against TBI in CB1R knockout mice. In addition, our results indicate that peroxisome proliferator-activated receptor-γ (PPARγ) is a downstream signaling molecule of 2-AG and CB1R as silencing of PPARγ in astrocytes eliminates the protective effects against TBI in mice lacking MAGL in astrocytes and overexpression of human PPARγ in astrocytes prevents TBI-induced neuropathological changes and cognitive decline in wild-type mice. Our results reveal a previously undefined cell type-specific role of 2-AG signaling in alleviation of TBI-induced neuropathology and synaptic and cognitive deficits, suggesting that astrocytic MAGL is a promising therapeutic target for TBI-induced AD-like neurodegenerative disease.

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Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR407.25/X20

Topic: C.10. Brain Injury and Trauma

Support: NS111378
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NS116838

Title: Humanin (HN) attenuates TBI pathology by influencing bioenergetic processes

Authors: *P. THAPAK, Z. YING, F. GOMEZ-PINILLA;
Univ. of California Los Angeles, Los Angeles, CA

Abstract: Background: Reduction in the bioenergetic capacity of the mitochondria to sustain cell functions is central to the traumatic brain injury (TBI) pathogenesis, resulting in oxidative stress, inflammation, synaptic dysfunction, and cognitive impairment. Recently, humanin (HN), a mitochondrially encoded micro-peptide, has shown promise to mitigate these fundamental molecular events in the brain, portraying HN as a potential therapeutic agent for the management of TBI. **Methods:** C57BL/6J mice (n=6) were exposed to moderate lateral fluid percussion injury and HN (40 µg/kg, i.p.) was administered 1 and 6 hr post-TBI. Memory was assessed 3 weeks post-injury. Protein and mRNA levels were assessed in the ipsilateral hippocampus using western blot and RT-PCR. Cytokine array was performed in plasma. Seahorse was used to access mitochondrial activity. **Results:** HN administration counteracted a TBI-related reduction in mitochondrial oxygen consumption rate. Mice exposed to TBI showed a significant decline in spatial memory in the Barnes maze which was counteracted by HN intervention. HN restored levels of synaptic proteins (synapsin 1 and p-CREB) important for learning and memory. HN

also counteracted TBI-related elevations of pro-inflammatory cytokines in plasma (TNF-α, INF-y, IL 17, IL 5, MCP 5, GCSF, RANNETS, sTNFRI) as well as in the hippocampus (gp-130 and p-STAT3). Histone acetylation is intimately associated with mitochondria bioenergetics and subsequent epigenetic modifications. Ongoing studies indicate that TBI increased mRNA levels of NLRP3, and HDAC2, and reduced SIRT3 levels in the ipsilateral hippocampus. These changes were counteracted by HN intervention. **Conclusion:** This study showed that HN intervention has a neuroprotective effect on molecular systems underlying mitochondria bioenergetics and cognitive function. Overall, these findings delineate the neuroprotective potential of HN against TBI.

Disclosures: P. Thapak: None. **Z. Ying:** None. **F. Gomez-Pinilla:** None.

Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

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Program #/Poster #: PSTR407.26/X21

Topic: C.10. Brain Injury and Trauma

Support: NIH NS 111378
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NIH NS 116838

Title: Thyroid hormone alleviates blood-brain barrier disruption after TBI

Authors: *F. GOMEZ-PINILLA[1,2], M. KHANDELWAL[1];
[1]Dept. Integrative Biol. and Physiol., [2]Dept. Neurosurgery, UCLA Brain Injury Res. Ctr., UCLA, Los Angeles, CA

Abstract: Traumatic brain injury is a life threatening with lifelong neurological disabilities. Blood-brain barrier (BBB) leakage is one of the major events that occur after TBI that threatens brain integrity and function. Astrocytes, surrounding endothelial and pericyte cells lining the ventricles are critical for controlling the influx and efflux of biological substances essential for maintaining BBB integrity, brain's metabolic activity as well as cellular functions. Our current study focusses on candent questions that are poorly explored. (A) Does TBI affect tight junction proteins like ZO-1, occludin-1. (B) Impact of TBI on hyperpermeability (MMP-9). (C) Role of A1 and A2 astrocytes 7 days post-TBI. (D) Specific interventions that can alleviate BBB disruption after TBI. Based on the results of single cell genomics data (Arneson et. al., Nature Communications, 2018), thyroid hormone BBB transporter gene is differentially expressed after TBI, such that here we used thyroid hormone to regulate BBB integrity post-TBI. Mice were subjected to fluid percussion injury (FPI) followed by acute T4 treatment (1 hour and 6 hours post-TBI). Barnes maze studies revealed that mice showed impaired spatial memory 7 days post-TBI while T4 treatment reversed the damaging outcomes. We also found BBB leakage in 7 days post-TBI mice evidenced by Evans blue staining and T4 administration counteracted these

changes. The gene expression of key markers for endothelial (CD31) and pericytes (CD13) were also found to be affected by TBI and changes were counteracted by T4 treatment. The ratio of C13:C31 plays critical role in determining the pericyte coverage and measures microvascular integrity. Our data shows that TBI compromised BBB integrity with subsequent neuroinflammation 7 days post-TBI. T4 showed a promising therapeutic action by improving BBB leakage and integrity in the TBI pathology.

Disclosures: F. Gomez-Pinilla: None. **M. Khandelwal:** None.

Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

Location: WCC Halls A-C

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Program #/Poster #: PSTR407.27

Topic: C.10. Brain Injury and Trauma

Support: WP911F-17-2-0222

Title: Investigating Protein Expression Within the Hyperacute Phase Following Impact in an Ex Vivo Porcine Brain Model

Authors: *B. HOFFE[1], G. KANG[2], R. BANTON[3], T. PIEHLER[3], O. E. PETEL[2], M. R. HOLAHAN[1];
[1]Neurosci., [2]Mechanical and Aerospace Engin., Carleton Univ., Ottawa, ON, Canada; [3]U.S. Army Res. Lab., Aberdeen Proving Ground, MD

Abstract: Brain tissue as a material presents unique properties with a multitude of cell types and densities, varying degrees of axonal fiber diameters and blood vessels. All of these neural components are contained within a very viscous environment that, within a gyrified brain, displays a high degree of cavitation. Cavitation in the depths of the sulci may make this area particularly vulnerable to biomechanical forces following an impact. Post-mortem analyses of human brain tissue have indeed shown a pattern of pathological outcomes with repeated impacts in midline structures and the apex of certain sulci where cavitation is prominent. The movement and subsequent forces loaded on to the brain have been shown to produce a variety of biomechanical responses that impair neurophysiological functioning at the cellular level. Excitotoxicity, brought on by biomechanical load, has been shown to result in synaptic dysfunction, as increased intracellular Ca2+ concentrations over-activate various kinases and proteases responsible for cellular survival. Previous work in our lab has shown that after a drop impact, there was a marked decrease in staining of the microtubule stabilizing protein MAP2, as well as preferential shift towards mushroom-type spines within the hyperacute phase post-impact (minutes to hours) in the *ex vivo porcine cortex*. For the current work, Western blot assays were conducted on predetermined regions of interest (ROI) within the porcine cortex. These ROIs consisted of both sulcal arms and sulcal depths to investigate the vulnerability of these regions to increased strain and altered protein expression. To follow up the changes observed from previous

work using the Golgi-Cox method and spine morphology changes, we analyzed excitatory synaptic remodeling markers Matrix metallopeptidase 9 (MMP-9), and neurogranin. Along with these synaptic markers, we investigated the activity of calpain-2, a microtubule protease involved in modulating the dynamics of microtubule associated proteins implicated in the progression of pathology resulting from multiple impacts. These outcomes were paired with strain data collected from markers implanted in the brain tissue to compare protein expression to strain placed on the tissue. These results further clarify the hyperacute protein dynamics following impact in gyrified brain tissue.

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Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

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Topic: C.10. Brain Injury and Trauma

Support: NIH 2P20GM109098-06A1
T32 AG052375

Title: Cerebral hypoperfusion impairs traumatic brain injury recovery in mice

Authors: *B. WHITEHEAD, D. R. CORBIN, N. ZHANG, K. KARELINA, Z. M. WEIL; West Virginia Univ., Morgantown, WV

Abstract: Traumatic brain injuries (TBI) produce cognitive and physical impairments and can induce lasting cerebrovascular dysfunction and increase the risk of neurodegenerative disease as patients age. Cardiovascular and metabolic dysfunction is common among patients with a history of TBI and can further increase the risk of secondary deterioration. One physiological process that is predictive of neurodegeneration, and exacerbated by both TBI and metabolic disease is cerebral hypoperfusion. We hypothesized that mild surgical hypoperfusion in mice would worsen both vascular outcomes and cognitive impairments after TBI. To test this hypothesis, we induced chronic hypoperfusion in adult Swiss-Webster mice via bilateral carotid artery stenosis (BCAS) or sham procedure for 30 days prior to either sham injury or moderate TBI, resulting in the following groups: sham-sham (control); BCAS-sham; sham-TBI; BCAS-TBI. We established baseline cerebral blood flow (CBF) via laser speckle flowmetry (LSF), with additional measurements at 30 days post-BCAS, and again 2 weeks following TBI. Cognitive dysfunction was assessed on Barnes maze one week following TBI. Finally, brains were collected following the final LSF session for immunohistochemical (IHC) analyses of vascular dysfunction including Fibrin(ogen) and IgG buildup within vessels and vascular density measurements. Results indicate that mice in the BCAS-TBI group had significantly reduced CBF by two weeks post-TBI compared to control animals. BCAS-TBI mice also exhibited significantly greater latencies to

reach the escape hole in the Barnes maze than control or BCAS-only mice . IHC analyses revealed that BCAS mice had decreased vascular density in the CA1 region of the hippocampus as well as the corpus callosum compared to non-BCAS mice. Additionally, BCAS-TBI mice exhibit significant vascular accumulation of Fibrin(ogen) and IgG compared to other groups. Together, these data suggest that TBI-induced vascular dysfunction can be worsened by a preexisting cerebral hypoperfusion, as evidenced by further CBF reduction, protein aggregation within vessels, loss of vessels, and impaired cognitive performance.

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Poster

PSTR407. Brain Injury: Animal Models and Pharmacology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR407.29/X23

Topic: C.10. Brain Injury and Trauma

Support: KSCHIRT grant (#14-12A)
Neurobiology of CNS Injury and Repair (5T32Ns077889)
Graduate Training in Integrative Physiology (1T32GM118292-04)

Title: Sex-based differences in hippocampal neurogenesis after TBI

Authors: *H. WILLIAMS[1,2], A. GLOVER[1,2], K. E. SAATMAN[1,2];
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Abstract: Sex-based differences in hippocampal neurogenesis after TBI

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Moderate or severe contusion brain injury robustly increases cellular proliferation within the dentate gyrus, resulting in the generation of new neurons. The development, integration, and long-term survival of posttrauma-born neurons is poorly understood. To date, traumatic brain injury (TBI) neurogenesis studies have almost exclusively utilized male rodents, resulting in a significant gap in knowledge as to how this aspect of neuroplasticity may differ for females. To evaluate sex-dependent neurogenic responses to TBI, male and female Ascl1-CreERT2; R26R CAG-floxStopTom reporter mice were used to label and track neural progenitor cells (NPCs) born after injury. Mice received a controlled cortical impact (CCI) followed by tamoxifen injections on days 2 and 3 postinjury to permanently label NPCs born early after TBI. Injured (n=10 male, n=9 female) and naïve (n=8 male, n=12 female) mice survived 6 weeks after receiving two tamoxifen injections. Significantly fewer tdTom+ neurons were observed in the hippocampal dentate gyrus ipsilateral to impact compared to naïve controls ($p<0.005$), with

equivalent numbers for males and females. However, CCI-injured females exhibited a significant increase in tdTom+ neuron numbers in the contralateral hippocampus when compared to CCI-injured males ($p<0.0001$) or to naïve females ($p<0.005$). Sholl analysis of dendritic arbor complexity revealed a modest increase in branch complexity in neurons of female naïve mice compared to males. TBI-related changes to the dendritic arbor differed for females and males. Numbers of mossy fiber boutons formed by tdTom+ neurons extending axons to the CA3 region were reduced after TBI, with a more pronounced reduction in males. This reduction in bouton density appeared to be offset by an increase in bouton surface volume in females. TBI causes impairment in multiple aspects of hippocampal neurogenesis. Surviving posttrauma-born neurons show axonal and dendritic structural changes that suggest impaired connectivity. Responses consistent with compensatory plasticity were observed in female mice following TBI.

Disclosures: H. Williams: None. **A. Glover:** None. **K.E. Saatman:** None.

Poster

PSTR408. Spinal Cord Injury: Methodologies

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR408.01/X24

Topic: C.11. Spinal Cord Injury and Plasticity

Support: DARPA Grant, Contract No. N660012024046

Title: Illuminating changes in spinal cord oxygenation and hemodynamics using optical biosensing

Authors: *K. RASCHDORF[1], M. KHOSHNAM TEHRANI[2], S. ASKARI[2], A. ZAIDI[2], N. MANOUCHEHRI[2], M. WEBSTER[2], K. SO[2], F. SAHRAGARD[2], G. FRANK[2], A. WARNER[2], J. ETHRIDGE[2], F. STREIJGER[2], V. SIVAJI[3], J. B. ZIMMERMANN[4], D. GRASSE[3], B. SHADGAN[2], B. K. KWON[2];
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Abstract: Introduction: Traumatic spinal cord injury (SCI) results in local and systemic vascular changes, which make the spinal cord extremely vulnerable to ischemia, hypoxia, and energy dysfunction. Therefore, current clinical guidelines for the early management of spinal cord injury patients emphasize mean arterial pressure (MAP) augmentation for the first seven days post-SCI. However, to assess the effectiveness of MAP augmentation on spinal cord perfusion, we must develop new techniques to monitor the injury site continuously and in real-time. To address this clinical need, we embarked upon developing a miniaturized, implantable optical biosensor based on near-infrared spectroscopy (NIRS) to monitor spinal cord oxygenation and tissue hemodynamics from the injury penumbra. This study's objective was to compare the newly developed spinal cord NIRS system against 1.) an invasive, combined partial

pressure of oxygen (PO_2)/blood flow sensor and 2.) a second, research-grade NIRS sensor in response to a series of physiologic perturbations. **Methods**: We performed n=8 non-survival studies on uninjured female Yucatan miniature pigs. The NIRS system was laid onto the dura, and a pair of intraparenchymal OxyFlow sensors were inserted into the ventral aspect of the spinal cord tissue underneath the NIRS sensor. A second, research-grade NIRS device (PortaLite-Mini, Artinis) was placed caudal to this set-up. Subsequently, animals were challenged with multiple episodes of moderate-severe hypoxia and pharmacologically induced changes in MAP. **Results/Conclusion**: Our results suggest that the newly developed NIRS sensor can monitor intra-operative changes in spinal cord oxygenation and hemodynamics induced by various physiological perturbations, leading the way towards clinical translation. We are currently analyzing the data to establish the utility of NIRS monitoring and determine the dynamic relationship between spinal cord oxygenation and perfusion patterns with commonly tracked clinical indices such as fluctuations in MAP.

Disclosures: K. Raschdorf: None. **M. Khoshnam Tehrani:** None. **S. Askari:** None. **A. Zaidi:** None. **N. Manouchehri:** None. **M. Webster:** None. **K. So:** None. **F. Sahragard:** None. **G. Frank:** None. **A. Warner:** None. **J. Ethridge:** None. **F. Streijger:** None. **V. Sivaji:** None. **J.B. Zimmermann:** None. **D. Grasse:** None. **B. Shadgan:** None. **B.K. Kwon:** None.

Poster

PSTR408. Spinal Cord Injury: Methodologies

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR408.02/X25

Topic: C.11. Spinal Cord Injury and Plasticity

Support: W81XWH1910413

Title: Perineuronal Nets in the Spinal-intact and Injured Porcine Spinal Cord.

Authors: *S. JARLSDÓTTIR[1], J. WANG[1], N. MANOUCHEHRI[1], M. WEBSTER[1], K. SO[1], J. ETHRIDGE[1], A. WARNER[1], A. BILLINGSLEY[1], R. NEWSOME[1], F. STREIJGER[1], C.-Y. LIN[2], Y.-S. LEE[2], B. K. KWON[1,3];
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Abstract: Introduction: Perineuronal nets (PNN) are specialized extracellular matrix structures that encapsulate the soma and the proximal dendrites of a subset of motor neurons and spinal interneurons with a widely known role in synaptic stabilization. Therefore, manipulating PNN may provide the key to reactivating plasticity and restoring function, both of which are severely impaired following traumatic spinal cord injury (SCI). The objective of his study was characterizing the spatial expression of PNN in a porcine model of SCI. A more detailed characterization of the injury-induced changes within PNNs in the chronic phase of SCI, will

extend our knowledge of the pathophysiological events after SCI and further, help us determine how the porcine model correlates with human SCI to ensure its translational and predictive value. **Methods:** Following a T10 spinal cord contusion/compression SCI, lumbar porcine spinal cord samples were dissected, fresh-frozen and cryo-sectioned into 20µm thick coronal sections. Wisteria Floribunda agglutinin (WFA) and 5HT immunofluorescence was used to visualize PNNs and serotonergic fibres, to address post-traumatic changes in the lumbar spinal cord, distal to the initial injury. In addition, we evaluated the distribution of PNNs in intact porcine spinal cords. **Results:** Distal to the injury site, almost none of the large alpha motor neurons in the lumbar ventral horn were surrounded by PNNs. Instead, most of the framed motor neurons had small cell bodies, which are putatively gamma motor neurons. No bouton-like dots were observed surrounding the soma of motor neurons, instead 5HT was observed in the cytoplasm of motor neurons, illustrating a decrease in supraspinal synaptic input. Occasionally, isolated serotonergic fibres were seen in the motor pool area of the lumbar ventral horn. **Conclusion:** In conclusion, we demonstrated significant changes among PNNs and serotonergic boutons surrounding the motor neurons in the lumbar spinal cord in the porcine model of thoracic SCI. A better understanding of such long-lasting consequences distal to the injury site may be essential in the development of novel treatments to promote neuroplasticity and meaningful functional repair.

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Poster

PSTR408. Spinal Cord Injury: Methodologies

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR408.03/Y1

Topic: C.11. Spinal Cord Injury and Plasticity

Support: MSFHR Award AWD-00482

Title: Tracing the path; an emergent need to evaluate spinal cord optical properties

Authors: *G. FRANK[1,2], F. SAHRAGARD[2], K. RASCHDORF[2], N. MANOUCHEHRI[2], M. WEBSTER[2], K. SO[2], S. ASKARI[2], F. STREIJGER[2], B. SHADGAN[2], B. K. KWON[2,3]; [2]Intl. Collaboration On Repair Discoveries (ICORD), [1]Univ. of British Columbia, Vancouver, BC, Canada; [3]Vancouver Spine Surgery Inst., Vancouver, BC, Canada

Abstract: Introduction: The ability of near-infrared spectroscopy (NIRS) to non-invasively quantify blood oxygenation and volume in biological tissues with high temporal resolution, has made it increasingly popular in the last decade. While most NIRS applications have focused on the brain, investigators have recently begun to investigate NIRS to interrogate hemodynamics and oxygen delivery within the spinal cord. However, translation of such an approach to the

spinal cord remains a challenge due to the unique properties of the tissue and the effect of the extracellular layers surrounding the spinal cord. Therefore, the objective of this study was to evaluate the effect of the dura, cerebrospinal fluid (CSF) and spinal cord tissue on near-infrared light behavior. **Methods:** Intact, formalin-fixed, porcine spinal cord was harvested and cut to 40, 60, and 80 µm thickness in both axial and longitudinal orientations. A 1 mm diameter laser beam, at both 635 and 780 nm wavelengths, was passed through the samples and the intensity and direction of the transmitted light were simultaneously assessed. Furthermore, a Monte Carlo simulation was constructed to simulate light propagation through the spinal cord, CSF layer and dura mater using light wavelengths between 650 and 950 nm. **Results:** While axial samples resulted in a circular scattering profile, longitudinal samples caused an ovoid scattering profile with the long axis oriented perpendicular to axon orientation. Additionally, the Monte Carlo simulation predicted a significant amount of scattering caused by the dura mater. A large proportion of this scattered light tended to progress through the non-scattering CSF layer without entering the spinal cord. The presence of the dura mater paired with the CSF layer caused a 10-fold reduction in the peak fluence (optical energy density) within the spinal cord. Peak fluence appears to decay exponentially with increasing CSF thickness before plateauing at ~1.2 cm thickness with a corresponding 100-fold reduction in peak fluence. **Conclusion:** These results highlight experimental evidence of the directionally dependence of light propagation within the spinal cord. Peak fluence within the spinal cord is also strongly affected by the thickness of the surrounding CSF layer. Such knowledge is key for the success of accurate quantitative NIRS analysis and optical imaging techniques.

Disclosures: G. Frank: None. **F. Sahragard:** None. **K. Raschdorf:** None. **N. Manouchehri:** None. **M. Webster:** None. **K. So:** None. **S. Askari:** None. **F. Streijger:** None. **B. Shadgan:** None. **B.K. Kwon:** None.

Poster

PSTR408. Spinal Cord Injury: Methodologies

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR408.04/Y2

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Department of Defense - Spinal Cord Injury Research Program (SCIRP)

Title: Assessment of neurogenic bladder dysfunction using wireless catheter-free pressure sensors in a porcine model of spinal cord injury

Authors: *A. W. DOELMAN[1], J. ETHRIDGE[2], A. WARNER[2], A. ARORA[2], C.-C. TSAI[2], M. WEBSTER[2], K. SO[2], N. TEARLE[2], A. BILLINGSLEY[2], F. STREIJGER[2], S. J. A. MAJERUS[3,4], M. S. DAMASER[4,5], B. K. KWON[2,6];
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Cleveland, OH; [5]Biomed. Engin., Cleveland Clin., Cleveland, OH; [6]Orthopaedics, Vancouver Spine Surgery Inst., Vancouver, BC, Canada

Abstract: Neurogenic lower urinary tract dysfunction remains a leading cause of morbidity after spinal cord injury (SCI). Urodynamics (UDS) is presently the clinical standard for the assessment of bladder dysfunction after SCI. However, this method presents many well-recognized limitations which may interfere with bladder function and its subsequent interpretation. To address this, we tested a wireless intravesical pressure sensor (the "UroMonitor"), as well as an implanted transmural pressure sensor to permit continuous bladder pressure recording without the use of catheters. The aim of the presented study was to compare the UroMonitor and transmural telemetric systems to conventional catheter-based pressure measurement and to characterize neurogenic bladder dysfunction during UDS and ambulatory urodynamic monitoring in a large animal model of SCI.

Yucatan minipigs (n=9) were used under IACUC and veterinary oversight. SCI was induced via contusion-compression impact at the 10^{th} thoracic level. A transmural telemetric sensor was implanted 4-weeks prior to SCI. UDS experiments were performed before and 4-, 8-, and 11-weeks after SCI. UroMonitor was inserted transurethrally before each UDS experiment under general anesthesia.

We demonstrated that the UroMonitor and transmural sensors reliably identified >90% of voiding and non-voiding contractions during UDS assessment before and after SCI. UroMonitor and transmural pressure recordings showed strong, statistically significant correlation to conventional UDS catheters. The amplitude of bladder contractions measured from wireless sensors were within ~4 cmH2O of the present clinical standard. During ambulatory monitoring, voided volumes were found to be significantly reduced relative to those collected during UDS in pre-SCI animals. Further, bladder contraction amplitudes were found to be greater during ambulation relative to UDS assessment in SCI pigs.

Wireless, catheter-free devices may offer an alternative to traditional bladder assessment techniques permitting more natural, comfortable observation of lower urinary tract dysfunction after neurological injury in pre-clinical animals and humans. The present study tested two devices that can reliably identify and quantify bladder contractions before and after SCI in a large animal model with a high degree of accuracy.

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Poster

PSTR408. Spinal Cord Injury: Methodologies

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR408.05/Y3

Topic: C.11. Spinal Cord Injury and Plasticity

Support: US Department of Defense / Translational Research Award, Sponsor Reference Number: W81XWH2010480; SC190120

Title: Methodological framework for ultrasound, MRI, and histological detection and quantification of intraparenchymal hemorrhage in a porcine model of SCI

Authors: *A. ALLARD BROWN[1], K. SO[1], N. MANOUCHEHRI[1], M. WEBSTER[1], J. ETHRIDGE[1], A. WARNER[1], A. BILLINGSLEY[1], R. NEWSOME[1], K. BALE[2], A. YUNG[2], M. SENEVIRATNE[1], J. CHENG[1], J. WANG[1], S. BASNAYAKE[1], F. STREIJGER[1], P. KOZLOWSKI[2], B. K. KWON[1,3];
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Abstract: Introduction: After traumatic spinal cord injury (SCI), intraparenchymal hemorrhage (IPH) is a common phenomenon that is associated with worsened neurologic outcome. A primary concern is that the blood itself and its breakdown products can be toxic to the injured spinal cord. Therefore, accurate evaluation and quantification of IPH following SCI are key to defining the effect of treatments on IPH progression and secondary neuronal injury. Thus, the purpose of our study was 1) to detect IPH by magnetic resonance imaging (MRI) and high-frequency ultrasound (US), 2) to associate IPH on MRI and US with histology and 3) to quantitatively monitor the time course of IPH following SCI. **Methods:** A porcine model of SCI was employed in this study using female Yucatan pigs. At 6 hours (n=3) or 7 days post-SCI (n=29), *in vivo* US and *ex vivo* MR images were captured. Spinal cord sections were stained with Hematoxylin and Eosin (H&E) for visualization of red blood cells and Prussian blue for iron. US and MR images were then co-registered with the corresponding histology images. **Results:** On MRI, IPH was visible as hypointense regions which visually matched regions stained for red blood cells and iron deposits, both at 6 hours and 7 days post-SCI. The appearance of IPH on US typically presented as a hyperechoic area. However, the correspondence with histology was observed at 6 hours post-SCI only, predominantly close to the epicenter of the impact. Overall, the size of the IPH developed rapidly after injury and was largest at the injury site and extended roughly 8 mm in both the rostral and caudal segments. Central IPH was usually seen within the gray matter of the spinal cord at the center of the impact and a variable proportion of adjacent white matter. In one case, remote IPH was detected as far as 2 cm caudal to the epicenter. **Conclusion:** Our semi-automated method provides a plausible pipeline for the segmentation and quantification of IPH from MR, US and histology images. Moreover, the study illustrated the power of using multiple imaging systems to investigate the progression of IPH following SCI, an approach that will hopefully become more frequently adopted for translational SCI research.

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Poster

PSTR408. Spinal Cord Injury: Methodologies

Location: WCC Halls A-C

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Program #/Poster #: PSTR408.06/Y4

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH S10 OD025085
NIH R01 NS092961
DOD grant SC160154

Title: Ai-assisted machine learning analysis of 3d video recordings of grasping behavior of monkeyspre- and post-spinal cord injury

Authors: D. H. DUQUE[1], N. ZHENG[2], T.-W. LEE[1], P.-F. YANG[2], J. C. GORE[2], ***L. M. CHEN**[2];
[1]Biomed. Engin., Vanderbilt Univ., Nashville, TN; [2]Radiology and Radiological Sci., Vanderbilt Univ. Med. Ctr., Nashville, TN

Abstract: Spinal Cord Injuries (SCIs) compromise tissues including nerves responsible for relaying and integrating signals between the brain and the body. These injuries frequently result in behavioral deficits in sensory and motor functions at and below the level of spinal cord injury, typically arising from physical trauma. Targeted injuries to the cervical spinal cord in monkeys can specifically impede skilled hand use. In our previous studies, we employed a machine-learning-based video analysis system - DeepLabCut - to quantify hand-reaching and grasping behavior. We detected subtle abnormalities in finger posture in monkeys with cervical spinal cord injuries, even when their end-point hand performance had returned to pre-injury level. However, our analysis was limited to a frontal view, overlooking relevant information from side and top perspectives. In the current study, we designed a 3D camera recording system to quantify the kinematic deficits of hand reaching and grasping behavior following injuries to C5-level. Four squirrel monkeys were trained to retrieve sugar pellets from seven different devices, six of which featured wells of increasing difficulty. Each behavioral session was recorded simultaneously from top, side, and front views and included one video per completed task trial. A single behavioral trial allowed evaluation of hand and finger behaviors during three dynamic phases: reaching, grasping, and retrieving. Using DeepLabCut, we tracked and quantified individual finger movement, speed and trajectory during grasping and retrieval, changes in finger orientation, alterations in hand shape and orientation, and task completion time. These parameters were quantified over a twelve-week post-injury period. We discovered that impairments in motor and sensory skills were more pronounced with deeper well depths and increased task difficulties. Our results demonstrate that an AI-assisted machine-learning-based video analysis system can robustly identify subtle behavioral impairments in finger posture and hand movement speed and trajectory, which are not discernible through conventional end-point performance analyses. This method enables separate quantification of motor and somatic sensory deficits. The insights obtained from this study will shed light on the compensatory mechanisms employed by monkeys to successfully complete the assigned tasks.

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Poster

PSTR408. Spinal Cord Injury: Methodologies

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR408.07/Y5

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Depth cameras provide accurate estimates of center of pressure measurement in seated leaning tasks for people with thoracic incomplete spinal cord injury and non-injured controls

Authors: *M. N. HATCH[1], R. MATTHEW[2];
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Abstract: ABSTRACT*Objectives:* There exists a major gap in the field regarding trunk stability in people with spinal cord injury (SCI). This is somewhat surprising given the impact that trunk stability (or lack thereof) can have on one's activities of daily living and its ranked importance by people with SCI-- even above walking. However, existing trunk stability measures for SCI remain under-developed, outdated, and cost and space prohibitive. Small depth camera systems, such as the Microsoft Kinect, show promise for motion analysis and clinical testing in non-SCI populations for gait, Sit-to-Stand, and stair climbing. Here, we assess the feasibility of a single 3-dimensional (3D) depth camera to predict Center of Pressure (CoP), the gold standard for trunk/seated posture assessment, across various seated leaning task in persons with SCI and healthy controls. ***Methods/ approach:*** Five individuals with thoracic SCI and ten healthy controls were recruited for this study. Subjects were asked to perform seated lateral leaning tasks while being simultaneously recorded by a force plate and a 3D Microsoft Kinect sensor, twice, and to their fullest extend (CoP end excursion) while maintaining control. Data were post-processed in MATLAB using a custom allometrically-scaled skeletal model. Max CoP excursion and velocity were computed from both the force platforms and the estimated CoP from the depth camera. Bland-Altman was used to determine reliability between the devices.***Results:*** All participants, including those with SCI, completed the seated leaning tasks with no issues. A total of 53 leaning actions were recorded and processed for this study. CoP estimates from the 3D sensor displayed excellent correlations force plate metrics with R2 values of 0.92, 0.80, 0.74, and reliability coefficients of 2.4cm, 2.6cm/s, and 4.6cm/s for the maximum CoP excursion, maximum CoP outward speed, and inward speeds respectively. ***Conclusion(s):*** These initial results indicate that estimation of CoP from a single 3D camera system is comparable to standard force plate analyses. Given that 3D sensors are more cost and space efficient, they may be more feasible and suitable for trunk stability assessment in individuals with SCI.

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Poster

PSTR408. Spinal Cord Injury: Methodologies

Location: WCC Halls A-C

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: US Department of Defense / Translational Research Award, Sponsor Reference Number: W81XWH2010480; SC190120

Title: Mr imaging of intraparenchymal hemorrhage following cervical sci and the effect of map augmentation

Authors: *T. MALOMO[1], F. STREIJGER[1], M. DVORAK[1,2], C. G. FISHER[1,2], T. AILON[1,3], S. PAQUETTE[1,3], J. STREET[1,2], N. DEA[1,3], R. CHAREST-MORIN[1,2], C. DANDURAND[1,3], B. KWON[1,2];
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Abstract: Introduction: Current practice guidelines for traumatic spinal cord injuries (SCI) recommend augmenting MAP to a target of 85-90mmHg for the first 7 days post-injury in acute SCI patients to maintain perfusion to the injured and ischemic spinal cord. While MAP augmentation with vasopressors may improve blood flow and reduce ischemia in the injured cord, it may also induce undesirable increases in intraparenchymal hemorrhage (IPH) within the injured spinal cord. Therefore, the present MRI-based clinical study determined whether MAP augmentation with vasopressors influences IPH within the injured spinal cord (ClinicalTrials.gov Identifier: NCT04758377). **Method:** Ethical approvals were obtained from the IRB and CREB of Vancouver General Hospital. Informed consent was obtained from each patient. Per institutional protocol, the target MAP of 85-90 mmHg was achieved using norepinephrine, vasopressin and midodrine infusion as required. T2WI images were collected on a 1.5T MRI scanner at baseline (>24 hours post-SCI), 2, 4, 7 and 14 days post-SCI. IPH progression (delta hemorrhage) was calculated between the first and subsequent MRI scans on Day 2, then from days 2-4, 4-7, and 7-14 post-SCI. Using simple linear regression and linear mixed effect models, we evaluated the associations between IPH progression and both time-weighted average MAP (TWA-MAP) and mean measures of MAP. **Results:** To date, we have enrolled 8x cervical SCI patients. At the baseline time point, all patients demonstrated IPH. On days 2 and 4, the extent of IPH progressed, followed by a reduction on days 7 and 14. During the first 48 hours after injury, TWA-MAP significantly correlated with delta IPH ($p=<0.0001$). Further analysis revealed a threshold-driving association whereby starting at a MAP of 85 mmHg and increasing the binary cut-off by 5 mmHg increments, TWA-MAP became a predictor of IPH progression in the first 2 days after SCI at a MAP upper threshold >90 mmHg ($p=0.035$). **Conclusion:** These results represent an important finding that to improve blood flow and reduce ischemia in the injured cord by augmenting MAP, clinicians may inadvertently promote undesirable bleeding within the spinal cord, thereby increasing the size of IP hemorrhage at the injury site.

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Poster

PSTR408. Spinal Cord Injury: Methodologies

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Program #/Poster #: PSTR408.09/Y7

Topic: C.11. Spinal Cord Injury and Plasticity

Support: R01 NS116068 (JCG)

Title: Contusion Spinal Cord Injury Model Characterization for Autonomic dysreflexia: A pilot study

Authors: *S. KAUR, H. VAUGHT, E. AKIN, J. GENSEL, S. PATEL;
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Abstract: Contusion Spinal Cord Injury Model Characterization for Autonomic dysreflexia: A pilot study
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AbstractComplete high thoracic spinal cord injury (SCI) often leads to autonomic dysreflexia (AD), a condition that manifests as acute, episodic hypertension with and without bradycardia. AD is characterized by excessive discharge of sympathetic preganglionic neurons (SPN) in the intermediolateral cell column (IML) that are reflexively activated by noxious stimuli below the injury level. Most animal models of AD utilize a complete spinal cord transection at the T3 spinal level or above, however, anatomically complete spinal transection is relatively uncommon clinically, and individuals with incomplete injury still experience AD. We hypothesized that rats will develop AD over time following severe contusion SCI at the T3 spinal level. Thus, we designed an 8-week study with two different injury severities and evaluated the development of AD. Adult female Wistar rats were subjected to T3 contusion SCI with two different forces (300 kdyn (5s dwell time) and 400 kdyn (5s dwell time). Injured rats were subjected to weekly behavioral testing using Basso Beatie Bresnahan (BBB) locomotor rating scale for hindlimb function as well as the tail spasticity test for eight weeks. To evaluate changes in blood pressure, a telemetric probe was implanted in the descending aorta 2 weeks after the injury. Spontaneous AD events were recorded 3, 5, and 7 weeks post-injury. We also monitored induced AD by colorectal distension (CRD) at 4, 6, and 8 weeks post-SCI. We observed significant locomotor dysfunction and the development of spasticity regardless of injury severity. Both injury severities also resulted in significant increases in mean arterial pressure (MAP) but no change in heart rate (HR) after AD by CRD. This evoked AD was not significantly different between injury

severities. There was no significant difference in spontaneous AD events between injury severities. Tissue histology will be correlated with the magnitude of induced AD for both SCI severities. Collectively, our data support severe SCI contusion as a correlate for SCI-induced AD in humans.

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Poster

PSTR408. Spinal Cord Injury: Methodologies

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Topic: C.11. Spinal Cord Injury and Plasticity

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DoD SCIRP-IIRA Grant SC210266

Title: Magnetic resonance imaging-based tractography to evaluate mid-cervical neural tracts in the acute and chronically injured spinal cord of the rat

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Abstract: Hemisection of the C2 spinal cord (CH2x) is an experimental model of incomplete cervical spinal cord injury (SCI) commonly used to study mechanisms of respiratory neuroplasticity and recovery. Extensive characterization of this model provides physiological and anatomical evidence that recovery of ipsilateral phrenic motor activity may arise from commissural axon tracts and/or propriospinal relays. Magnetic Resonance Imaging (MRI) of *ex vivo* spinal cords provides a powerful tool for visualizing neural tracts in high-resolution, enabling detailed characterization of neural pathways without need for sectioning/staining. Advanced methods such as Diffusion Tensor Imaging (DTI) and Susceptibility Weighted Imaging (SWI) enable characterization of cysts/hematomas, secondary injury progression, and efficacy of therapeutic interventions. We sought to determine if MRI and DTI tractography techniques could detect: 1) mid-cervical commissural (crossed-spinal) fiber pathways, and 2) growth/sprouting of neural fibers in the lesion vicinity after acute vs. chronic C2Hx. In ongoing studies, we are evaluating intact rats (n=9) and rats with acute (1 wk; n=8) and chronic C2Hx (>16 wks; n=7) using a 7 tesla MRI scanner (MR Solutions, Guilford, UK) with a coil

specifically designed for ex vivo rat spinal cord imaging. Axial and sagittal SWIs enable characterization of hematoma volume and extent of secondary injury, while T2-weighted spin-echo images enable evaluation of fluid-filled cysts/cavities. The DTI protocol uses a b-value of 2000 and is applied in 66 directions. Echo-time is 25 ms, and recycle time is 6 s to allow spins to fully relax, resulting in a total experimental time of 14.3 h. DTI processing is performed using DSI Studio software; DTI metrics and tractography are derived from these data. Preliminary analyses suggest alterations in DTI matrices (fractional anisotropy, mean diffusivity, and axial and radial diffusivity) with both acute and chronic injury *vs.* intact controls, particularly at the site of injury and within 2 mm rostral and caudal. The extent of tract abnormalities detected by DTI differ in acute *vs.* chronic C2Hx, with differing microstructural and density changes in descending tracts. Notably, after chronic C2Hx, the injury site is largely filled with viscous cysts and blood (iron hemosiderin), with evidence of significant fiber sprouting. We observe no evidence of injury or hematoma expansion with chronic injury. These data provide evidence of spontaneous axonal sprouting near a C2Hx lesion, and provide a baseline for studies assessing the impact of therapeutic interventions aimed at neuroprotection and/or axonal growth.

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Poster

PSTR408. Spinal Cord Injury: Methodologies

Location: WCC Halls A-C

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Program #/Poster #: PSTR408.11

Topic: C.11. Spinal Cord Injury and Plasticity

Support: DoD CDMRP Grant W81XWH-16-1-0655

Title: Evaluation of spinal cord injury-induced neuropathic pain in a pig model

Authors: L. E. SCHNEIDER, T. L. NIEDZIELKO, ***C. L. FLOYD**;
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Abstract: Introduction: Neuropathic pain can be a debilitating occurrence after spinal cord injury (SCI). Treatment options for neuropathic pain remain suboptimal and discovery of novel therapeutics is limited, to some extent, by challenges in evaluating neuropathic pain in animal models. Most pain studies are conducted in rodents and focus evaluation predominantly on reflexive outcomes. Thus, there is an unmet need to develop novel animal models of neuropathic pain in non-rodent models that can be used as a translational tool to accelerate the development of new treatments for SCI-NP. The goal of this project was to assess neuropathic pain outcomes using methods back-translated from human clinical medicine to a clinically relevant porcine model of SCI. We also evaluated the effect of sex as a biological variable on outcomes. Methods: Adult male and female gonad-intact, adult pigs (n=8 each sex) were weight-matched and then received a mid-thoracic (T10) contusion-compression SCI. Starting at 2 weeks and continuing

for 12 weeks after SCI, pigs were evaluated using quantitative sensory testing (QST) methods and responses rated with the porcine evoked pain scale (PEPS) that we developed. The PEPS includes assessments of both reflexive (spinal) and affective/cognitive (supraspinal) outcomes. Results: Pain-like responses to QST stimuli were compared between pre-injury and post-injury responses. We found that over 80% of both male and female pigs exhibit a neuropathic pain-like phenotype after SCI, with percentage responding varying by QST modality. For mechanical detection threshold, we found that both male and female pigs exhibited a robust reduction in the withdrawal threshold. Similarly, both male and female pigs exhibited a loss of function in pressure pain threshold after SCI as compared to pre-injury values. For temperature sensitivity, both male and female pigs showed significantly greater pain responses in the heat pain threshold test, with significantly more female pig showing pain-like responses than males. Responses on the cold pain threshold test did not reach statistical significance. Conclusion: Taken together, these data show that using methods back-translated from the human clinical setting, pigs after SCI exhibit a neuropathic-pain phenotype that is similar to that exhibited in the human clinical setting. The data also show that the predictive validity of the pig model may be high and suggest that this porcine model may serve as a translational tool to accelerate development of novel therapies.

Disclosures: L.E. Schneider: A. Employment/Salary (full or part-time):; Emory University. **T.L. Niedzielko:** A. Employment/Salary (full or part-time):; Emory University. **C.L. Floyd:** A. Employment/Salary (full or part-time):; Emory University, Atlanta VA.

Poster

PSTR408. Spinal Cord Injury: Methodologies

Location: WCC Halls A-C

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: EU FLAG-ERA JTC 2021 (RESCUEGRAPH)
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TÜBİTAK 221N399

Title: Low-cost impactor device for contusion-type spinal cord injury

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Abstract: Recent neuromodulation therapies are promising to help with some of the sensorimotor losses in spinal cord injury. One novel approach focuses on the functional electrical stimulation (FES) of the peripheral nervous system to induce neuroplasticity in addition to the

classical gains in sensory and motor function. We developed a low-cost impactor device to produce displacement-controlled and reproducible spinal cord contusions in rats, based on a linear servo actuator. The device probe includes force, acceleration, and displacement sensors to characterize the biomechanical parameters during the application of the injury. In preliminary experiments, optimal parameters were determined (contactor diameter: 2.3 mm, indentation peak force: 0.9-1 N, peak displacement: 1.5-1.75 mm, duration: 0.3-0.5 s) to produce moderate injury as observed with the Basso, Beattie and Bresnahan (BBB) locomotor rating scale at D1 post-op (T8-T9 laminectomy and contusion). High-speed video recordings of rats walking on a platform were obtained pre-op and at several time points post-op for subsequent gait analyses. Although the animals recovered considerably without particular treatment after a month, there were asymmetries in some gait parameters (e.g. stance duration, duty factor, relative paw position) which remained even up to 3 months. Similarly, evoked epidural field potentials recorded on S1 cortex of the right and left hemispheres were asymmetric to contralateral mechanical stimulation of the limbs (tactile and muscle receptors). We are currently studying these neuroplastic changes in a larger sample size, and the possibility to facilitate recovery by neuromodulation.

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Poster

PSTR408. Spinal Cord Injury: Methodologies

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Topic: C.11. Spinal Cord Injury and Plasticity

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NIH NINDS grant R01NS117749- 01 to GS
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Title: Transduction of dorsal root ganglia neurons with AAV2-hSyn-hM3Dq-mCherry constructs via direct microinjection targets large diameter afferents and minimally transduces IB4+ neurons

Authors: *J. CAPALDI[1], H. SOBOTKA-BRINER[5], S. NICE[2], J. EISDORFER[6], G. MOUKARZEL[5], T. J. CAMPION[3], G. SMITH[7], G. KOMA[4], A. SPENCE[4];
[1]Bioengineering, Temple Univ., Levittown, PA; [3]Shriners Hosp. Pediatric Res. Ctr., [4]Bioengineering, [2]Temple Univ., Philadelphia, PA; [5]Merck Corp., Landsdale, PA; [6]Rutgers Univ., Camden, NJ; [7]Shriners Pediatric Res. Ctr., Lewis Katz Sch. of Medicine, Temple Univ., Philadelphia, PA

Abstract: Transducing peripheral sensory neurons in a targeted manner has a number of uses across neuroscience. One example is to uncover the mechanisms by which epidural stimulation

improves recovery from a spinal cord injury. In this instance, it is unknown precisely which populations of neurons are critical to improving recovery from an injury, and it is important to trace which second and higher order neurons are influenced by afferent stimulation. Transduction of dorsal root ganglion (DRG) neurons with genetically encoded tools such as DREADDs packaged in an AAV is one way to achieve this. In using this approach it is important to establish that small diameter nociceptive afferents are not targeted to large extent. In order to validate this, we carried out an immunohistochemical (IHC) stain for IB4 to quantify the fraction of cells transduced by the virus that are IB4 positive. The right side L2-L5 DRGs of rats were injected with AAV2-hSyn-hM3Dq-mCherry. After 8 weeks, animals were euthanized and tissue harvested. Labelled DRG neurons were counted to determine the total number of cells infected by the virus and the subset that were IB4 positive (which are thought to be include many small diameter nociceptors). Histology revealed that the fraction of transduced cells that were IB4+ was small, at 10.9 ± 8.3% (mean ± s.e.m, n=8 DRGs from 5 rats, range 1 to 3 DRGs per rat). This small fraction of IB4 positive cells indicates that the AAV2 was effective in targeting large diameter neurons, and corroborates our recent finding that afferent activation with this approach does not cause changes in thermal nociception assays *in vivo*. These results suggest that transduction of DRG neurons through direct injection with AAV2 is a suitable method to target large diameter afferents for SCI studies.

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Poster

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Topic: C.11. Spinal Cord Injury and Plasticity

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Title: Infrared videography of a subcutaneous marker as a simple, inexpensive method to overcome skin motion artifact in rodent kinematics

Authors: *A. J. SPENCE[1], C. A. PATIL[1], B. RAUSCHER[2], G. MOUKARZEL[1];
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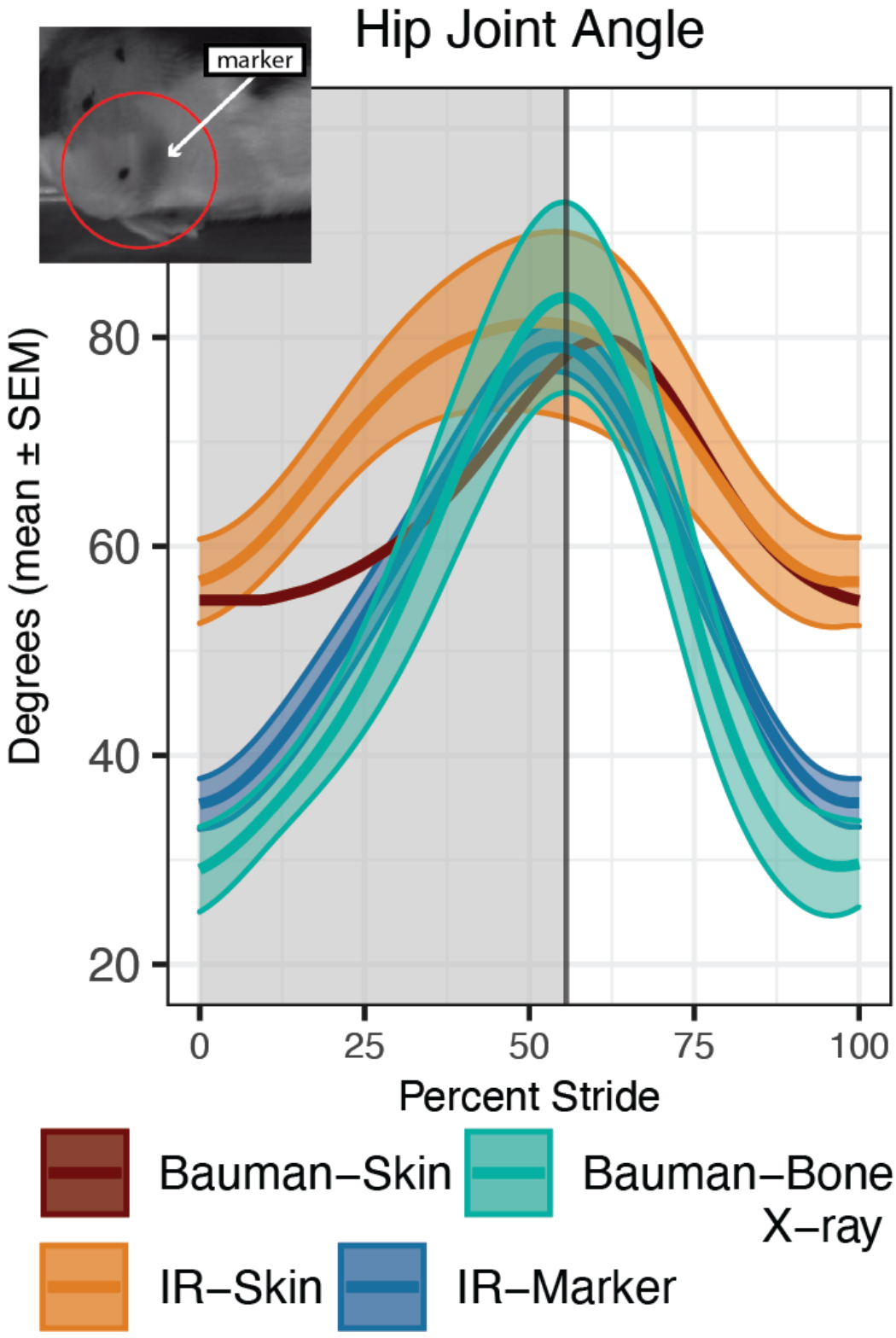
Abstract: Here we show that infrared videography of a subcutaneous marker can dramatically reduce skin motion artifact in rodent kinematics. The method yields data similar to gold standard x-ray fluoroscopy systems at a fraction of the cost, does not affect the animals' locomotion, and results in markers that persist for at least 16 weeks. Knee joint movement under the skin causes errors in rat hip and knee angles of up to 50-75% (Bauman and Chang, 2009). Fluoroscopy,

while the gold standard, requires expensive x-ray systems (>$200k), and has a limited capture volume and short exposure times. We compared joint angles computed using our method versus skin derived markers in the same animals, as well as x-ray imaging of bone derived angles from the literature. No significant difference at the $p<0.05$ level was reported between skin and kinematic data from our method two weeks after surgery (n=7 rats; speed 32 cm/s). DeepLabCut detected our marker at ten weeks at similar rates to two weeks, and the marker remained visible out to 26 weeks. The difference in mean hip angle between our data (2 weeks post surgery; 48 cm/s) and x-ray data was reduced from 17° ± 6.0 to 3.1° ± 2.4; RMS error across the mean hip angle waveform was reduced from 20° to 5.3° (Fig 1: teal: x-ray gold standard; blue: IR marker; orange: skin). Skin derived kinematics overestimated the mean and underestimated the standard deviation of hip angle, while they overestimated the knee angle at touchdown (0 and 100% stride). The surgery required is minimally invasive and short (10 min), infra-red cameras are relatively inexpensive (~$1500), and the data processing workflow standard. Our results reveal between-animal or between-marker application errors result from placement of the knee marker on the skin, and large subcutaneous knee joint movement. Because the knee moves so substantially beneath the skin, no skin marker placement can be accurate throughout the stride. As the method is inexpensive and scalable to many animals and long bouts of locomotion, it will aid in establishing large databases of shared kinematic data with less between-lab variability.



Disclosures: **A.J. Spence:** None. **C.A. Patil:** None. **B. Rauscher:** None. **G. Moukarzel:** None.

Poster

PSTR408. Spinal Cord Injury: Methodologies

Location: WCC Halls A-C

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Program #/Poster #: PSTR408.15/Y13

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Unbiased and replicable assessment of locomotion changes for a rat spinal cord injury model using advanced gait analysis technology.

Authors: *J. BASTIDAS, M. LANG, R. BARRETT, S.-C. KIM, S. MONGIA, J. BELTRAN, D. BRUNNER, T. HANANIA;
PsychoGenics, Inc., Paramus, NJ

Abstract: Preclinical investigations into novel treatments for Spinal Cord Injury (SCI) have shown promise, but replicating these findings has been challenging thereby emphasizing the need for unbiased and reproducible methodologies in SCI research. In this study, we employed a graded thoracic (T8) SCI model using the IH impactor with 3 different force levels (170, 200, and 230 Kdyn) in female Lewis rats. Additionally, we evaluated the efficacy of well-known neuroprotective compounds (Rolipram, Riluzole, and Minocycline) in a 200 Kdyn SCI model. Our assessments included behavioral tests (BBB scale, horizontal ladder test), lesion size measurement and plasma biomarkers (GFAP, NF-H, Tau). We also utilized PsychoGenics' proprietary technology, NeuroCube® (NC), for automatic gait analysis. The NC system is a fully automated platform that uses computer vision and machine learning algorithms for gait analysis, limiting the possibilities for bias and error. A comprehensive collection of gait data was obtained, revealing clear distinctions between naïve rats and those with SCI in various gait parameters (increased base width, increased stride length, and decreased stand duration, and paw intensity). Furthermore, many of these gait changes displayed a strong correlation with BBB score and lesion size, indicating consistency and sensitivity in detecting changes based on injury severity in a SCI model. Particularly noteworthy were the significant changes and the strongest correlations observed in the front-limb gait parameters. The most robust changes in gait were found in SCI cohorts that underwent injury force of 200 and 230 Kdyn. When assessing the effects of various neuroprotective compounds, we found that only Rolipram showed a modest but significant change in BBB score and ladder test, while both Rolipram and Riluzole showed overall significant effects on gait geometry and dynamics. The sensitivity of the NeuroCube system and the richness of the data generated from evaluating multiple gait endpoints renders it very valuable in detecting efficacy of novel therapeutics. By considering these interconnected parameters within a multidimensional assessment, gait data can be analyzed and understood more comprehensively, resulting in enhanced sensitivity compared to unidimensional assessments.

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Psychogenics. **S. Mongia:** A. Employment/Salary (full or part-time):; Psychogenics. **J. Beltran:** A. Employment/Salary (full or part-time):; Psychogenics. **D. Brunner:** A. Employment/Salary (full or part-time):; Psychogenics. **T. Hanania:** A. Employment/Salary (full or part-time):; Psychogenics.

Poster

PSTR408. Spinal Cord Injury: Methodologies

Location: WCC Halls A-C

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: Defense Advanced Research Projects Agency (DARPA) under the Bridging the Gap Plus program N660012024075
The views, opinions and/or findings expressed are those of the author and should not be interpreted as representing the official views or policies of the Department of Defense or the U.S. Government.

Title: The Acute Cerebrospinal Management Implant: an Intelligent Catheter for Drainage of Cerebrospinal Fluid

Authors: J. KALTER[1], S. BABIN[1], A. DEVINNEY[1], L. DIAZ[1], G. COLES[1], K. BAHATI[1], K. MOORMANN[1], D. DAVIDAR[2], D. ROUTKEVITCH[2], K. KEMPSKI LEADINGHAM[2], A. HERSH[2], A. MANBACHI[2], N. THEODORE[2], ***F. TENORE**[1];
[1]Res. and Exploratory Develop., Johns Hopkins Univ. APL, Laurel, MD; [2]Johns Hopkins Univ., Baltimore, MD

Abstract: In the United States, nearly 250,000 people are affected with traumatic spinal cord injury (SCI), and nearly 17,000 new cases are diagnosed annually. Although once considered untreatable, significant advancements over the past century in the medical and surgical care of SCI have helped improve outcomes and reduce the likelihood of early mortality. These outcome improvements are largely due to early surgical decompression to reduce tissue injury and aggressive maintenance of elevated mean arterial pressure (MAP) to maintain spinal cord perfusion. Extensive physical rehabilitation is critical to maximizing neurological function after injury. Nonetheless, patients with traumatic SCI often face lifelong loss of motor, sensory, bowel, and bladder function. Ischemia, oxidative stress, loss of autoregulation, inflammation, and excitotoxicity follow and constitute the secondary phase of injury, the focus of this research. This secondary phase contributes to neuronal death, gliosis, hemorrhage, and cord edema, which can worsen the initial damage of the primary phase and last days to weeks. During this phase, spinal cord vascular resistance increases due to a rise in intrathecal pressure (ITP), which in turn decreases perfusion to the spinal cord. Recently, SCI patients have been treated with lumbar drains to reduce ITP and improve perfusion. Sampling the cerebrospinal fluid (CSF) for biomarkers in real time may potentially improve monitoring and response to therapy, including when considering newer modalities such as focused ultrasound stimulation. We have developed

a custom 4-lumen catheter capable of traditional CSF draining but also of measuring CSF biomarkers using fiber optics threaded through three peripheral lumens. These sensing fibers provide us with real-time continuous data related to intrathecal pressure, CSF temperature as well as chemical biomarkers, including total CSF protein concentration, which is often elevated following SCI as a result of cellular trauma and upregulation. We report on collection of pre-clinical data from a porcine model of spinal cord injury for multiple consecutive days for durations ranging from 15 minutes to four hours, examining the trends of the biomarkers in real-time and across multiple days. We observe rapid changes to CSF pressure in response to saline injections through the catheter and upon initial draining of CSF. Additionally, we collected real-time total protein data over the duration of the study and observed changes to its concentration over time. We believe these real-time data can be used to observe the evolution of the acute phase of spinal cord injury as well as impact of therapies during this phase.

Disclosures: **J. Kalter:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Johns Hopkins Applied Physics Laboratory. **S. Babin:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Johns Hopkins Applied Physics Laboratory. **A. Devinney:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Johns Hopkins Applied Physics Laboratory. **L. Diaz:** None. **G. Coles:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Johns Hopkins Applied Physics Laboratory. **K. Bahati:** None. **K. Moormann:** None. **D. Davidar:** None. **D. Routkevitch:** None. **K. Kempski Leadingham:** None. **A. Hersh:** None. **A. Manbachi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Johns Hopkins University. **N. Theodore:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Johns Hopkins University. **F. Tenore:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Johns Hopkins Applied Physics Laboratory.

Poster

PSTR408. Spinal Cord Injury: Methodologies

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Program #/Poster #: PSTR408.17/Y15

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH T32GM136577
DARPA N660012024075

Title: Non-contrast ultrasound for the determination of blood flow changes following spinal cord injury

Authors: *D. ROUTKEVITCH, E. BACA, Z. SOULE, N. KATS, A. M. HERSH, K. M. KEMPSKI LEADINGHAM, N. THEODORE, N. V. THAKOR, A. MANBACHI;
Johns Hopkins Univ., Baltimore, MD

Abstract: Background There is currently a lack of evidence guiding cardiovascular control of spinal cord injury (SCI) in critical care, resulting in secondary damage that occurs hours to days after injury. Previously, contrast-enhanced ultrasound has been used to quantify spinal cord perfusion to investigate changes in blood flow after SCI, but the clinical translatability is limited. Therefore, we present the following use of non-contrast ultrasound to measure blood flow after spinal cord injury and relate it to injury severity. **Methods** An i22LH8 probe was used in conjunction with the Aplio i800 ultrasound machine (Canon, USA) to capture superb microvascular imaging (SMI) videos of 24 Sprague-Dawley *in vivo* rat spinal cords after T11-T13 laminectomy. A contusion spinal cord injury was delivered at various impact forces: mild (100 kDyn), moderate (175 kDyn), and severe (250 kDyn). Post-injury images were taken, and the resulting images were analyzed to obtain blood flow as a function of distance from the injury. Simple parameters were computationally extracted from these curves and compared amongst the experimental groups. These parameters included injury extent through distance as well as flow at sites around the injury, including the epicenter (umbra), neighboring tissue (penumbra), and distal healthy tissue. **Results/Discussion** Umbra flow showed significant decrease from pre- to post-injury ($p<0.0001$), and trends of decreasing flow as severity increased. Injury extent showed significant increase from the mild to severe injuries ($p<0.05$). Flow in the penumbra, both rostrally and caudally, was significantly elevated as compared to pre-injury values, both in raw flow (rostral: $p<0.05$; caudal: $p<0.01$) and difference from umbra (rostral: $p<0.0001$; caudal: $p<0.0001$). However, only the rostral penumbra had significant differences between injury types. The raw flow in the rostral penumbra was greater in the severe injury when compared to moderate ($p<0.01$). The distal flow was unchanged from pre-to post-injury. **Conclusions** Non-contrast ultrasound can detect differences in blood flow between pre- and post-injury images. Certain parameters were also affected by severity of injury, including injury spatial extent and flow in the rostral penumbra. Further work will focus on the biological and functional significance of the described injury zones.

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Poster

PSTR408. Spinal Cord Injury: Methodologies

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Program #/Poster #: PSTR408.18/Y16

Topic: C.11. Spinal Cord Injury and Plasticity

Support: University of Southern Califonia Neurorestoration Center
Hellman Foundation

Title: Functional Ultrasound Imaging Reveals Dynamics of Micturition in the Human Spinal Cord

Authors: *K. A. AGYEMAN[1], D. J. LEE[4], S. SAKELLARIDI[2], J. RUSSIN[5], W. CHOI[5], A. ABEDI[5], R. V. EDGERTON[5], C. LIU[5], V. N. CHRISTOPOULOS[3];
[1]BIoengineering, [2]Univ. of California Riverside, [3]Univ. of California, Riverside, UC Riverside, Riverside, CA; [4]USC, [5]USC, Los Angeles, CA

Abstract: The spinal cord was traditionally neglected in the study of neural function because it was predominantly viewed simply as a pathway between the brain and the peripheral nervous system. As a result, its anatomy and physiology are not well understood as those of the brain. While strong evidence from pre-clinical work exists for the presence of regulatory neural-network-circuits that are responsible for vital motor, sensory and autonomic functions, its demonstration in humans has been challenging. The facial enclosures and the small cross-sectional dimension of the spinal cord combined with susceptibility to artifacts make it an unfavorable target for traditional neuroimaging techniques. The lack of knowledge in humans consequently, results in sub-optimal therapeutic strategies for treating dysfunctions associated with disease or injury to the spinal cord - with devastating health and cost burdens. For instance, current care for urinary dysfunction that affect over 30 million Americans relies mostly on symptomatic management with the use of catheters to empty the bladder**.** Given this context, there is a distinct need for developing neurotechnologies that make the functional study of the human spinal cord more accessible.
Here, we investigate the human spinal cord hemodynamic response during bladder filling and emptying, by leveraging the superior spatiotemporal properties of functional ultrasound imaging (fUSI). We characterize the spatial and temporal responses to micturition using simultaneous power Doppler (pD) spinal cord signal acquired through a partial lamina opening and intravesical bladder pressure recordings from 6 patients. Utilizing 2 bladder-filling cycles and 1 emptying cycle, interspersed by hold periods, we identified spinal cord regions in which the pD signal is strongly correlated with the bladder pressure ($r = 0.86 \pm 0.03$, Mean ± SE). We further combined classwise principal component analysis (cPCA) with linear discriminant analysis (LDA) and decoded the bladder pressure dynamics with high accuracy (92.8 ± 7.2 %, Mean ± SE), as well as identified regions that encode the effects of micturition, based solely on the spinal cord pD signal.
Overall, our study provides the first in-human application of fUSI to characterize hemodynamic responses of the spinal cord during urodynamics and offers direct evidence of the existence of spinal cord networks that control micturition. The high accuracy for decoding the state of the bladder based on the pD signal provides proof-of-concept that fUSI is an essential component for investigating function and building spinal cord machine interface for patients with urinary dysfunctions.

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Poster

PSTR408. Spinal Cord Injury: Methodologies

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Program #/Poster #: PSTR408.19/Y17

Topic: C.11. Spinal Cord Injury and Plasticity

Support: USC Neurorestoration Center
Hellman Fellowship

Title: Decoding micturition from the spinal cord using functional ultrasound imaging

Authors: *S. SAKELLARIDI[1], K. AGYEMAN[1], D. LEE[2], E. KREYDIN[2], J. RUSSIN[2], W. CHOI[2], A. ABEDI[3], H. ZONG[4], V. EDGERTON[5], C. LIU[2], V. CHRISTOPOULOS[1]; [1]Bioengineering, UC Riverside, Riverside, CA; [2]Neurosurg., [3]USC, Los Angeles, CA; [4]Univ. of California Los Angeles, Los Angeles, CA; [5]Rancho Res. Inst., Downey, CA

Abstract: Micturition characterizes the process in which neural pathways coordinate the activity of smooth muscles in the bladder to expel urine. Disease or injury of the nervous system can cause the re-emergence of involuntary urination, leading to urinary incontinence (UI). Elucidating the functional circuities in normal bladder control is key to identify abnormalities in UI patients. Our understanding of the brain circuits involved in micturition has grown immensely over the past decades. Yet, assessing the functional attributes of spinal cord in relation to bladder function has proven to be a challenge. This study sought to utilize functional ultrasound imaging (fUSI) to characterize the spinal cord hemodynamics in response to micturition in rats. fUSI is an emerging neuroimaging technology that represents a new platform with high sensitivity, spatial coverage and spatiotemporal resolution. Based on power Doppler (pD) imaging, fUSI measures changes in blood flow by detecting backscattered echoes from red blood cells moving within its field of view. After inducing anesthesia, animals (N=8) underwent multi-level laminectomy at T12-L2 to expose the lumbosacral spinal cord. This window allows the position of a 15 MHz ultrasound probe in a sagittal plane. A transducer was inserted and secured to the bladder dome to record the pressure dynamics. A catheter connected to an infusion pump was inserted in the same location to fill the bladder with saline at a constant rate of 0.1 ml/min. We acquired fUSI images for 50 minutes during which the animals were spontaneously voiding. Voiding is associated with typical abrupt changes in bladder pressure, which was directly verified by observing (through camera) outflow of urine from urethra. We measured pD changes of the spinal cord with respect to baseline – 10 min before starting to fill up the bladder. We identified region-specific hemodynamical changes associated with bladder pressure changes during filling. We further extended these results to predict an impending voiding. We used principal component analysis (PCA) as a feature extraction to optimally discard shared information between the two classes (class 0: pre-voiding, class 1: voiding). We then used linear discriminant analysis (LDA) and found that the PCA-transformed images predict an impending voiding with cross-validated accuracy 89.4% ± 3.3% (MEAN ± SE) about 4 seconds before the actual voiding. These results provide the first proof-of-concept that fUSI is a viable modality for developing ultrasonic spinal cord machine interface technologies to restore bladder function in patients with urinary incontinence (UI) – a technology that currently does not exist.

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Poster

PSTR409. Peripheral Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR409.01/Y18

Topic: D.02. Somatosensation – Pain

Support: R21HD091559
Johns Hopkins Sheikh Khalifa Stroke Institute

Title: Peripheral Modulation of Muscle Stiffness, Spasticity, and Motor Impairment After Cerebral Injury

Authors: *P. ARFA FATOLLAHKHANI[1], N. GOPAL[1], M. BIRD[1], N. L. SURESH[2], P. CELNIK[1], P. RAGHAVAN[1];
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Abstract: Muscle stiffness and spasticity cause severe disability in approximately 12 million people after neurologic injury of cerebral or spinal origin, such as a stroke. Muscle stiffness is thought to be caused by neural reflex-induced muscle overactivity. However, we hypothesized that non-neural alterations in the composition of the extracellular matrix of the muscle due to the accumulation of hyaluronan can produce muscle stiffness. Here we describe the effects of treatment of muscle stiffness using intramuscular hyaluronidase injections on hyperreflexia and motor impairment. Fourteen individuals with upper limb muscle stiffness after cerebral injury participated in a single-center, double-blind, randomized, placebo-controlled, Phase II trial of human recombinant hyaluronidase injections. All subjects received both human recombinant hyaluronidase and placebo injections in a random order in multiple upper limb muscles and were evaluated at baseline and 1-2 weeks after each injection visit. Resistance to passive movement was assessed using the modified Ashworth Scale (MAS), and reflex-induced muscle activity was measured using surface EMG from the medial biceps during tendon tap (n=45 per assessment) using an electronic hammer. Upper limb motor impairment was evaluated using the Fugl-Meyer Assessment (FMA). At baseline, the affected side showed a larger reflex EMG amplitude to tendon tap (mean ± SD= 9.24±10.58 V on the affected side vs. 1.81±2.28 V on the contralateral side). On the last post-injection visit, the muscle reflex EMG amplitude decreased by 3.31±12.68 V (36%) on the affected side. Moreover, on the last post-injection visit, compared to baseline, the MAS score for elbow extension decreased by 0.61±0.63 and the upper limb FMA increased by 7.29±5.72 on the affected side. The results suggest that peripheral modulation of muscle stiffness using hyaluronidase injections can reduce resistance to passive movement, hyperreflexia, and motor impairment after cerebral injury, suggesting that secondary changes in muscle composition contribute to these phenomena.

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Poster

PSTR409. Peripheral Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR409.02/Y19

Topic: D.02. Somatosensation – Pain

Support: NIH Grant 1R61AT012279-01

Title: Hyaluronic acid accumulation in shoulder muscles of patients with active versus latent post stroke shoulder pain

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Abstract: Recent studies suggest that the accumulation of Hyaluronic acid (HA) in muscles may contribute to myofascial post stroke shoulder pain (PSSP). PSSP can be classified as active or latent based on the severity of pain reported during shoulder external rotation and palpation of tender nodules in the pectoralis major (PMA) and infraspinatus (INF) muscles. T1rho MRI imaging of muscles has provided a novel avenue for investigating HA accumulation in muscles. In this study we assessed the T1rho relaxation time in the PMA and INF muscles of the affected and unaffected sides, as well as its relationship to active versus latent PSSP. 10 patients with unilateral PSSP (6 with Latent and 4 with Active PSSP) completed the T1rho MRI scans. Patients underwent 3T MRI with 3D-T1rho mapping at spinlock times of 0, 10, 20, 30 and 40 ms. T1rho relaxation times we derived using a non-linear decay model and mean T1rho values were extracted for the regions of interest for both the affected and unaffected sides. Both PMA and INF muscles had higher mean T1rho values on the affected side (PMA: 28.86±2.78, INF: 29.23±3.04) compared to the unaffected side (PMA: 27.87±2.11, INF: 26.26±1.89). Patients with active PSSP exhibited higher mean T1rho values in both muscles (PMA: 29.59±1.40, INF: 31.30±1.50) compared to those with latent PSSP (PMA: 28.37±3.46, INF: 27.85±3.09). These preliminary findings suggest that the level of HA accumulation may be associated with the severity of PSSP. Further investigations will lead to a better understanding of the underlying pathophysiology of myofascial pain in patients with stroke.

Disclosures: **A. Etemadimanesh:** None. **J. Huang:** None. **A. Ghasemi:** None. **P. Debs:** None. **R. Menon:** None. **L.M. Fayad:** None. **P. Raghavan:** None.

Poster

PSTR409. Peripheral Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

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Program #/Poster #: PSTR409.03/Y20

Topic: D.02. Somatosensation – Pain

Support: NIH grant R61AT012279

Title: Quantifying myofascial shear strain in chronic post-stroke shoulder pain with ultrasound shear strain measurements

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Abstract: Reduced shoulder joint mobility as a result of a stroke may lead to hyaluronic acid (HA) accumulation in muscles such as the pectoralis major and minor (PMA and PMI). We hypothesize that the accumulation of HA stiffens the muscles and prevents shear motion between the PMA and PMI muscles, eventually triggering post-stroke shoulder pain (PSSP). This study uses ultrasound shear strain imaging to quantify and compare the inter-layer relative motion between PMA and PMI muscles on the paretic and non-paretic sides of patients with PSSP. Our study included 7 PSSP patients with one shoulder predominantly affected by stroke. A bimanual arm trainer moved the patient repeatedly into shoulder external rotation at a rate of 0.5 Hz starting from a position of full internal rotation with a range of 30° excursion for each cycle. We acquired three ultrasound cine loops of 20 seconds from each side of a patient, using a Clarius L15 scanner held by a Sawyer robot, placed to access the pectoralis muscles. A cross-correlation-based speckle tracking technique was used to estimate the inter-frame lateral displacements, which were temporally accumulated to render the cumulative displacements. We included or excluded a particular trial based on the acquired data's usability and the reliability of displacement estimates, based on an independent rating by two co-authors with ultrasound speckle tracking expertise (MA, MALB). Shear strains between PMA and PMI muscles were calculated from the cumulative displacement fields. The shear strain (%) between the PMA and PMI muscles on the paretic side (median: 11.8) was generally lower than on the non-paretic side (median: 19.5). However, there were some discrepancies where the non-paretic side showed a lower shear strain and correlated with higher pain levels. Additional investigations are being conducted to understand the relationship between shear strain, myofascial pain, and HA accumulation. The results provide preliminary evidence of reduced shear strain in patients with post stroke shoulder pain suggesting a myofascial etiology. Ultrasound shear strain may serve as a biomarker for myofascial dysfunction and pain.

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Poster

PSTR409. Peripheral Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR409.04/Z1

Topic: D.02. Somatosensation – Pain

Title: Differentiating clinical characteristics of active versus latent myofascial shoulder pain post stroke

Authors: *J. HUANG[1], A. ETEMADIMANESH[2], S. BONWIT[2], R. NICKL[2], P. RAGHAVAN[3];
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Abstract: Post-stroke shoulder pain (PSSP) is a common condition significantly affects the quality of life for stroke patients. A large proportion of shoulder pain is thought to be myofasical. We sought to characterize patients with active versus latent myofascial pain based on clinical pain questionnaires and physical exam. Twelve patients with post-stroke shoulder pain completed the study. The patients were classified as having active vs. latent pain based on their subjective rating of pain during shoulder external rotation and the presence of palpable tender nodules in the muscle. Patients with active pain scored >/= 5/10 on the visual analog scale, where as patients with latent pain scored < 5/10. Six patients were classified as having active PSSP and 6 were classified as having latent PSSP on enrollment. The clinical assessments included HEAL core clinical pain questionnaires, physical examination (pain-pressure threshold using algometer, muscle stiffness assessment using the PACT device. Pain intensity and interference assessed using the PEG pain screening tool, the pain catastrophizing scale, geriatric depression scale, and the PHQ-2 showed higher levels of pain intensity, catastrophizing, and depression in patients calssified as having active PSSP compared to those classified as having latent PSSP. They also showed higher pain ratings when using an algometer and higher levels of stiffness using the PACT device, on both the stroke affected and unaffected sides compared to those with latent PSSP. The results suggest that compared to latent PSSP which appears to be largely localized to the affected side, active PSSP is more generalized and bilateral, suggesting possible underlying systemic inflammatory processes.

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Poster

PSTR409. Peripheral Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR409.05/Z2

Topic: D.02. Somatosensation – Pain

Support: CIHR Grant FRN-162434

Title: The ER chaperone ER Oxidoreductin (ERO1) plays a critical role in determining nociceptive sensitivity

Authors: *A. D. MAGUIRE[1,2], J. RAO[3,1], G. TENORIO[4,1], B. J. KERR[4,3,2,1];
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Abstract: Neuropathic pain (NP) is a debilitating condition caused by injury or disease of the somatosensory nervous system. Conventional pain treatments such as opioids are accompanied by severe side effects and are often ineffective against NP. Thus, there is a pressing need to develop more effective therapies. In many cases, NP originates in the peripheral nervous system (PNS) where primary pain-sensing neurons, termed nociceptors, become hyperexcitable and sensitized to stimuli. By understanding and manipulating neuronal hyperexcitability we may be able to develop new treatments for NP. Here, we focused on how excitability can be affected by endoplasmic reticulum (ER) and mitochondrial calcium dynamics. Particularly through calcium transfer from the ER to mitochondria at the mitochondrial associated membrane (MAM). The enzyme ER oxidoreductin 1 (ERO1) plays an important role in this process as it stabilizes ER IP3 receptors, allowing for efficient calcium transfer to mitochondria. We hypothesized that inhibiting ERO1 and thus reducing calcium transfer at the MAM would dampen nociceptor hyperexcitability and thereby reduce pain. First, we determined that the ERO1 antagonist EN460 can reduce pain. Using an acute model of nociception (tail flick test) and an acute inflammatory model (hind paw formalin injection), we found that 10 mg/kg intraperitoneal EN460 drastically reduced pain in male and female mice in both models. Additionally, we assessed the effects of EN460 on primary sensory neurons in culture. Because high levels of sprouting and complexity have been linked to NP, we assessed neurite outgrowth in the presence of EN460. We found that EN460 reduced the mean outgrowth, processes, and branches per cell, predominantly in female neurons. This suggests that EN460 may be able to impact the peripheral structural changes that underly NP. Finally, to confirm that EN460 can alter MAM calcium transfer we used a seahorse oxygraph and found that mitochondrial ATP production is decreased after EN460 treatment. Using a fluorescent live cell stain, we also found that mitochondrial reactive oxygen species (ROS) are decreased with EN460. Both results, much like the changes in outgrowth, were found to be stronger in female cells. Our findings suggest that ERO1 inhibition with EN460 can meaningfully impact calcium transfer at the MAM and sensory neuron plasticity. Based on these results we hypothesize that NP will be reduced with EN460 treatment, and that this effect may be stronger in females.

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Poster

PSTR409. Peripheral Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

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Program #/Poster #: PSTR409.06/Z3

Topic: D.02. Somatosensation – Pain

Support: CIHR Project Grant FRN183837
Canada Research Chair in Glial Neuroimmunology

Title: Fate-mapping CX3CR1+ cells to determine resident macrophage dynamics in the DRG in neuropathic pain

Authors: *M. S. HO;
Univ. of Alberta, Edmonton, AB, Canada

Abstract: Fate-mapping CX3CR1+ cells to determine resident macrophage dynamics in the DRG in neuropathic pain
Authors M.F.S. HO[1], A.O.V. FARIA[1], S. KHAN[1], J.R. PLEMEL[1,2,3,*], B.J. KERR[1,4,5,*]
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*contributed equally. **Disclosures M.F.S. Ho:** None **A.O.V. FARIA:** None **S. KHAN:** None **J.R. Plemel:** None **B.J. Kerr:** None
Abstract Typically rated as "the worst pain imaginable" by patients, neuropathic pain (NeP) results from damage to the somatosensory nervous system. NeP presents in many chronic pain conditions including but not limited to peripheral nerve injury (PNI) neuropathies such as from phantom limb pain, trigeminal neuralgia, and post-chemotherapy pain in cancer patients. Peripheral neuropathies are common yet debilitating and very difficult to treat due to a poor understanding of the complex underlying mechanisms. One fundamental initiator of NeP is the innate immune response to injury; the macrophages of the neuroimmune system initiate the NeP's well-characterized phenotype of prolonged hypersensitivity. Recent studies have found the acute impacts of tissue-resident macrophages (TRMs) at the dorsal root ganglion (DRG) under PNI; however, its roles throughout the shift from acute establishment to chronic maintenance of NeP remain to be known. To address a cell-specific targeting approach, our study employs a fate-mapping strategy with transgenic mouse lines to better define the localization, morphology, and dynamics of the TRMs in the spared nerve injury (SNI) model of PNI-NeP. Using $CX3CR1^{CreER}$ mice crossed with Ai9 mice (mice presenting the constitutive promoter line $ROSA26^{tdTomato}$), we follow the DRG resident macrophages over the acute-to-chronic timecourse and co-stain the DRGs to measure for proliferation and cell death. We observe that the TRMs increase in population density acutely at 7 days post injury (DPI) and then a decrease in density by 120DPI. Moreover, we observe that TRMs are morphologically diverse throughout the NeP timecourse. Ultimately, our findings provide evidence that TRMs dynamically change throughout the timecourse of the SNI model of PNI-NeP. By studying how DRG-resident macrophages change throughout NeP, we can understand the pathophysiology of NeP chronicity, thus providing context for future targets for immune-based neuropathic pain therapies.

Disclosures: M.S. Ho: None.

Poster

PSTR409. Peripheral Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR409.07/Z4

Topic: D.02. Somatosensation – Pain

Support: CIHR Project Grant FRN162434

Title: Neuroplasticity and pain: Examining the influence of exercise on nociception

Authors: *D. N. VILLARREAL[1], T. FRIEDMAN[1], A. D. MAGUIRE[2], G. TENORIO[3], B. J. KERR[3];
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Abstract: Exercise is praised for its extensive health benefits, but for people experiencing chronic pain, exerting oneself with movement seems counterintuitive and may be challenging to adhere to. The advantages of exercise on chronic pain states are severely understudied. Inflammatory conditions, specifically those that result in chronic pain, have been shown to increase neurite outgrowth in the sensory ganglia of mice and affect their electrophysiological properties. Whether this outgrowth exacerbates pain remains to be fully elucidated. Our data suggest that sensory neurons from adult mice show increased outgrowth in response to an aerobic exercise regimen. We now aim to study the transition between acute and chronic pain in mice and how it is affected by aerobic exercise.
We employed a 'hyperalgesic priming' model to study the transition from acute to chronic pain. Mice are injected subcutaneously with tumor necrosis factor-alpha (TNFα, 5 ng) to mimic an acute noxious stimulus. Once sensory thresholds resolve, they are treated with a second injection of the inflammatory-mediator prostaglandin (PGE2, 100 ng), leading to a prolonged hypersensitivity state. Before priming, naïve mice were given two-weeks of voluntary wheel-running to analyze the effects of aerobic exercise. Control mice received either a saline injection or no running treatment. We hypothesize that active mice will exhibit increased neurite outgrowth, as observed in previous pilot studies. However, with the addition of a noxious stimulus, the mice will experience prolonged pain due to the simultaneous structural changes in the sensory neurons.
Sensory neurons were stained with βIII tubulin to visualize the cytoskeletal structure, and neurite outgrowth analysis was conducted using MetaXpress 3.1. Our data indicate that wheel running promotes neurite outgrowth, but the addition of an acute TNFα injection significantly impairs the growth-promoting activity of wheel running. Pain assessment revealed that non-running mice recovered seven days after the first TNFα injection while running prolonged this pain for 72 hours. Our data indicate that aerobic activity alters the growth of sensory neurons, and a noxious

stimulus counteracts this change in growth status. Sex differences in these responses are currently being explored.

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Poster

PSTR409. Peripheral Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

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Program #/Poster #: PSTR409.08/Z5

Topic: D.02. Somatosensation – Pain

Support: T32 GM 8306-29
NIH/NINDS grant R01NS1132257 (DS)

Title: A role of CARTp/GPR160 in peripheral sensitization

Authors: *R. M. SCHAFER[1,2], L. A. GIANCOTTI[1,2], D. J. DAVIS[3], D. SALVEMINI[1,2]; [1]St. Louis Univ., St. Louis, MO; [2]Inst. for Translational Neurosci., St. Louis, MO; [3]Animal Modeling Core, Univ. of Missouri, Columbia, MO

Abstract: Chronic neuropathic pain occurs when there is damage or lesion to the somatosensory nerves. Understanding the mechanisms that cause neuropathic pain at the peripheral and central level offers possibilities to develop novel therapeutics as current therapeutics are inadequate and long-term use of opioids have adverse side-effects and abuse potential. We previously reported that activation of the G-protein coupled receptor 160 (GPR160) in the spinal cord by its ligand cocaine- and amphetamine-regulated transcript peptide (CARTp) contributes to the development of central sensitization associated with neuropathic pain states. The role of CARTp/GPR160 in the development of peripheral sensitization is not known and was explored in this study. Intraplantar (i.pl.) injection of CARTp in mice (n=7 males and n=4 females) evoked time-depended development of behavioral hypersensitivities (mechano- and cold-allodynia) that were completely lost in *Gpr160* knockout mice (n=3 males and n=5 females), suggesting GPR160 is dependent on CARTp-mediated hypersensitivities. *In vivo* and *in vitro* studies have shown CARTp signals via a $G_{i/o}$-GPCR. Similarly, an i.pl. injection of pertussis toxin (an ADP-ribosylating toxin that inactivates $G_{i/o}$ proteins) blocked CARTp-mediated behavioral hypersensitivities (n=4 males), supporting $G_{i/o}$-coupled signaling as a mode of action. In some studies, CARTp has been reported to exert its effects by modulating the N-methyl-D-aspartate receptor (NMDAR). The development of peripheral sensitization following an i.pl. injection of CARTp was not attenuated by the NMDAR antagonists, D-AP5 and MK-801 (n=3-6/group males). Therefore, CARTp-induced peripheral sensitization is not mediated by NMDAR activation; this supports our additional *in vitro* findings that CARTp is not a ligand for the NMDA receptor. In contrast, we have previously reported that, in the central nervous system, CARTp/GPR160-induced central

sensitization is mediated through mitogen activated protein kinases (MAPK) activation. We now extend these findings and show that at the molecular level, CARTp/GPR160-induced peripheral sensitization is driven by the MAPK, extracellular signal-regulated kinase (ERK). Thus, i.pl. injection of U0126, a well characterized ERK inhibitor, attenuated CARTp-induced peripheral sensitization (n=3 males). Our results suggest CARTp mediates peripheral sensitization in a $G_{i/o}$-coupled manner that is dependent on GPR160 and ERK.

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Poster

PSTR409. Peripheral Mechanisms of Neuropathic Pain I

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Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR409.09/Z6

Topic: D.02. Somatosensation – Pain

Support: NIDDK, ZIADK31116
Saint Louis University Startup Funds

Title: P2y$_{14}$ receptor in the spinal cord plays a role in a murine model of chronic neuropathic pain

Authors: *F. MUFTI[1,2], Z. CHEN[1,2], M. CAMPOLO[3], L. A. GIANCOTTI[1,2], E. ESPOSITO[3], J. ZHANG[1,2], S. CUZZOCREA[3], K. A. JACOBSON[4], D. SALVEMINI[1,2];
[1]Pharmacol. and Physiol., St. Louis Univ., Saint Louis, MO; [2]Inst. for Translational Neuroscience, St. Louis Univ., Saint Louis, MO; [3]Clin. and Exptl. Med. and Pharmacol., Univ. of Messina, Messina, Italy; [4]Mol Recog Sec, LBC, NIDDK-NIH, BETHESDA, MD

Abstract: Chronic neuropathic pain is a major health issue, but the underlying mechanisms are not well understood. Moreover, the available treatment options are not effective in all cases, and some have serious side effects. Recently, we identified the purinergic G protein-coupled receptor, P2Y$_{14}$ receptor (P2Y$_{14}$R), as a potential therapeutic target. We demonstrated that systemic administration of P2Y$_{14}$R antagonists reversed mechanical hypersensitivity in a traumatic nerve injury model of neuropathic pain. However, the sites of action of the pharmacological effects caused by targeting P2Y$_{14}$R and the mechanism of action remain to be understood. To study that we used a peripheral nerve injury model of neuropathic pain, in which mice go through unilateral chronic constriction injury of the sciatic nerve (CCI). Proteomic analyses of the dorsal horn of the spinal cord at time of peak neuropathic pain (mechano-/cold allodynia hypersensitivities) after CCI revealed significant increase in P2Y$_{14}$R expression. Intrathecal (i.th.) injection of a selective P2Y$_{14}$R antagonist (PPTN) reversed neuropathic pain behavior sensitivities in CCI mice (n=5) identifying the spinal cord as a potential site of P2Y$_{14}$R activity. P2Y$_{14}$R antagonists are not ligands for and did not activate opioid receptors. Noteworthy, direct activation of P2Y$_{14}$R in the spinal cord with i.th. injection of UDP-Glucose or

a highly selective and potent $P2Y_{14}R$ agonist (MRS2690) in naïve mice (n=3 UDP-Glucose, n= 5 MRS2690) was sufficient to recapitulate neuropathic pain behavioral phenotypes. On a mechanistic level, this depended on pertussis toxin-sensitive ($G\alpha_{i/o}$-linked) and $P2Y_{14}R$-mediated extracellular signal-regulated kinase (ERK) and p38 pathways. Naïve mice (n=4 per group) failed to develop UDP-Glucose or MRS2690 induced mechanical hypersensitivity when pertussis toxin was injected i.th. beforehand. In addition, i.th. injection of ERK inhibitor (n=5) prior to MRS2690 blocked the development of mechanical hypersensitivity in naïve mice, while i.th. injection of p38 inhibitor (n=4) attenuated the development of MRS2690 induced mechanical hypersensitivity. We hypothesize that $P2Y_{14}R$ in the spinal cord is a non-opioid based therapeutic target for neuropathic pain and its antagonists produce analgesic effects through the mitogen-activated protein kinases (MAPKs) pathways. Thus, targeting $P2Y_{14}R$ represents an innovative, non-opioid receptor target for neuropathic pain therapeutic development to address the current and growing patient needs.

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Poster

PSTR409. Peripheral Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR409.10/Z7

Topic: D.02. Somatosensation – Pain

Support: Center for Neuroplasticity and Pain (CNAP), supported by the Danish National Research Foundation (DNRF121)

Title: Assessment of Psychometric Function Slopes in Individuals with Type 1 Diabetes and Diabetic Peripheral Neuropathy

Authors: *C. MORCH[1], J. MØLLER RØIKJER[2], S. S. CROOSU[3], J. B. FRØKJÆR[3], T. M. HANSEN[3], L. ARENDT-NIELSEN[4], N. EJSKJÆR[2];
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Abstract: Introduction: Diabetic peripheral neuropathy (DPN) is a common complication of type 1 diabetes mellitus (T1DM), causing sensory impairments. These impairments are reflected in increased electrical and thermal thresholds jeopardizing nocifensive behavior. Paradoxical, concurrent to decreased pain perception often occurs. The aim of this study was to estimate the slope of the psychometric function to electrical stimulation to the foot as a proxy for perceptual accuracy. Methods: A total of 60 participants with T1DM (20 without (-)DPN, 20 with painless

(+)DPN, and 20 with painful (P)DPN) and 20 healthy controls (HC) were included in an initial cohort (MEDON: NCT04078516). The perception threshold to a 1ms rectangular electrical pulse was assessed as part of the assessment of peripheral nerve excitability using perception threshold tracking. Two types of specialized electrodes were used to preferentially active small and large sensory nerve fibers, respectively. During the experiment, the perception thresholds were estimated using a step up/down method. The slope and perception thresholds were estimated post-hoc by fitting the psychometrical function as a sigmodal function. The log-transformed slopes were compared between groups and nerve fiber types using a rmANOVA. Results: The excitability assessment could not be completed in 7 participants; therefore, data was reported from 73 participants. The slopes of the psychometric function were different between the groups ($p < 0.001$) being lowest for PDPN (0.14 [0.08; 0.24]mA^{-1}), followed by +DPN (0.31 [0.19; 0.52] mA^{-1}), -DPN (0.74 [0.46; 1,19] mA^{-1}) and HC (1.04 [0.64; 1.67] mA^{-1}). The slopes were generally not different between fiber types except for the +DPN group where, the slopes were lower for the large fibers (0.21 [0.11; 0.38] mA^{-1}) than the small fibers (0.48 [0.28; 0.82] mA^{-1}; $p<0.003$, Sidak) Discussion: In addition to an increased perception threshold which we have previously reported, this study showed lower slopes of the psychometric function in groups with more severe DPN, indicating an impaired discriminability of sensory stimuli. Individuals with lower psychometric function slopes are less accurate and reliable in distinguishing between stimuli there making perceptual protective judgments is impaired. Therefore, estimation of the psychometric function slope and thresholds appears to be valid tools for estimation the perceptual accuracy in persons with DPN.

Disclosures: **C. Morch:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); stock in Inventors Way. **J. Møller Røikjer:** None. **S.S. Croosu:** None. **J.B. Frøkjær:** None. **T.M. Hansen:** None. **L. Arendt-Nielsen:** None. **N. Ejskjær:** None.

Poster

PSTR409. Peripheral Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR409.11/Z8

Topic: D.02. Somatosensation – Pain

Support: NINDS R01NS119263

Title: Mechanism of sensory neuron sensitization via $Na_V1.8$ in anti-CV2/CRMP5 autoimmune neuropathy

Authors: ***N. L. A. DUMAIRE**[1], L. SALIH[1], L. FRANÇOIS-MOUTAL[1], J. HONNORAT[2], A. MOUTAL[1];
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Abstract: Mechanism of sensory neuron sensitization via Na$_V$1.8 in anti-CV2/CRMP5 autoimmune neuropathy Nicolas Dumaire[1, 2], Lyuba Salih[1, 2], Liberty François-Moutal[1, 2], Jérôme Honnorat[3], Aubin Moutal[1, 21]Department of Pharmacology and Physiology, and [2]Institute for Translational Neuroscience, Saint Louis University, Saint Louis, Missouri, [3] Team Synaptopathies and autoantibodies, Mechanisms in Integrated Life Sciences Institute, Lyon, France

Paraneoplastic neurological syndromes (PNS) are rare manifestations observed in cancer patients caused by autoantibodies. Upon immune-mediated apoptosis of tumor cells, naïve lymphocytes are exposed to the intracellular content which can trigger an autoimmune reaction. Recently classified "high-risk autoantibodies" such as CV2/Collapsin Response Mediator Protein 5 (CRMP5)-associated PNS are accompanied with a myriad of neurological symptoms, including peripheral painful neuropathy. The mechanisms by which autoantibodies can cause pain in more than 80% of patients with anti-CV2/CRMP5 are unknown. We found that anti-CV2/CRMP5 reacts with the sensory neurons of both rat and human dorsal root ganglion (DRG) expressing the pain voltage gated sodium channel Na$_V$1.8. In cultured DRG neurons, human anti-CV2/CRMP5 autoantibodies increased Na$_V$1.8 membrane localization. This suggested that the target of autoantibodies, CRMP5, could regulate Na$_V$1.8 trafficking. We found a single interaction domain between Na$_V$1.8 and CRMP5 in the first intracellular loop of the sodium channel. Using a peptide encompassing this unique amino acid sequence, we were able to dissociate the CRMP5/Na$_V$1.8 interaction. Consequently, the membrane localization of Na$_V$1.8 was potentiated in cultured sensory neurons. This shows that CRMP5 binding to Na$_V$1.8 regulates the trafficking of the channel. We next asked if anti-CV2/CRMP5 painful neuropathy relies on the increase of Na$_V$1.8 trafficking and function. Injection of human anti-CV2/CRMP5 antibodies in the paw, lowered the threshold to mechanical stimuli. This sensitization was prevented by concomitant administration of a Na$_V$1.8 inhibitor. Together, our results show that pain sensitization in anti-CV2/CRMP5 painful neuropathy is mediated by a dysregulation of Na$_V$1.8 trafficking, probably resulting from a loss of Na$_V$1.8/CRMP5 interaction.Funding: NINDS R01NS119263

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Poster

PSTR409. Peripheral Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

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Program #/Poster #: PSTR409.12/Z9

Topic: D.02. Somatosensation – Pain

Support: NCATS UL1TR003015
NCATS KL2TR003016

Title: Evaluation of the role of α6 nicotinic receptor subunit in chronic pain using α6-GFP transgenic mice

Authors: *D. WEI[1], R. PARKER[2];
[1]Small Animal Clin. Sci. VA-MD Col. of Vet. Med., Radford, VA; [2]Small Animal Clin. Sci. VA-MD Col. of Vet. Med., Blacksburg, VA

Abstract: Novel treatments for chronic pain are needed as current treatment options are either inconsistent in efficacy across patients, or they have negative side effects. Nicotinic acetylcholine receptors (nAChRs) are attractive targets for pain studies because the various subunits assemble to form pentameric receptors expressed in many neuronal cell types, and there is a wide range of ligand sensitivity depending on the receptor subtype. We are studying the role of nAChRs during chronic pain to determine if they may be appropriate drug targets in the treatment of pain. One specific nicotinic receptor subunit of interest is the α6 subunit, which has been shown to be downregulated during the development of allodynia (Wieskopf et al., 2015; PMID: 25972004). We evaluated the α6-GFP transgenic mouse by performing von Frey withdrawal testing after unilaterally treating the mice with a spared nerve injury (SNI), injection of complete Freund's adjuvant, or a sham treatment (Mackey et al., 2012; PMID: 22836257). The tester is blinded to treatment. The 50% withdrawal threshold is then measured using the up-down method and the threshold is compared between the ipsilateral and contralateral limbs. In the SNI model, using a mixed effects model, we found that there are no significant differences between the transgenic (Tg) and wildtype (WT) mice (n_{Tg} = 11 and n_{WT} = 9); furthermore, no significant sex differences were observed (n_{males} = 11 and $n_{females}$ = 9). We did measure a change in withdrawal threshold in the treated limb up to four weeks after surgery (n=20), p = <0.0001. We quantitated α6 gene expression in dorsal root ganglion (DRGs) by qPCR (n = 9; n_{Tg} = 4, n_{WT} = 5) and saw no significant difference between WT and Tg animals when analyzed via two-way ANOVA. This preliminary data confirms that the α6-GFP mouse is a good model to evaluate α6 nAChR gene expression during allodynia using immunohistochemistry (IHC) or whole cell patch clamp electrophysiology. Quantitative IHC is used to measure α6-GFP expression in CGRP positive and negative cells. Additionally, we are recording nicotinic receptor currents in freshly isolated DRG after induction of allodynia. Therefore, we may evaluate the role of the α6 nAChR subunit during the induction of allodynia.

Disclosures: D. Wei: None. **R. Parker:** None.

Poster

PSTR409. Peripheral Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR409.13/Z10

Topic: D.02. Somatosensation – Pain

Support: NIDDK/UW-Madison K12 Urologic Research (KURe) Career Development Grant K12DK100022
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UW-Madison Office of the Vice Chancellor for Research and Graduate

Education
Wisconsin Alumni Research Foundation

Title: Bladder inflammation alters CGRP and TRPV1 expression in paw afferent-rich dorsal root ganglia in a mouse model of visceral pain

Authors: *E. L. TRAN, S. A. STUEDEMANN, L. A. HAHN, L. K. CRAWFORD; Pathobiological Sci., Univ. of Wisconsin Madison, Madison, WI

Abstract: Bladder pain syndrome, which can stem from bladder inflammation, is often accompanied by referred somatic pain that is poorly understood and may be underdiagnosed in patients. In mice, bladder inflammation, or cystitis, produces secondary mechanical hypersensitivity in the hind paw skin, mirroring lower limb hypersensitivity seen in clinical studies of bladder pain patients. Yet, the mechanisms underlying this “referred pain” in mouse models of cystitis remain unclear. Literature suggests calcitonin gene related peptide (CGRP) and transient receptor potential channel V member 1 (TRPV1) increase in bladder afferents after cystitis. However, paw afferents are seldom examined in cystitis studies, despite the compelling paw phenotypes. We hypothesized that cystitis increases TRPV1 and CGRP in hind paw afferents. The L3-L5 dorsal root ganglia (DRG), rich in paw afferents, were analyzed 48 hours after induction of the intravesicular acrolein model of cystitis in adult female mice. Image analysis of multiplex immunofluorescent staining was conducted by experimenters blinded to treatments groups. Compared to vehicle-treated controls, mice with cystitis exhibited fewer CGRP-positive neurons ($p = 0.0493$, mixed effects model); however, there was a simultaneous increased density of CGRP expression amongst CGRP-positive cells, as measured by a shift towards higher area fraction ($p = 0.042$, Kolmogorov-Smirnov (KS) test). Cystitis also increased the density and intensity of TRPV1 expression amongst the TRPV1-positive cell population ($p = 0.0106$, KS test). While there was no difference in TRPV1-positive neuron counts, there was a significant shift towards increased cell size of TRPV1-positive neurons ($p = 0.0048$, KS test), suggestive of phenotype switching. Collectively, these data strongly suggest that cystitis induces molecular changes in L3-L5 DRGs, potentially altering paw afferents. In parallel, we used dual retrograde tracer injections to determine if bladder and paw afferents co-populate any of the T13 to S1 DRG in our mouse lines. While mice demonstrated zero bladder afferents in L3 and sparse bladder afferents in the L4 and L5 DRGs, the next caudal L6/S1 DRG was a site where both bladder and paw afferents were more routinely found, supporting the possibility of peripheral, intraganglionic mechanisms for referred hind paw pain. Ongoing studies examine cystitis-induced changes in L6/S1 DRG and correlate molecular findings to severity of hind paw pain. Further understanding of paw-innervating DRG will elucidate biological mechanisms of referred pain in bladder disease, perhaps highlighting the DRG as a promising target for improved pain therapies.

Disclosures: E.L. Tran: None. **S.A. Stuedemann:** None. **L.A. Hahn:** None. **L.K. Crawford:** None.

Poster

PSTR409. Peripheral Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR409.14/Z11

Topic: D.02. Somatosensation – Pain

Support: Craig H. Neilsen Foundation 882060

Title: Increased Nav1.8 Resurgent Currents Contribute to Nociceptive Neuron Hyperexcitability Induced by Spinal Cord Injury

Authors: *Y. XIAO[1], Y. PAN[1], N. LIU[2], T. CUMMINS[1];
[1]Dept. of Biology, Sch. of Science, Indiana University-Purdue Univ. Indianapolis, Indianapolis, IN; [2]Spinal Cord and Brain Injury Res. Group, Stark Neurosciences Res. Institute, Dept. of Neurolog. Surgery, Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Chronic neuropathic pain associated with spinal cord injury (SCI) is a major medical problem. Studies have shown that moderate thoracic (T10) SCI can substantially up-regulate excitability of small diameter dorsal root ganglion (DRG) neurons in rodents. This suggests that SCI pain may arise from pathological changes in peripheral neurons. However, the underlying molecular mechanisms remain unknown. In this study, we show that SCI significantly increases classical TTX-resistant sodium currents (Nav1.8 and Nav1.9), but not TTX-sensitive sodium currents, in rat small DRG neurons. We also show that SCI almost doubles the proportion of small DRG neurons that produce TTX-resistant resurgent currents, which are unusual currents typically evoked during the repolarization phase of action potentials. These currents have a very slow onset and decay, indicating that they are mainly carried by Nav1.8. SCI increases the amplitude of the slow resurgent currents but fails to affect voltage-dependence of activation of the currents. We also show that the increase in Nav1.8 induced by SCI can be reversed by ZL0177, a small molecule that mimics the critical residues in FHF4A binding to the C-terminus of sodium channels. In SCI small DRG neurons, ZL0177 not only decreases the classical and resurgent currents of Nav1.8, but also reduces the percentage of the neurons with Nav1.8 resurgent currents. Furthermore, we show that ZL0177 significantly downregulates hyperexcitability of small DRG neurons induced by SCI. Therefore, our data demonstrate that Nav1.8 dysfunction contributes to SCI-induced hyperexcitability of nociceptive neurons and that the FHF-binding site on Nav1.8 C-terminus might be a promising therapeutic target for the treatment of SCI pain.

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Poster

PSTR409. Peripheral Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR409.15/Z12

Topic: D.02. Somatosensation – Pain

Support: NS055251
R25GM083270
R01HL126887

Title: Voltage-gated $Ca_v2.2$ calcium ion channels mediate CFA- induced heat hypersensitivity via interleukin signaling

Authors: *A.-M. SALIB[1,2], M. J. CRANE[3], A. M. JAMIESON[3], D. LIPSCOMBE[2];
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Abstract: Voltage-gated calcium ion channels (Ca_v) are essential for the detection and transmission of noxious stimuli. Previously, our lab demonstrated that transient capsaicin-induced heat hypersensitivity in peripheral Trpv1 nociceptor nerve endings in skin depends on $Ca_v2.2$ channel activity (DuBreuil et al., 2021). Here, we show that Cav2.2 channels are also critical for the development of prolonged Complete Freund Adjuvant (CFA) induced heat hypersensitivity. Within one day of intraplantar CFA injection, wild type (WT) mice exhibit robust heat and mechanical hypersensitivity (n=8), while global $Ca_v2.2$ knockout mice (KO) only develop mechanical allodynia (n=12). Using CFA as a model of prolonged neuroinflammation, we show that $Ca_v2.2$ channels have a highly specialized role in the development of heat, but not mechanical, hypersensitivity, further supporting our previous studies using a capsaicin model of transient neuroinflammation. Within one day of intraplantar CFA injection, we measured increased levels of ten different cytokines in hindpaw interstitial fluid at the site of injection. Here, we show that the cytokine imprint, particularly levels of interleukins IL-1α and IL-6, are altered in mice lacking $Ca_v2.2$ (KO) and in wild type (WT) mice co-injected with CFA and the highly selective $Ca_v2.2$ inhibitor, ω-conotoxin GVIIA (n=8/group). The precise mechanism by which $Ca_v2.2$ channels mediate heat hypersensitivity in peripheral nerve endings is not fully understood, but it is likely that $Ca_v2.2$ channels are the major source of intracellular calcium that triggers vesicular release of proinflammatory mediators from nerve endings. The vesicular cargo of sensory neurons includes signaling molecules such as neuropeptides (e.g., CGRP and Substance P), glutamate, and damage-associated molecular patterns such as ATP. Reducing the release of these signaling molecules from neurons drives changes in plasma extravasation, dendritic cell migration, adhesion of immune cells to the vasculature, and the production and release of proinflammatory cytokines from stimulated leukocytes. Our data show that $Ca_v2.2$ channels in skin are critical for neuroimmune signaling cascades that trigger both transient and prolonged forms of heat hypersensitivity. Our ongoing studies will establish the relationship between $Ca_v2.2$ channel activation and interleukin release, and the resulting downstream effects of interleukins on the development of transient and prolonged hypersensitivity.

Disclosures: **A. Salib:** None. **M.J. Crane:** None. **A.M. Jamieson:** None. **D. Lipscombe:** None.

Poster

PSTR409. Peripheral Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR409.16/Z13

Topic: D.02. Somatosensation – Pain

Support: R01 NS070711 (CLS)
R37 NS108278 (CLS)

Title: Contributions of keratinocytes to traumatic nerve injury induced mechanical and cold hypersensitivity

Authors: *C. M. MECCA, A. R. MIKESELL, O. ISAEVA, C. L. STUCKY;
Cell. Biol. Neurobio. and Anat., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Neuropathic pain is a debilitating health condition that affects nearly 10% of the general population and is often associated with severe cutaneous mechanical and cold allodynia. Despite its prevalence, neuropathic pain remains one of the most challenging types of chronic pain to manage effectively. The study of non-neuronal peripheral keratinocytes in neuropathic pain may uncover novel peripheral analgesic targets that reduce the potential for undesired central nervous system (CNS) side effects and provide increased efficacy in treating pain. Recent evidence suggests that keratinocytes, a major cell type in the epidermis, play an important role in the initial processing of mechanical and thermal information and may contribute to neuropathic pain. The aim of this study is to evaluate the role of keratinocytes to touch and cold pain after traumatic nerve injury, where the keratinocytes themselves are not directly injured. To investigate this, a surgical model of traumatic nerve injury, the tibial spared nerve injury (tSNI), was used. We found that optogenetic inhibition of keratinocytes can attenuate tSNI-induced cold and mechanical allodynia but does not alter behavioral responses to radiant heat. Furthermore, extrudate collected from cultured keratinocytes isolated from tSNI mice promotes (1) behavioral mechanical hypersensitivity when injected into naïve mouse paws and (2) increased calcium responses to a low potassium test stimulus (20mM KCl) in naïve sensory neuron cultures in calcium imaging experiments. Together, these findings highlight the importance that keratinocytes may play in hypersensitivity after nerve injury.

Disclosures: C.M. Mecca: None. **A.R. Mikesell:** None. **O. Isaeva:** None. **C.L. Stucky:** None.

Poster

PSTR409. Peripheral Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

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Program #/Poster #: PSTR409.17/Z14

Topic: D.02. Somatosensation – Pain

Title: Monocyte-derived cells control the induction of hyperalgesic priming through intracellular NAAA-regulated lipid signaling

Authors: Y. FOTIO, ***A. MABOU TAGNE**, E. SQUIRE, H.-L. LEE, F. AHMED, D. PIOMELLI;
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Abstract: Chronic pain is a burdensome condition necessitating improved management strategies. Understanding the mechanisms underlying chronic pain development is crucial for identifying therapeutic targets. The hyperalgesic priming (HP) model, involving prolonged hypersensitivity following an initial inflammatory insult, sheds light on the molecular mechanisms contributing to pain chronification. We investigated the role of N-acylethanolamine acid amidase (NAAA), a cysteine hydrolase, in HP induction. HP was induced by injecting a low dose of IL-6 into the hind paw of male and female mice, causing acute hypersensitivity to heat stimuli lasting less than 24 hours. Subsequent administration of PGE2 one week later prolonged heat hyperalgesia in primed mice, while non-primed animals experienced transient hyperalgesic effects. Pharmacological inhibition of NAAA using ARN19702 was employed to assess its contribution to HP. Preemptive ARN19702 administration relieved IL-6-induced heat hyperalgesia yet had no effect on long-lasting hypersensitivity caused by PGE2 in primed animals. However, post-IL-6 injection ARN19702 administration prevented HP development, as indicated by the absence of lasting hyperalgesic effects of PGE2 in primed mice. Genetic loss-of-function experiments in NAAA-deficient mice confirmed these results. Investigation with the peripherally-preferring NAAA inhibitor, ARN726, suggested that NAAA likely drives HP in peripheral tissues. Increased NAAA activity disrupted PEA-regulated PPAR-α signaling in the periphery, while PEA administration effectively halted HP induction. Monocyte-derived cells were identified as the cellular substrate for NAAA-mediated HP induction. Mice lacking NAAA in CD11b+ cells exhibited reduced IL-6-induced hyperalgesia and failed to develop lasting hypersensitivity to PGE2, while NAAA overexpression in these cells resulted in persistent hypersensitivity. Disruption of PPAR-α signaling in monocyte-derived cells abolished HP, emphasizing intact PPAR-α signaling requirement. Together, these findings underscore the indispensable role of peripheral NAAA-regulated PPAR-α signaling in monocyte-derived cells for HP induction. Insights gained into the underlying mechanisms of HP may contribute to novel therapeutic approaches for chronic pain management.

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Poster

PSTR409. Peripheral Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

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Program #/Poster #: PSTR409.18/Z15

Topic: D.02. Somatosensation – Pain

Support: NIDDK Intramural Research Program ZIA DK043304-29

Title: Exploring the role of Gβ5/R7-RGS complex in nociceptors of the trigeminal ganglion

Authors: *N. MOKHASI, J. STAPLES, J. ZHANG, K. DEGNER, M. PANDEY, W. SIMONDS;
Natl. Inst. of Diabetes and Digestive and Kidney Dis., Bethesda, MD

Abstract: The trigeminal ganglion contains cell bodies of the sensory nerves that innervate the face and head to mediate itch, pain and other sensations. Chronic damage to these nerves may cause painful conditions like trigeminal neuralgia. Clinical treatment of trigeminal pain often relies on anticonvulsant drugs with significant neurological side effects. Lack of effective and convenient treatments of the disease highlights the need for more effective and targeted therapeutic approaches. GNB5 encodes the protein Gβ5, a divergent member of the G protein β family, that binds with each member (RGS6, 7, 9 and 11) of the R7 subfamily of the regulators of G signaling (R7-RGS) to form obligate Gβ5/R7-RGS complexes. The Gβ5/R7-RGS complex proteins are expressed primarily in neuronal cells and function as GTPase-Activating Proteins to dampen signaling downstream of Gi/o-coupled receptors. Studies in our lab of conditional knockout of Gnb5 from sensory neurons mediated by Advillin-Cre in *Gnb5*$^{fl/fl}$ mice have shown significant reduction in mechanical, thermal and chemical nociception. However, Rgs7-cre mediated conditional Gnb5 knockout only diminished mechanical nociception. Additionally, treatment with 2- hydroxysaclofen (a GABA-B receptor antagonist) abolished the decrease of mechanical nociception in both Advillin- Cre$^{+/-}$; *Gnb5*$^{fl/fl}$ and Rgs7-Cre$^{+/-}$; *Gnb5*$^{fl/fl}$ mice implicating the involvement of GABA-B receptor signaling in Gβ5/Rgs7-associated mechanical nociception. To further understand the cellular and molecular mechanisms of Gβ5/R7-RGS complex-mediated nociception in trigeminal ganglion, we conducted bioinformatic analysis of publicly available scRNAseq datasets using the established sensory neuron markers, in order to find the cell-type expression patterns of Gnb5 and R7-RGS transcripts. We found high levels of Gnb5 expression in all sensory neuron cell types of the trigeminal ganglion while the expressions of members of R7-RGS subfamily showed distinctive patterns among different cell types. Rgs7 and Rgs11 were found highly expressed in non-peptidergic neuron clusters, while Rgs9 was mainly localized to peptidergic clusters. Rgs6 showed little or no expression across the trigeminal ganglion. Using RNAscope Hi-Plex in-situ hybridization techniques, we aim to verify these findings and explore the potential mechanisms of the behavioral test results.

Disclosures: N. Mokhasi: None. **J. Staples:** None. **J. Zhang:** None. **K. Degner:** None. **M. Pandey:** None. **W. Simonds:** None.

Poster

PSTR409. Peripheral Mechanisms of Neuropathic Pain I

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Program #/Poster #: PSTR409.19/Z16

Topic: D.02. Somatosensation – Pain

Support: NIH grant NS065926
Hoba Therapeutics ApS

Title: Meteorin Treatment Resolves Cisplatin Induced Peripheral Neuropathic Pain in Mice

Authors: *L. HE[1], I. SANKARANARAYANAN[1], T. M. MADSEN[2], K. A. PETERSEN[2], G. MUNRO[2], T. J. PRICE[1];
[1]Univ. of Texas at Dallas, Dept. of Neurosci., Richardson, TX; [2]Hoba Therapeut. ApS, Copenhagen, Denmark

Abstract: Cisplatin is a potent alkylating agent that can induce chemotherapy-induced peripheral neuropathy (CIPN) and chronic pain. This severely limits its usefulness as a cancer treatment and negatively impacts patient outcomes. Evidence suggests that CIPN arises as a consequence of molecular alterations in dorsal root ganglion (DRG) neurons and their neighboring satellite glial cells that lead to functional changes generating hyperexcitability. CIPN is also associated with a more distal loss of intraepidermal nerve fibers in the skin. Meteorin is a secreted protein that plays a fundamental role in the development of the nervous system. Previous studies have shown that systemic treatment with recombinant mouse meteorin (rmMeteorin) produces robust, long-lasting antinociception in rodent models of peripheral neuropathic pain and paclitaxel induced peripheral neuropathy. To investigate a broader role for Meteorin therapy in CIPN, we treated female ICR mice with 2 cycles of cisplatin (2mg/kg, 5 x i.p.) or vehicle through Days 1-15 in the current study. Hind paw withdrawal thresholds to mechanical stimulation were assessed using Von Frey filaments. Once hypersensitivity was established, rmMeteorin (1.8mg/kg, 5 x s.c.) or vehicle was administered every other day from Day 18, and withdrawal thresholds routinely assessed until Day 79. Lumbar DRGs and hind paw skin were collected from 4 mice for each treatment group on Day 34 for immunohistochemical processing. Cisplatin-induced mechanical hypersensitivity was significantly reversed after a second injection of rmMeteorin and persisted throughout, and even beyond the dosing duration. Moreover, cisplatin-mediated changes in DRG expression of the gap junction protein Connexin43 and the satellite glial cell marker glutamine synthase were both restored by rmMeteorin administration. Intraepidermal nerve fibers in the skin were also protected from cisplatin-induced loss by rmMeteorin. The resolution of preclinical behavioral and cellular correlates of CIPN in cisplatin mice with rmMeteorin treatment, supports similar findings obtained in mice with paclitaxel-induced neuropathic pain.

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Poster

PSTR409. Peripheral Mechanisms of Neuropathic Pain I

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Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR409.20/Z17

Topic: D.02. Somatosensation – Pain

Support: European Union's Horizon 2020 grant agreement No 956477

Title: Pro-inflammatory macrophages transfer of miR-155 enriched extracellular vesicles to dorsal root ganglia neurons

Authors: F. PICCO[1], L. ZEBOUDJ[2], S. OGGERO[2], V. PRATO[3], N. GAMPER[4], *M. MALCANGIO[5];
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Abstract: Peripheral nerve damage is associated with up-regulation of non-coding RNAs such as miR-21 in sensory neurons and accumulation of macrophages in the dorsal root ganglia (DRG). We have previously reported that sensory neurons communicate with macrophages by shuttling exosomes that regulate nociceptive mechanisms under neuropathic pain conditions (PMID: 29176651). Here, we investigated whether macrophages release extracellular vesicles (EVs) containing specific microRNAs which are transferred to sensory neurons. We polarised bone marrow derived macrophages (BMDMs) to M1-like phenotype by lipopolysaccharide incubation (LPS 100 ng/ml for 16 h), isolated EVs in supernatants by ultracentrifugation at 100,000 g at 4ºC for 1 h and performed EV size distribution analysis (NS300 Nanoparticle Tracker). In both vehicle and LPS supernatants we quantified $2*10^8 \pm 0.4$ particles/ml and mean size of 150 ± 7.3 nm (n=4 replicates). Next, using Western blot we quantified BMDM-derived EV markers and found expression of tumour susceptibility gene 101 (TSG-101; exosome marker), but not annexin-A1 (ectosome marker) and calnexin (intracellular marker that rules out extracellular contamination). Thus, these results suggest that BMDMs release mainly exosomes after LPS stimulation, and we then evaluated whether such exosomes contain a signature microRNA (miR). Using RT-qPCR of six selected miRs we found that LPS induced intracellular upregulation of miR-155 (427 ± 74 -fold increase over internal Spike-in control) that was also found in the EVs (24 ± 7 -fold increase; n=5-6 replicates). Relevantly, miR-155 was not enriched in cultured DRG neuron-released EVs following incubation of LPS (100 ng/ml, 3 h) and capsaicin (1µM, 3 h) whilst as expected miR-21 was found in capsaicin-released EVs (2 ± 0.3 -fold increase; n=3 replicates). Having found miR-155 in EVs derived from LPS polarized BMDMs, we next transfected BMDMs with pHluorin-CD63 plasmid, isolated CD63-pHluorin-tagged EVs and exposed DRG neurons for 24h with these EVs (100 µg). We analyzed possible EV occurrence in neurons by AiryScan high resolution confocal microscopy and observed that some neurons (marker Tuj1) contained pHluorin-EVs. Moreover, we detected higher levels of miR-155 (4 ± 0.7 -fold increase), interleukin-6 (*Il-6*, 53 ± 20 -fold increase) but not *glycoprotein gp130* or miR-21 in DRG neurons incubated with LPS-BMDM-derived EVs (n=4 replicates). Our findings suggest that miR-155 is enriched in EVs released by M1-like macrophages and such EVs are taken up by neurons and modulate gene expression.

Disclosures: **F. Picco:** None. **L. Zeboudj:** None. **S. Oggero:** None. **V. Prato:** None. **N. Gamper:** None. **M. Malcangio:** None.

Poster

PSTR409. Peripheral Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR409.21/Z18

Topic: D.02. Somatosensation – Pain

Support: 1PO1NS119159-01A1

Title: Effects of systemic oxytocin administration on UVB-induced nociceptive hypersensitivity and tactile hyposensitivity in mice.

Authors: *M. BOADA, **S. GUTIERREZ**, J. EISENACH;
Anesthesiol., Wake Forest Univ. Baptist Med. Ctr., Winston Salem, NC

Abstract: UVB radiation induces cutaneous inflammation, leading to thermal and mechanical hypersensitivity. Here we examine the mechanical properties and profile of tactile and nociceptive peripheral afferents functionally disrupted by this injury and the role of oxytocin (OXT) as a modulator of this disruption. We recorded intracellularly for L4 afferents innervating the irradiated area (5.1 J/cm2) in 4-6 old week male mice (C57BL/6J) after administering OXT IP, 6 mg/Kg. The distribution of neurons characterized was shifted by UVB radiation to a pattern observed after acute and chronic injuries and reduced mechanical thresholds of A and C- high threshold mechanoreceptors while reducing the sensitivity of tactile afferents (low threshold mechano receptors). UVB radiation did not change somatic membrane electrical properties or fibers conduction velocity. OXT systemic administration rapidly reversed these peripheral changes toward normal in both low and high-threshold mechanoreceptors and returned the distribution of characterized neurons toward normal. OXT and V1aR receptors were present on the terminals of myelinated and unmyelinated afferents innervating the skin. We conclude that UVB radiation, similar to local tissue surgical injury, cancer metastasis, and peripheral nerve injury, alters the distribution of low and high threshold mechanoreceptors afferents and sensitizes nociceptors while desensitizing tactile units. Acute systemic OXT administration partially returns all of those effects to normal.

Disclosures: **M. Boada:** None. **S. Gutierrez:** None. **J. Eisenach:** None.

Poster

PSTR409. Peripheral Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR409.22/Z19

Topic: D.02. Somatosensation – Pain

Support: JSPS KAKENHI 22K21034

Title: Involvement of Lysophospholipids in trigeminal ganglion in neuropathic pain of orofacial region

Authors: *R. KURISU, Y. YAMAZAKI, S. MAEDA;
Tokyo Med. and Dent. Univ., Bunkyo-ku / Tokyo prefecture, Japan

Abstract: Object: The mechanism of neuropathic pain in the orofacial region is still unclear, and treatment is often difficult. Neuropathic pain refers to a type of chronic pain that is caused by damage or dysfunction in the nervous system. Lysophosphatidic acid (LPA), one of the lysophospholipids, is involved in neuropathic pain. In this study, we aimed to elucidate the involvement of LPA in facial mechanical allodynia that develops after infraorbital nerve injury (IONI) in a rat model of infraorbital nerve injury.
Methods: We used male Sprague Dawley rats in all experiments. The rats were deeply anesthetized with an intraperitoneal (i.p.) injection of a mixture containing butorphanol (2.5 mg/kg; Meiji Seika Pharma, Tokyo, Japan), midazolam (2.0 mg/kg; Sandoz, Tokyo, Japan), and medetomidine (0.15 mg/kg; Zenoaq, Fukushima, Japan). The top of the head was shaved, and the rats were then placed in a stereotaxic apparatus. After exposing the skull, a small hole with a diameter of 1 mm was drilled directly above the ipsilateral trigeminal ganglion to the infraorbital nerve injury (IONI) or sham operation. The guide cannula was extended into the trigeminal ganglion through the hole and was fixed. Following cannulation, the rats were allowed to recover for seven days before conducting experiments. One week later, the left infraorbital nerve was partially ligated under deep anesthesia to induce the IONI model in rats treated with LPA (IONI-LPA). Other groups included naïve rats treated with LPA (naïve-LPA), IONI rats treated with saline (NS) (IONI-NS), and naïve rats treated with NS (naïve-NS). The mechanical head-withdrawal threshold (MHWT) of the left whisker pad skin was measured using von Frey filaments every other day before and up to 14 days after the injury. Lysophosphatidic acid (LPA; 1 µl, 1 mM) or vehicle (NS) was administered daily into the trigeminal ganglion under 2% isoflurane anesthesia from day 1 to day 7 after suborbital nerve injury, and the MHWT in the moustache area was measured until day 14 after injury.
Results: MHWT in the IONI-LPA group was significantly lower than that in the naïve-NS group from day 2 to day 10 after injury. The naïve-LPA group showed significantly lower MHWT than the naïve-NS group from day 2 to day 10 and day 14 after injury. There was no significant difference between the IONI-LPA and naïve-LPA groups.
Discussion: This result suggests that LPA may be involved in facial mechanical allodynia that develops after infraorbital nerve injury. Further research is needed to explore the underlying mechanisms and potential interventions targeting LPA in the management of neuropathic pain.

Disclosures: R. Kurisu: None. **Y. Yamazaki:** None. **S. Maeda:** None.

Poster

PSTR409. Peripheral Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR409.23/Z20

Topic: D.02. Somatosensation – Pain

Support: TFFI

Title: Small molecules with neuroprotective effects against in vitro bortezomib-induced axonopathy and altered cytosolic calcium responses in dorsal root ganglia sensory neurons

Authors: *J. VALENCIA LESMES[1], L. MURPHY[1], I. UTKINA-SOSUNOVA[4], S. PRZEDBORSKI[4], D. BAUTISTA[2], E. LUMPKIN[3];
[1]Mol. and Cell Biol., UC Berkeley, Berkeley, CA; [2]UC Berkeley, BERKELEY, CA; [3]UC Berkeley, Berkeley, CA; [4]Columbia Univ., New York, NY

Abstract: Chemotherapy-induced peripheral neuropathy (CIPN) is a dose-limiting adverse outcome of many cancer chemotherapeutics that results in permanent symptoms and disability in up to 40% of cancer survivors. Individuals with CIPN can experience numbness, pain and/or burning sensations in the feet and hands. Among chemotherapeutics, Bortezomib (BTZ) is commonly used to treat multiple myeloma. To date, there is no standard for prevention and/or treatment of BTZ-induced CIPN (BIPN). We developed a high-content, live-cell imaging assay to validate potentially neuroprotective hits from an FDA-approved small molecule library screen (Utkina-Sosunova et al., 2022). Adult mouse dorsal root ganglia (DRG) neurons from 8-9 week old *Pirt^{Cre}; tdTomato/+* males were cultured in 96-well plates for 4 days *in vitro* (DIV4) followed by 48 h of either vehicle (0.1% DMSO), 3 nM BTZ treatment, or BTZ plus a small molecule of interest. Fluorescence imaging was performed at 24-h intervals to assess neurite outgrowth and BTZ-induced axonopathy. On DIV6, cultured cells were loaded with Fluo-4 AM for live-cell calcium imaging to assess neuronal responsiveness to a nociceptive stimulus (1 μM capsaicin) and depolarization (70 mM K^+). As previously observed, BTZ reduced the neurite length per cell by 63 ± 11% compared with vehicle-treated control neurons (DMSO 3039.0 ± 240.5 μm/cell, BTZ 1120.0 ± 341.8 μm/cell; P=0.01, Student's t test, two-tailed; n=3 biological replicates, n=4-8 technical replicates). Two compounds from this secondary screen showed neuroprotection in this assay, with reductions of neurite lengths of 29.1 ± 8% and 35.4 ± 10.4% (n=3 biological replicates, n=4 technical replicates). In live-cell calcium imaging experiments, BTZ increased the resting cytosolic calcium concentration, decreased the number of capsaicin responding neurons, and decreased the calcium response to high K^+-evoked depolarization. The two neuroprotective compounds partially reversed these effects. We are now poised to test these validated molecules in human DRG neurons for the treatment of BIPN. *These authors contributed equally. REFERENCES I. **UTKINA-SOSUNOVA**, H. LI, E. TATISHEV, C. KARAN, S. PRZEDBORSKI; Cell culture-based platform for high throughput screening of biomolecules with neuroprotective effect against bortezomib-induced peripheral neuropathy (BIPN). Program No. 201.07. 2022 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2022. Online.

Disclosures: **J. Valencia Lesmes:** None. **L. Murphy:** None. **I. Utkina-Sosunova:** None. **S. Przedborski:** Other; Reviewing Editor for eLife, Scientific Board Member of Luciole Pharmaceuticals, Inc.. **D. Bautista:** None. **E. Lumpkin:** None.

Poster

PSTR409. Peripheral Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR409.24/Z21

Topic: D.02. Somatosensation – Pain

Support: NRF-2020R1A2C1008084

Title: Modulation of TRPV1 Activation via Direct Binding of GLP-1R Antagonist, Exendin 9-39

Authors: ***E. GO**[1], H. JO[1], M. RAHMAN[1], Y. JO[2], Y. KIM[1], C. PARK[1];
[1]Gachon Univ. of Med. and Sci., Yeonsu-gu, Korea, Republic of; [2]Dept. of Anesthesiol. and Pain Med., Gil Med. Center, Gachon Univ., Yeonsu-gu, Korea, Republic of

Abstract: Transient receptor potential vanilloid 1 (TRPV1) channels, polymodal nociceptors activated by factors such as capsaicin (CAP), protons, or heat, are known to contribute significantly to chronic pain states. Upregulation of TRPV1 channels under such conditions reduces stimulation thresholds and exacerbates pain perception, manifesting as hyperalgesia or allodynia. Despite ongoing efforts to discover drugs targeting these channels, side effects like hyperthermia have hampered clinical development. In light of recent studies revealing the antinociceptive effects of glucagon-like peptide 1 (GLP-1) analogs in neuropathic pain through GLP-1 receptor (GLP-1R) activation, we investigated the role of Exendin 9-39, a GLP-1R antagonist, in modulating TRPV1 activation. Our finding showed that Exendin 9-39 exhibited the greatest inhibitory effect (IC50 28.18 ± 3.92 nM) on CAP-induced TRPV1 channel activation among all tested GLP-1R agonists. Additionally, cell-attached and inside-out patch clamp recordings indicated that Exendin 9-39 likely binds an extracellular site of human TRPV1 channels, thereby preventing activation. Given the hyperthermia side effect associated with previously developed TRPV1 antagonists, we examined the impact of Exendin 9-39 on the proton activation mode of the TRPV1 channels. Exendin 9-39 demonstrated minimal impact on TRPV1 even when applied at concentrations 30 times greater than its IC50 for CAP, indicating its potential to act as a modality-selective antagonist of the TRPV1 channels. Lastly, the analgesic effect of Exendin 9-39 was tested in an acute mouse pain model. Intraplantar administration of Exendin 9-39 following a CAP injection resulted in a dose-dependent alleviation of CAP-induced spontaneous pain. This finding supports the potential candidacy of Exendin 9-39 as a therapeutic agent in pain management.

Disclosures: **E. Go:** None. **H. Jo:** None. **M. Rahman:** None. **Y. Jo:** None. **Y. Kim:** None. **C. Park:** None.

Poster

PSTR409. Peripheral Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR409.25/Z23

Topic: D.02. Somatosensation – Pain

Support: NIH Grant R01 NS070711-10A1
NIH Grant R27 NS108278-01A1

Title: Elevated heme drives evoked and ongoing pain behaviors in WT mice by sensitization of dorsal root ganglia neurons

Authors: *S. ZORN[1], V. L. EHLERS[2], C. L. STUCKY[3];
[1]Med. Col. of Wisconsin, MILWAUKEE, WI; [2]Med. Col. of Wisconsin, Milwaukee, WI; [3]Cell Biology, Neurobio. & Anat., Med. Col. of Wisconsin Neurosci. Doctoral Program, Milwaukee, WI

Abstract: Individuals with sickle cell disease (SCD) suffer from complex stimulus-evoked and ongoing pain that is mediated in part by the hyperexcitability of their peripheral sensory neurons. However, it is unclear whether acute or chronic elevation of cell free heme, a key pathological feature of SCD, contributes to the aberrant activity of sensory neurons. Thus, determining whether heme drives pain behavior and enhanced excitability of dorsal root ganglia (DRG) primary sensory neurons involved in pain signal transduction is critical for the development of targeted pain therapies for SCD. In the current experiments, we use evoked and ongoing pain behavior assays and in vitro calcium imaging to evaluate the hypothesis that acute increases in heme causes direct sensitization of nociceptive DRG neurons in wildtype (WT) mice. Our preliminary data reveal that similar to pain behaviors in the mouse model of SCD, hind paw injection of heme into WT mice induces mechanical and cold hypersensitivity. These data suggest that axon terminals in the skin become sensitized when exposed to elevated heme. We next administered heme to primary cultured DRG neurons in vitro and observed calcium flux across the neuronal cell membrane that was dependent on extracellular calcium. Together, these data suggest that heme opens calcium channels on sensory neurons to drive acute pain behaviors. As ongoing activity of nociceptive neurons may cause pain signal transduction in the absence of external stimuli, we will next use current clamp electrophysiology to determine whether application of heme to cultured DRG neurons is sufficient to induce action potential generation. We will further determine whether exposure to heme enhances the intrinsic excitability of mechanical and cold-sensitive neurons that may lead to our observed acute onset mechanical and cold hypersensitivity in heme-injected mice. Completion of these experiments will shed light on the contributions of cell free heme to SCD pain and illuminate downstream effectors that may be targeted to provide analgesic relief for this historically understudied and debilitating disease.

Disclosures: S. Zorn: None. **V.L. Ehlers:** None. **C.L. Stucky:** None.

Poster

PSTR409. Peripheral Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR409.26/Z22

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01 DA041529

Title: Early life pain alters the response to an immune challenge in adult male and female rats

Authors: *M. GOMEZ, M. MACIK, H. HARDER, M. PANDIT, A. Z. MURPHY;
Neurosci. Inst., Georgia State Univ., Atlanta, GA

Abstract: Premature infants are more likely to be admitted to the Neonatal Intensive Care Unit (NICU) where they experience upwards of 10-18 painful procedures each day, often without anesthesia or analgesia. Preclinical and clinical studies have shown that neonatal pain disrupts normal CNS development in multiple ways that persist into adulthood. We have previously reported that early life pain results in an exaggerated febrile response to an immune challenge (administration of lipopolysaccharide; LPS) in adulthood. Administration of LPS induces the release of inflammatory cytokines in the periphery to stimulate prostaglandin E2 (PGE2) production. Centrally, PGE2 binding to the EP3 receptor (EP3R) within the hypothalamic median preoptic area (MnPO) induces a pyrogenic (fever) response. Microglia also change their morphology and release cytokines into the CNS as part of the immune response. Here, we investigate if early life pain (ELP) alters PGE2 signaling within the MnPO and the involvement of microglia in this response. Male and female rats were exposed to a short-term inflammatory insult induced by intraplantar administration of 1% carrageenan on the day of birth (P0). In adulthood (P60-P90), Thermicron iButtons were implanted to monitor core body temperature; 14 days later, LPS was injected to elicit an immune response. Rats were sacrificed at one of 3 time points post-LPS: 24 hours, peak fever, or 2 hours (fever initiation). Brain tissue was analyzed via immunohistochemistry for VGAT, VGLUT, Fos, prostaglandin receptor 3, and Cd11b within the MnPO and Cox-2 within the organum vasculosum of the lamina terminalis (OVLT). A whole brain survey of Fos activation patterns was also conducted to analyze the global brain response to LPS. Reconstructions and analysis of morphological changes in Cd11b+ microglia were conducted using Imaris. LPS administration resulted in an elevated febrile response in ELP males and females compared to controls and increased sickness behaviors in ELP females. Immunohistological analysis revealed sex and treatment differences in cellular activation in several brain regions and increased receptor and transporter expression in the MnPO in ELP rats. Cox-2 expression is also increased at peak fever in ELP rats. Together, these studies are consistent with clinical studies reporting that children experiencing unresolved pain during the perinatal period show increased severity of sickness behavior and altered immune signaling following exposure to a pathogen and will provide a foundation for future studies examining the biological underpinnings.

Disclosures: **M. Gomez:** None. **M. Macik:** None. **H. Harder:** None. **M. Pandit:** None. **A.Z. Murphy:** None.

Poster

PSTR410. Central Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR410.01/Z24

Topic: D.02. Somatosensation – Pain

Support: NIH Grant (Y.G) R01NS110598

Title: Spinal adenosinergic signaling mechanisms inhibit lamina I NK1 receptor-expressing neurons following electrical stimulation of Aβ-fibers after nerve injury

Authors: *N. C. FORD[1], X. CUI[1], S.-Q. HE[1], C. ZHANG[1], G. ZHU[1], X. DONG[2], S. N. RAJA[1], Y. GUAN[1];
[1]Anesthesiol. and Critical Care Med., [2]Solomon H. Snyder Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Background: Peripheral nerve stimulation (PNS) is an emerging candidate for effective opioid-sparing pain treatment. The Gate Control Theory asserts that spinal pain signal transmission is gated by inhibitory interneurons which can be activated by Aβ-fiber impulses. However, the detailed mechanisms that contribute to synaptic inhibition after PNS, such as 50 Hz Aβ-fiber stimulation, remain partially understood. Although adenosine A1 and A3 receptors (A1Rs/A3Rs) are expressed in the spinal cord, their roles in PNS-induced inhibition of spinal projection neuron synapses were unclear.
Methods: We used a multidisciplinary approach to investigate the influence of PNS-induced A1 and A3R signaling onto lamina I NK1R-GFP neurons, ~80% of which are projection neurons. Moreover, we examined the impact of endogenous adenosine metabolism modulation on PNS-induced synaptic inhibition. *NK1R-GFP* knockin mice were developed to facilitate the identification of NK1R+ neurons in the spinal cord. Neuropathic pain was induced by tibial spared-nerve injury (SNI-t) to the left hindlimbs of NK1R-GFP mice. Immunohistochemistry, RNA scope, and western blot analysis were used to investigate A1R and A3R expression in NK1R+ neurons in the spinal cord from naïve and neuropathic mice. Patch-clamp electrophysiology was used to elucidate the influence of A1 and A3R signaling and 50 Hz PNS on C-fiber-evoked excitatory post-synaptic currents (C-eEPSCs) in NK1R-GFP neurons.
Results: NK1R-GFP+ neurons in lamina I express A1 and A3Rs, and SNI-t significantly increased the level of both in the spinal cord. Importantly, 50 Hz PNS inhibited C-eEPSCs, which was significantly attenuated by bath-application of both A1 and A3R antagonist DPCPX (1 μM) and MRS1523 (30 nM). In contrast, blocking the adenosine deaminase-dependent conversion of adenosine to inosine with bath-applied deoxycoformycin (dCF; 1 μM) significantly increased the synaptic inhibition of NK1R-GFP neurons by 50 Hz PNS.
Conclusion: Nociceptive transmission in lamina I NK1R-GFP neurons may be dampened by 50

Hz PNS through activation of A1 and A3Rs. Moreover, inhibiting adenosine deaminase which would increase the level of adenosine accumulated in the synapse enhanced the overall synaptic inhibition produced by 50 Hz PNS. Thus, AR activation may serve as a key mechanism for the synaptic inhibitory effects of 50 Hz PNS, the effects of which can be amplified with enzyme inhibitors to increase its clinical efficacy for the management of neuropathic pain.

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Poster

PSTR410. Central Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR410.02/Z25

Topic: D.02. Somatosensation – Pain

Support: Duke University Anesthesiology Fund
NIH grant R01-DE29342

Title: General anesthesia-activated neurons in the central amygdala regulates pain and pain-induced anxiety in mice

Authors: *J. ZHAO[1], A. MCGINNIS[1], M. YUAN[1], J. MATHEW[1], F. WANG[2], R.-R. JI[1]; [1]Duke Univ., Durham, NC; [2]MIT, Cambridge, MA

Abstract: General anesthesia, such as isoflurane, produces analgesia (loss of pain) and loss of consciousness via different mechanisms, but the underlying mechanisms are not well understood. Recent study showed that a distinct population of GABAergic neurons in the central amygdala can be activated by general anesthesia and play an important role in analgesia. To further characterize the electrophysiological properties and analgesic effects of these general anesthesia activated neurons, we used 1.2% isoflurane to induce c-Fos activation in the mouse brain and validated the c-Fos expression by *in situ* hybridization. Our results showed that several brain regions, including the central amygdala (CeA), paraventricular nucleus of the thalamus (PVT), paraventricular nucleus of the hypothalamus (PVN), and supraoptic nucleus (SON), were robustly activated by isoflurane. Using three-color fluorescence *in situ* hybridization, we found that c-Fos$^+$ neurons in CeA were mainly GABAergic neurons (Vgat$^+$), while c-Fos$^+$ neurons in other regions were mainly glutamatergic neurons (Vglut2$^+$). Additionally, we confirmed that c-Fos$^+$ neurons in CeA were mostly protein kinase C (PKC)-delta (Prkcd$^+$) neurons and not somatostatin (Sst$^+$) neurons. Our *ex vivo* electrophysiological recordings in brain slices revealed that c-Fos$^+$ neurons in CeA had increased neuronal excitability and exhibited a distinct action potential pattern compared to c-Fos$^-$ neurons. To investigate the analgesic effects of the isoflurane-induced c-Fos$^+$ CeA neurons, we used a chemogenetic approach (DREADDs) to activate these neurons. Our results showed that chemogenetic activation of isoflurane-induced c-Fos$^+$ neurons in CeA was sufficient to increase pain thresholds in both naïve mice and mice with

nerve injury and neuropathic pain. In addition, chemogenetic activation of these neurons in CeA abolished pain induced anxiety-like behaviors. These findings suggest that targeting isoflurane-induced c-Fos$^+$ CeA neurons may provide therapeutic benefits for pain and its comorbidities management.

Disclosures: **J. Zhao:** None. **A. McGinnis:** None. **M. Yuan:** None. **J. Mathew:** None. **F. Wang:** None. **R. Ji:** None.

Poster

PSTR410. Central Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR410.03/Z26

Topic: D.02. Somatosensation – Pain

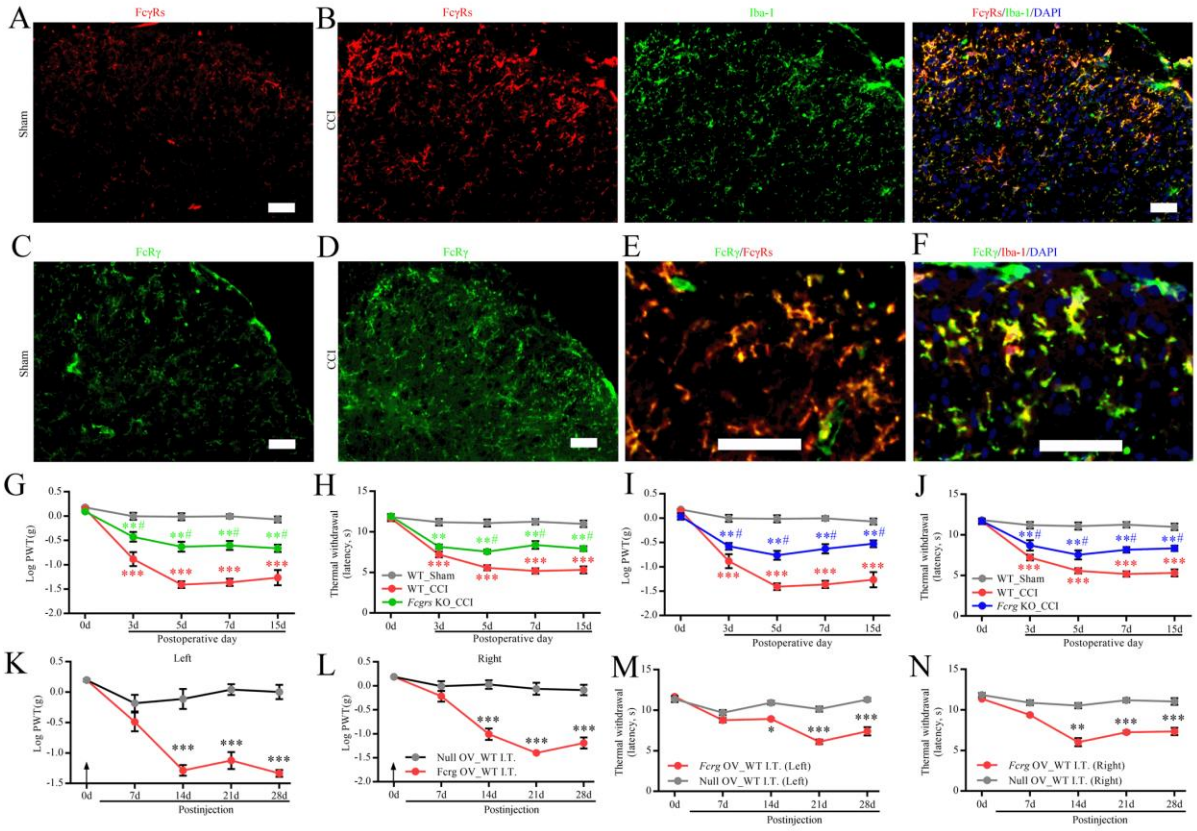
Support: National Natural Science Foundation of China Grant 81801114
National Natural Science Foundation of China Grant 82271260
National Natural Science Foundation of China Grant 81771205
National Natural Science Foundation of China Grant 82050004
CAMS Innovation Fund for Medical Sciences Grant 2021-I2M-1-025
Fundamental Research Funds for the Central Universities Grant 3332022036

Title: Activating Fc-gamma Receptors Mediate Sciatic Nerve Injury-induced Neuropathic Pain

Authors: *F. LIU[1], L. ZHANG[2], X.-S. YIN[1], W. QIU[1], C. MA[1];
[1]Inst. of Basic Med. Sci. Chinese Acad. of Med. Sci., Beijing, China; [2]Beijing Friendship Hospital, Capital Med. Univ., Beijing, China

Abstract: Neuropathic pain is difficult to treat in clinical practice, and the underlying mechanisms are insufficiently elucidated. Previous studies have demonstrated that Fc receptors may be involved in pain. However, the mechanisms of FcγRs in neuropathic pain remain to be explored. Here, we found that Fc-gamma receptors (FcγRs), including FcγRI, FcγRII, and FcγRIII, and Fc-epsilon receptor I gamma (FcεRIγ) mRNA were upregulated in the spinal dorsal horn (SDH) of a mouse model of sciatic nerve injury (CCI) using mRNA sequencing. FcεRIγ associates with activating Fc-gamma receptor on the cell surface to form a functional signaling complex and regulate immune responses. Our work also revealed that FcγRI, FcγRIII and FcεRIγ were expressed in microglial cells of the SDH after CCI. Meanwhile, sciatic nerve injury persistently and significantly increases the protein levels of FcγRI, FcγRIII and FcεRIγ in the SDH after neuropathic pain. Furthermore, knockout of the FcγRs or FcεRIγ-encoding gene *Fcer1g* significantly alleviated neuropathic pain in mice after CCI. Overexpression of FcεRIγ in the SDH by the adeno-associated virus (AAV)-*Fcer1g* vector evokes chronic pain in wild-type mice. These results indicate that the microglial complex of the SDH plays an important role in

the development of neuropathic pain in chronic constriction injury mice. The findings may provide novel insights into potential therapeutic targets for neuropathic pain.



Disclosures: **F. Liu:** None. **L. Zhang:** None. **X. Yin:** None. **W. Qiu:** None. **C. Ma:** None.

Poster

PSTR410. Central Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR410.04/Z27

Topic: D.02. Somatosensation – Pain

Support: CIHR PJT-162404

Title: Parvalbumin gates chronic pain through the modulation of tonic firing in inhibitory neurons

Authors: *H. QIU, L. S. MIRAUCOURT, A. DAVIDOVA, R. SHARIF NAEINI; Dept. of Physiol., McGill Univ., Montreal, QC, Canada

Abstract: Spinal cord dorsal horn inhibition is critical to the processing of sensory inputs, and its impairment leads to mechanical allodynia. How this decreased inhibition occurs and whether its restoration alleviates allodynic pain is poorly understood. Here, we show that the calcium (Ca^{2+})-binding protein, parvalbumin (PV), controls the activity of inhibitory PV-expressing neurons (PVNs) by enabling them to sustain high-frequency tonic firing patterns. Upon nerve injury, PVNs transition to adaptive firing and decrease their PV expression. Interestingly, decreased PV is necessary and sufficient to the development of mechanical allodynia and the transition of PVNs to adaptive firing. This transition of firing pattern is due to the recruitment of calcium-activated potassium (SK) channels and blocking them during chronic pain restores normal tonic firing and alleviates mechanical allodynia. Our findings indicate that PV is essential to the firing activity of PVNs and in preventing allodynia, these observations may lead to novel strategies for chronic pain relief.

Disclosures: H. Qiu: None. **L.S. Miraucourt:** None. **A. Davidova:** None. **R. Sharif Naeini:** None.

Poster

PSTR410. Central Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR410.05/Z28

Topic: D.02. Somatosensation – Pain

Support: NIH 094450
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NIH 119268
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Title: Acute and chronic neuropathic pain impact on corticostriatal circuit function

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Abstract: Chronic pain (>3 months) affects over 50 million Americans, limiting their life and daily activities, and creating a public health crisis. Although there are available treatments for those experiencing acute pain, there is a lack of understanding of the central circuits involved in chronic pain. One candidate involved in pain is the striatum which is known for sensorimotor integration and facilitating voluntary movement, particularly with the interplay between the GABAergic dopamine receptor 1 (D1N) cells and dopamine receptor 2 (D2N) -expressing principal neurons. The striatum can mediate analgesic action in orofacial pain by activating striatal D2N pathways. However, the striatal mechanisms underlying chronic neuropathic pain are largely unknown. The striatum also has inputs from the primary somatosensory cortex (S1) that contribute to pain processing. S1 is known to process somatic sensations including nociception and touch and its activity changes during pain. In addition to the spinothalamic tract,

S1 also has direct projections to the striatum, and plasticity changes in this pathway could point to a mechanism involved in the transition from acute to chronic pain. Our overarching hypothesis is that the transition from acute to chronic pain results in the heightened expression of pain-related behaviors driven by the hyperactivity of S1 leading to overactive striatal D2N populations to mediate analgesic action. We tested this hypothesis using D1-Cre::Ai14 and A2A-Cre::Ai14 mice in a neuropathic pain model using spared nerve injury and optogenetically stimulating the S1-D1N and S1-D2N pathways. We expect to find changes associated with acute and chronic pain states. Furthermore, we characterized specific striatal populations during pain behavioral assessments, and we expect to find differences in D1N and D2N bulk calcium signaling in response to innocuous and painful stimuli during the acute and chronic pain states. The collective results will provide an understanding of the functional role of corticostriatal pathways, and the activation of striatal D1N and D2N circuits, in the transition from acute to chronic pain.

Disclosures: A.J. George: None. **D.J. Margolis:** None. **V.E. Abraira:** None.

Poster

PSTR410. Central Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR410.06/AA1

Topic: D.02. Somatosensation – Pain

Support: NIH Grant NS109947
Wenner-Gren Foundations (Wenner-Gren Stiftelserna)

Title: Chronic Neuropathic Pain Alters Representation of Noxious Stimuli in the Primary Somatosensory Cortex: A Longitudinal Study in a Mouse Model of Infraorbital Nerve Constriction

Authors: *E. LENDARO[1], M. LEVY[2], A. PEARCE[3], V. PREVOSTO[2], F. WANG[4];
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Abstract: Nociception, the sensory nervous system's response to harmful stimuli, is fundamental to the perception of pain and essential for survival. Chronic pain conditions, such as neuropathic pain, can disrupt the processing of noxious and innocuous stimuli, leading to exacerbated perceptual correlates such as hyperalgesia, allodynia, and spontaneous pain.
In this longitudinal study, we investigate how infraorbital chronic nerve constriction, a mouse model of trigeminal neuropathic pain, affects the cortical processing of innocuous and noxious somatosensory stimuli applied to the face (whisker pad region). We focused on two sub-areas of the primary somatosensory cortex (S1): the barrel cortex (S1bc) and the dysgranular zone (S1dz). S1dz has been proposed to be a homolog of primate area 3a and implicated in the processing of proprioceptive inputs as well as in face nociception. However, how nociceptive, proprioceptive

and motor information are integrated in S1 during nocifensive behaviors and how it changes during chronic pain states is currently unknown.
We performed weekly wide-field and two-photon calcium imaging before and for one month following surgical induction of chronic neuropathic pain, tracking cortical responses at the mesoscale and cellular levels during the application of two distinct types of somatosensory stimuli: mechanical ("pin-prick" pole and 0.02g-1g von Frey filament) and thermal (0-200ms infrared laser pulse). At the same time, we tracked the animal movements during nocifensive behaviors and analyzed them using DeepLabCut. We found that infraorbital chronic nerve constriction decreases the threshold for wiping in response to mechanical stimuli, in agreement with previous findings. Similarly, the wiping threshold was also reduced for thermal stimuli. These results provide the behavioral foundation to identify the cortical correlates of nocifensive behaviors in S1, and to reveal the progressive neural changes associated with chronic neuropathic pain in the somatosensory cortex.
Understanding these cortical alterations will provide critical insights into the neural mechanisms underlying chronic neuropathic pain and potentially guide the development of new therapeutic strategies aimed at normalizing the cortical representations of nociception.
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Poster

PSTR410. Central Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR410.07/AA2

Topic: D.02. Somatosensation – Pain

Title: A stimulus-evoked EEG study for chronic pain patients and healthy controls

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Abstract: Previous work has implicated brain areas such as the prefrontal cortex (PFC), insular cortex (IC), anterior cingulate cortex (ACC), and primary somatosensory cortex (S1) in pain processing and perception. Furthermore, neural oscillations at alpha (7-13 Hz), low-gamma (30-60 Hz), and high-gamma (60-100 Hz) frequencies have been shown to reflect both evoked pain perception and chronic pain states. However, a suitable biomarker for chronic pain has not yet been developed. In this observational, cross-sectional study, EEG was recorded in patients with chronic lower back pain (CP) (n = 51) and healthy control (HC) subjects (n = 24). Pinprick stimuli at three intensity levels (low: 32 mN, medium: 128 mN, high: 256 mN) were applied to

the lower back region and to the back of the hand. Subjective pain scores (0-10) were reported. Kruskal-Wallis test with Post Hoc 1-way ANOVA test was applied for independent parameters to compare groups, using Bonferroni test for adjusted significance. CP subjects were able to distinguish medium and high stimuli from the low stimulus both on the lower back and hand ($p < 0.05$), whereas HC subjects were only able to distinguish high stimulus from low stimulus on the hand ($p < 0.05$), suggesting the presence of allodynia (heightened sensitivity) in CP subjects. Stimulus-evoked EEG data were source-localized to six regions of interest (medial-PFC, lateral-PFC, IC, rostral-ACC, caudal-ACC, S1) and event-related desynchronization and synchronization (ERDS) in the low-stimulus and high-stimulus conditions were compared in the frequency bands mentioned. Stimulus-evoked EEG data are then used to correlate with behavioral findings.

Disclosures: **G. Kenefati:** None. **M. Rockholt:** None. **D. Ok:** None. **M. McCartin:** None. **Q. Zhang:** None. **G. Sun:** None. **J. Maslinski:** None. **A. Wang:** None. **B. Chen:** None. **Z.S. Chen:** None. **J. Wang:** None. **L. Doan:** None.

Poster

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Program #/Poster #: PSTR410.08

Topic: D.02. Somatosensation – Pain

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the Shenzhen Innovation Committee of Science and Technology grants (ZDSYS20200811144002008)

Title: Distinct roles of differential populations of efferent neurons from the parabrachial nucleus in mechanical allodynia

Authors: J. HUO, X. LIU, F. DU, G. YIN, K. DUAN, ***D. DONG**, L. CHENG;
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Abstract: Mechanical allodynia (MA)-pain evoked by innocuous tactile stimuli-represents one prevalent symptom of chronic inflammatory and neuropathic pain. For some devastating patients, a local injury can lead to full-blown body-wise pain that lasts for long time, whereas for most patients the pain could be short-lasting or confined to the unilateral injury side. Our recent study reported a descending pathway that controls the latent sensitization induced by chemical

irritants, inflammatory reagents or nerve injury. This circuit starts with neurons located in the lateral parabrachial nuclei (lPBN) (marked by the expression of μ-opioid receptors (MORs)), which control the activity of hypothalamic neurons in the dorsal medial regions (dmH) that are marked by the expression of the prodynorphin (Pdyn) and that send bilateral projections to the spinal dorsal horn (SDH) ($lPBN^{MOR+}$→dmH^{Pdyn+}→SDH circuits). However, the underlying mechanisms for the induction of bilateral MA, particularly the contralateral MA, are, however, still remain unsolved. Here we observed that proenkephalin (Penk) neurons were highly expressed in lPBN and partially overlapped with MOR+ neurons in el-dvlPBN. We did behavioral experiments to study their functional roles in modulating MA. In contrast to ablation of MOR+ neurons, ablating or silencing the el-dvlPBN^{Penk+} neurons, or retro-ablating or chemogenetic silencing el-dvlPBN^{Penk+}→dmH projecting neurons had no significant effect on the lasting duration of bilateral MA, suggesting cell type-specific roles of $lPBN^{MOR+}$ neurons in negatively modulating the lasting duration of MA. However, retro-ablation/chemogenetic silencing the slPBN^{Penk+}→dmH projecting neurons before hind paw capsaicin injection completely prevented the induction of bilateral MA. In contrast, chemogenetic silencing the slPBN^{Penk+}→dmH
projecting neurons post-capsaicin injection had no significant effect on the expression of bilateral MA, suggesting that this population of neurons are required for the induction, but not expression of bilateral MA. Chemogenetic silencing of slPBN^{Penk} neurons post-capsaicin injection abolished bilateral MA, suggesting that slPBN^{Penk} neurons are required for the transmission of bilateral MA. In addition, chemogenetic activation of the slPBN^{Penk}→dmH projecting neurons opened the gate for bilateral MA in naïve mice. Overall, our study suggested distinct roes of differential populations of efferent neurons from the lateral parabrachial nucleus in mediating MA in mice.

Disclosures: **J. Huo:** None. **X. Liu:** None. **F. Du:** None. **G. Yin:** None. **K. Duan:** None. **D. Dong:** None. **L. Cheng:** None.

Poster

PSTR410. Central Mechanisms of Neuropathic Pain II

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Program #/Poster #: PSTR410.09/AA3

Topic: D.02. Somatosensation – Pain

Support: NS104297-04

Title: Sex differences in Central Amygdala response to Parabrachial Calcitonin Gene Related Peptide (CGRP) release

Authors: R. LORSUNG[1], J. ALIPIO[2], Y. JI[3], N. P. CRAMER[4], J. KOENIG[5], R. MASRI[6], ***A. KELLER**[5];
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and Neurobio., Univ. Maryland, Baltimore, Baltimore, MD; [5]Univ. of Maryland Sch. of Med., Baltimore, MD; [6]Dept. EPOD, Univ. Maryland, BALTIMORE, MD

Abstract: Calcitonin gene-related peptide (CGRP)-expressing neurons in the parabrachial nucleus (PB) densely project to several brain structures implicated in aversive-affective pain processing, including the central amygdala (CeA). We are testing the overarching hypothesis that these projections are causally involved in the affective component of chronic pain. We first tested whether CGRP release from PB potentiates post-synaptic responses. We performed whole-cell, voltage-clamp recordings in CeA slices from $CGRP^{Cre}$ mice in which an excitatory opsin was expressed in PB_{CGRP} terminals. Stimulation of these PB terminals in the CeA potentiated optically-evoked excitatory postsynaptic currents only in females. This potentiation lasted approximately one minute and was blocked by a CGRP-antagonist. This suggests that CGRP release from PB terminals potentiates PB glutamatergic inputs from PB to CeA of females, potentially tuning these synapses to respond more strongly to further aversive input. To investigate whether this sex difference arose from transcriptional sex differences in the CeA subpopulation expressing CGRP receptor (CGRPr+), we examined previously published single cell CeA datasets. GO term and DEG analysis between males and females revealed that female CGRPr+ CeA neurons are enriched for both asymmetric synapse markers, indicating a greater number of presumptive excitatory synapses, and nitric oxide signaling compared to males. As postsynaptic nitric oxide release can play a role in strengthening synapses via retrograde signaling, we tested whether CGRP-dependent potentiation in females affected presynaptic release by performing paired pulse ratio (PPR) analysis. PPR significantly decreased in female neurons following endogenous CGRP release from PB terminals in the CeA, indicating that CGRP induced an increase in presynaptic release probability. This suggests that CGRP-driven potentiation in female CeA neurons is at least partly due to a presynaptic mechanism. We also tested for sex-differences in the presynaptic PB neurons by quantifying CGRP expression in the PB using RNAscope. Females expressed slightly *less* CGRP than males, despite being more responsive to its endogenous release in the CeA *in vitro*. This suggests that females release less CGRP at baseline conditions, but are more responsive to elevations in CGRP signaling in the CeA, such as those that occur in chronic pain states. Taken together, our data suggests that the PB to CeA pathway demonstrates significant sex-differences in both expression of various markers as well as functional response to CGRP.

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Poster

PSTR410. Central Mechanisms of Neuropathic Pain II

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Program #/Poster #: PSTR410.10/AA4

Topic: D.02. Somatosensation – Pain

Support: NIH Grant R21TR004701
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Title: Single cell transcriptomic analysis of DNA methylation machinery in the prefrontal cortex of transient and persistent neuropathic pain mouse models

Authors: *S. LEE[1], M. RIEDL[2], L. VULCHANOVA[2], L. STONE[1];
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Abstract: Chronic pain is associated with molecular, cellular, structural, and functional changes throughout the neuroaxis including in the prefrontal cortex (PFC). PFC plays a crucial role in the processing and modulation of pain sensation and in the integration of sensory, cognitive, and emotional information. Epigenetic modifications including DNA methylation can impact gene regulation without altering the genetic code. DNA methylation is regulated by DNA Methyltransferases (DNMTs), and ten-eleven translocation (TET) enzymes and is read by Methyl-CpG-binding Domain (MBD) proteins. We previously reported changes in DNA methylation in rodent PFC following spared nerve injury (SNI) that are associated with the transition from acute to chronic pain. However, the cell phenotypes driving these changes have not been identified. In this study, we aimed to map changes DNA methylation machinery expression in PFC using a single nuclei RNA sequencing approach in mouse models of transient vs persistent neuropathic pain. To model transient and persistent neuropathic pain, the sciatic nerve crush and SNI models were induced in 10 to 12 week old C57BL6 male mice, respectively. Sham surgery consisted of exposing the nerve without damaging it. Mechanical and cold sensitivity on the ipsilateral hind paws were monitored for 6 weeks, then PFC was collected for single-nuclei RNA sequencing. Data was analyzed by the R package Seurat (v4.3). Expression levels of DNMTs, TETS and MBDs were compared between Sham vs SNI, Sham vs. Crush and Crush vs SNI groups within cellular phenotypic clusters. Increased mechanical and cold sensitivity persisted in SNI but the nerve crush mice showed a progressive recovery over time. Upregulation of MBD1 and Tet1 was observed in some non-neuronal cells (e.g., Oligodendrocytes, Endothelial cells) in SNI compared to both sham and crush groups. Tet3 expression was upregulated in excitatory neuron subpopulations (Slc17a7+) in SNI but downregulated in the crush model compared to SNI. DNMT3b expression was downregulated in inhibitory neurons (Gad1, 2+) in SNI but was similar in crush and sham. Together, dynamic changes of DNA methylation machinery in PFC following nerve injury could contribute to the establishment and persistence of chronic neuropathic pain through modulating the epigenetic genes.

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Poster

PSTR410. Central Mechanisms of Neuropathic Pain II

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Program #/Poster #: PSTR410.11/AA5

Topic: D.02. Somatosensation – Pain

Support: NIH R01NS107356

Title: Anterior cingulate cortex metabotropic glutamate receptor type-2 expressing neurons regulate complex pain behaviors: dependence on sex, estrous cycle, and injury.

Authors: *A. KIRRY, S. DAVIDSON;
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Abstract: Our lab previously determined that metabotropic glutamate receptor type-2 (mGluR2) containing pyramidal neurons of the anterior cingulate cortex (ACC) become hyperexcitable following neuropathic and inflammatory pain in mice. The consequence of pain-induced hyperexcitability of mGluR2 expressing neurons of the ACC is unknown but can be informed by examining the function of ACC mGluR2 expressing neurons in naïve and injured mice. We aim to determine the function of ACC mGluR2 neurons using a multidimensional behavioral approach which includes pain-related affective, motivational, and sensory assays. Sex and ovarian cycle influence the multidimensional experience of pain, and we included sex and estrous stage as a factor in analyses. The discovery of the neural nodes involved in pain-related affect, motivation, and sensory processes across sex and estrous cycle may lead to better pain therapies.
We utilized transgenic mice that express cre recombinase specifically in mGluR2 expressing cells. We delivered a viral vector containing a cre-dependent caspase to ablate ACC mGluR2 neurons. We also crossed our mGluR2-cre transgenic line with a cre-dependent channelrhodopsin transgenic line to optogenetically activate ACC mGluR2 neurons. Mice underwent a thermal pain tolerance assay and a complimentary array of pain threshold and affect related tests, including hot plate, Hargreaves, open field, and conditioned place preference. We found that estrous cycle interacts with thermal pain tolerance. Females in proestrus, a putatively high estradiol state, have higher pain tolerance for a sucrose reward. Ablation of ACC mGluR2 neurons prevents the increase in pain tolerance of proestrus females. Independent of sex, optogenetic activation of ACC mGluR2 neurons results in conditioned place aversion and produces antinociception. We next used neuropathic and inflammatory pain models to determine if ACC mGluR2 neurons are required for the development of affective and pain processing changes in chronic pain conditions. Initial studies suggest that ablation of ACC mGluR2 neurons blocks affect-related pain processing changes following chronic pain. These data show that ACC mGluR2 neurons participate in motivational responses to noxious stimuli in both sexes, and likely contribute to emotional and sensory dysregulation seen in pathological pain conditions.

Disclosures: A. Kirry: None. **S. Davidson:** None.

Poster

PSTR410. Central Mechanisms of Neuropathic Pain II

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Topic: D.02. Somatosensation – Pain

Support: DOD Grant W81XWH2010509

Title: Sexual dimorphism in the spinal effects of the VGF derived peptide TLQP-62

Authors: *T. XIE[1], R. E. SCHORN[2], E. MARRON FERNANDEZ DE VELASCO[3], G. L. WILCOX[4], C. A. FAIRBANKS[5], L. VULCHANOVA[2];
[1]Dept of Pharmaceutics, [2]Dept of Neurosci., [3]Dept of Pharmacol., [4]Dept Neurosci, Pharmacol, Dermatol, [5]Dept of Pharmaceutics, Pharmacology, Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: VGF (non-acromymic) is a neurosecretory protein implicated in neuroplasticity associated with depression, learning and memory, and chronic pain. Our previous work has demonstrated that VGF-derived peptides contribute to the establishment of hypersensitivity in mouse models of persistent pain. We have also shown that the peptide TLQP-62 potentiates calcium responses to glutamate (Glu) in a subset of spinal neurons in naïve male mice. This study aims to determine the mechanisms of this TLQP-62 effect. Work in the hippocampus has suggested that the actions of TLQP-62 are mediated in part by BDNF/TrkB signaling, and in spinal dorsal horn BDNF/TrkB signaling has been shown to participate in neuroplasticity mediated by the NMDA receptor subunit GluN2B. Therefore, we hypothesized that the potentiation of spinal Glu responses by TLQP-62 requires GluN2B. To address this hypothesis, we conducted imaging of calcium transients in transverse spinal cord slices from male and female GluN2B*fl/fl* mice, in which GluN2B can be deleted in a Cre-dependent manner. GluN2B*fl/fl* mice were injected intraspinally at the L3 and L4 spinal levels (300 nL/injection) at 3-4 weeks of age with AAV9-hSyn-GCaMP6s-Cre or control AAV9-hSyn-GCaMP6s-ΔCre, in which Cre-recombinase is inactive and does not induce GluN2B deletion. Imaging was performed 4 weeks after viral injections. GCaMP6s fluorescence intensity was captured as time-lapse images by single plane two-photon microscopy (Nikon A1RMP system). Cre-dependent deletion of GluN2B was confirmed by loss of ifenprodil inhibition of NMDAR-mediated calcium transients (n=3/sex, $P<0.01$, Student's t-test). Slices were sequentially exposed to the following treatments: 1) 100 uM Glu (10 s); 2) 50 nM TLQP-62 or aCSF (15 min); 3) 100 uM Glu (10 seconds); 4) 1mM Glu (10 s). The fold change in response amplitudes to the two 100 uM Glu treatments was calculated for each cellular profile and was found to be significantly reduced in TLQP-62-exposed cells from Cre- compared to ΔCre-injected male mice ($p < 0.05$, Student's t-test). Similarly, the proportion of profiles with potentiated responses, defined as fold change greater than the mean+2SD of profiles from aCSF treated slices, was significantly reduced in Cre- compared to ΔCre-injected mice (5/32 and 16/60, respectively; $p = 0.011$, Fisher's exact test). These Cre-dependent differences were not observed in cellular profiles of female mice. These results suggest that GluN2B mediates TLQP-62-induced potentiation of Glu responses in male but not in female mice. Ongoing experiments are investigating the contribution of TrkB to the spinal actions of TLQP-62.

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Poster

PSTR410. Central Mechanisms of Neuropathic Pain II

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Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR410.13/AA7

Topic: D.02. Somatosensation – Pain

Support: P50 DA044121

Title: Evaluating The Role of Perineuronal Nets in Mice With Chronic Pain

Authors: *S. CERMAK[1], G. SEKERKOVA[1], M. MARTINA[2], A. APKARIAN[1];
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Abstract: Approximately one in five individuals in the United States will experience chronic pain (CP), contributing to a significant annual economic burden of around $560-635 billion. The societal burden is just as drastic, causing a great deal of suffering in patients and taxing healthcare systems. Despite decades of advances in research, there is no cure to CP, and limited effective therapies exist. A mechanistic understanding of the neurobiological basis of chronic pain is essential to the development of effective treatments.Perineuronal nets (PNNs) are specialized extracellular matrix structures that surround certain neurons that may play a role in the development of chronic pain. Recently, PNNs have been implicated in pain in rodents. However, the dynamics of these changes in the brain are not well understood in neuropathic pain, particularly in corticolimbic brain regions- areas that have repeatedly been implicated in CP in both humans and rodent models.We use a spared-nerve injury (SNI) model of neuropathic pain in C57 mice to assess changes to PNNS over the development of CP. Control animals received sham surgery, in which the sciatic nerve was exposed rather than ligated. Both male and female mice were 8-12 weeks old. We quantified PNNs in corticolimbic brain regions at multiple time points, 7 days (7d) post-surgery, 14d, and 28d. We used immunostaining with Wisteria Floribunda Agglutin (WFA) to stain for PNNS, as well as anti-aggrecan and anti-Parvalbumin antibodies for double immunofluorescent staining.Quantification revealed increased PNNs in brain regions at multiple time points in animals with CP. Specifically, there were increased PNNs in both prelimbic and infralimbic components of the prefrontal cortex 7d post-surgery, but no change in anterior cingulate or motor cortex. We next characterized PNNs in key regions by assessing aggrecan-positive PNNs in this region, which were primarily aggrecan-negative. Ongoing research will confirm the trend of increasing PNNs at later time points. Establishing how PNNs change temporally and spatially would capture their importance into the mechanisms underlying CP and pave the way for novel clinical applications. Funded by P50 DA044121

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Poster

PSTR410. Central Mechanisms of Neuropathic Pain II

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Program #/Poster #: PSTR410.14/AA8

Topic: D.02. Somatosensation – Pain

Title: Virtual Reality treatment of severe neuropathic pain in an adolescent child

Authors: *J. H. SORENSEN[1], A. KNUDSEN[2], M. VLACHOU[3], I. MILIDOU[3,4], K. MEIER[2]; [1]Neurosurg., Danish Neurosci. Ctr. - Aarhus Univ. Hosp., Aarhus N, Denmark; [2]Neurosurg., [3]Neurol., Aarhus Univ. Hosp., Aarhus N, Denmark; [4]Pediatrics, Regional Hosp. West Jutland, Herning, Denmark

Abstract: *Introduction:* Virtual reality (VR) has successfully been used over the past years as an adjunct therapy in acute pain management. In this case report we present a thirteen-year-old boy where virtual reality (VR) was used as a successful intervention on both short and long term to treat serious chronic neuropathic pain following calcaneus extension orthopaedic surgery in a boy aged 13. After the surgery, he developed severe chronic allodynic pain on the lateral aspect of the right foot. Medical and psychological interventions were unsuccessful. Nerve blockade gave transient relief, and spinal cord or peripheral nerve stimulation was not possible.
Virtual Reality treatment: Inspired by mirror therapy we recommended the boy to experiment with various 3D VR games, which either stimulated his mobility, or other games in which he could see an embodiment of himself. One such engaging VR game was "Beat Saber" developed by Beat Games, where you wield two lightsabers, slicing geometric boxes flying against you, all the while you must avoid obstacles in the VR game space. In addition, the patient also used "VR chat" which is a virtual forum, where you can walk around in cityscapes and see yourself as an avatar. Especially a VR rendering, where the patient could see himself in a mirror, enabled him to touch his virtual (and real self) without pain.
Discussion: This case report documents the beneficial role of VR immersion and engagement as a possible new treatment modality for severe neuropathic pain after surgery. Different theories about the pain alleviation mechanism of VR are generally divided into two main types. One is that VR provides a short-term analgesic effect, which emanates from pain distraction and anxiety reduction, resulting from the VR immersion and diversion of attention to another stimulus. The other theory posit that neuroplasticity is the underlying mechanism contributing to the analgesic effect of VR by inducing a long-term structural change in neuron populations in sensory and motor brain regions. We hypothesize that the embodiment and immersion in virtual gaming and play in the present case modulated the central pain sensitization of the patient's right foot neocortical representation, thereby reducing and ultimately abolishing his chronic pain. The case shows promising prospects for the use of VR to treat chronic pain where current pharmacological and other medical treatments failed. The encouraging results presented in the current case study highlight the importance of further studies investigating the potential of immersive and embodying VR technologies in providing pain relief.

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Poster

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Topic: D.02. Somatosensation – Pain

Support: NSF1456818
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NIH NS118731

Title: Impaired GluD1-Cbln1 Trans-Synaptic Signaling in the Central Amygdala Contributes to Autophagy Deficits and Chronic Pain

Authors: *K. S. NARASIMHAN[1], T. KIRITOSHI[3], G. JI[3], V. NEUGEBAUER[3], S. DRAVID[2]; [1]Pharmacol. and Neurosci., [2]Creighton Univ., Omaha, NE; [3]Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: Chronic pain is a debilitating condition affecting more than 100 million Americans, with more than half of them showing comorbid depression and anxiety. Neuroplasticity in the central amygdala (CeA) is an important contributor to the emotional and affective aspects of pain. GluD1, a delta glutamate receptor is one of the ionotropic glutamate receptors that play a critical role in inhibitory and excitatory neurotransmission. We have recently demonstrated that in chronic pain, dysfunction of GluD1 and its binding partner Cbln1 mediated trans-synaptic signaling in CeA. Studies have indicated that the activation of autophagy, a cellular process for recycling and degradation, leads to the internalization of AMPARs (AMPA receptors) and enhances long-term depression (LTD). Furthermore, it has been demonstrated that GluD1 regulates autophagy mechanisms in multiple brain regions. Based on these findings, we hypothesized that the loss of GluD1 contributes to chronic pain by causing autophagy deficits and impairing LTD. To test this hypothesis, we conducted experiments using sham, complete Freund adjuvant (CFA)-induced, and spinal nerve ligation (SNL) surgery-induced pain models. Initially, we assessed mechanical hypersensitivity in the right paw of the animals using the Von-frey test and observed that both the CFA and SNL models induced hypersensitivity. Subsequently, we analyzed the levels of autophagic proteins in the synaptoneurosomes of the CeA using western blotting. Interestingly, we discovered hyperactive Akt-mTOR signaling in the CeA of animals with CFA and SNL-induced pain, compared to the sham group. This heightened mTOR signaling cascade inhibited beclin-1, disrupting the maturation of autophagosomes. Additionally, we observed increased p62 levels and a decline in the LC3-II/LC3-I ratio in the CeA of animals with CFA and SNL-induced pain, compared to sham mice. These alterations in autophagy deficits were strongly correlated with the downregulation of GluD1 and Cbln1 in the CeA. Furthermore, CFA and SNL models led to impaired LTD, as evidenced by elevated surface expression of GluA2 (AMPAR). These findings further support the connection between autophagy defects resulting from GluD1 downregulation and pain. Interestingly, when we administered recombinant Cbln1 (rCbln1) directly into the CeA through intracerebroventricular (i.c.v.) injection (250ng), we observed a reversal of both autophagy impairment and LTD.

Overall, our results suggest that the restoration of GluD1-Cbln1 impairment alleviates pain by reversing autophagic signaling deficits and restoring synaptic function.

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Poster

PSTR410. Central Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR410.16/AA10

Topic: D.02. Somatosensation – Pain

Support: NIH R61 NS127286

Title: The Role of Endogenous Dopamine Signaling in the Lateral Parabrachial Nucleus in Somatosensation

Authors: *H. KOO, J. WANG, R. PARIYAR, R. HAMMOND, J. CHUNG, J.-H. LA; Univ. of Texas Med. Br., Galveston, TX

Abstract: The lateral parabrachial nucleus (LPBN), a relay and modulatory station for various sensory information, is known to express dopamine D1 and D2 receptors and receive dopaminergic inputs. However, it remains unclear how endogenous dopamine signaling in the LPBN modulates somatosensation. Therefore, here we investigated the effects of the D1 antagonist SCH 23390 and the D2 antagonist Eticlopride, microinjected at the right LPBN in mice, on mechanical and thermal sensory responses measured as the threshold of paw withdrawal from von Frey filament stimulation and the latency to paw withdrawal from radiant heat, respectively. The D1 antagonist SCH 23390 (0.01-1 ug) did not significantly affect either mechanical or thermal sensory responses. In contrast, Eticlopride (0.3 and 1 ug) significantly inhibited both mechanical and thermal sensory responses. These findings indicate that endogenous dopamine in the LPBN activates D2 dopamine receptors, facilitating mechanical and thermal sensory responses. Therefore, interfering with this dopaminergic signaling may result in hyposensitivity to mechanical and thermal stimulation.

Disclosures: H. Koo: None. **J. Wang:** None. **R. Pariyar:** None. **R. Hammond:** None. **J. Chung:** None. **J. La:** None.

Poster

PSTR410. Central Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR410.17/AA11

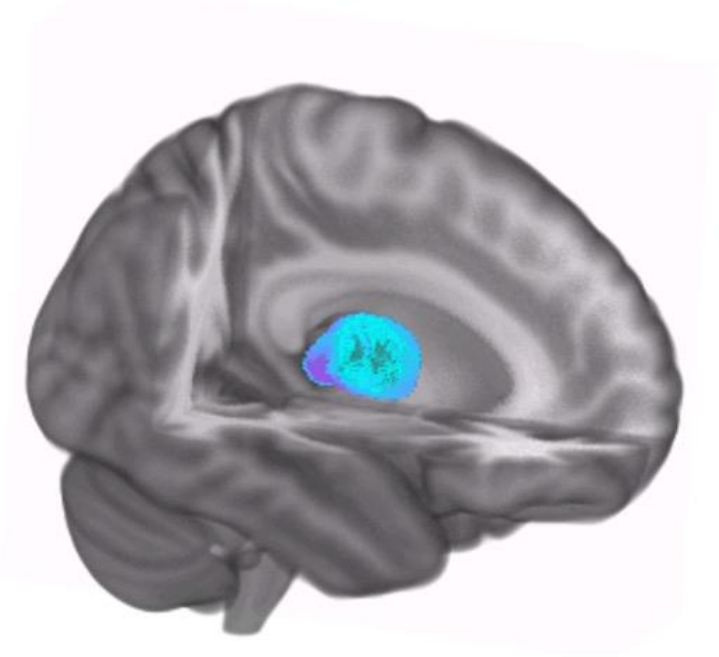
Topic: D.02. Somatosensation – Pain

Support: R21 NINDS 118162

Title: Clinical phenotype matters: Thalamic structural changes in neuropathic low back pain

Authors: *R. YUAN[1], J. D. MARKMAN[2], J. GEWANDTER[3], Z. ZHANG[5], P. GEHA[4]; [2]Neurosurg., [3]Anesthesia and Perioperative Med., [4]Psychiatry, [1]Univ. of Rochester Sch. of Med., Rochester, NY; [5]Statistics, Univ. of North Carolina, Chapel Hill, Chapel Hill, NC

Abstract: Chronic low back pain (CLBP) has a significant impact on the lives of millions, yet treatment options are limited. CLBP can be classified into musculoskeletal and neuropathic (sciatica) pain conditions, which are believed to have distinct mechanisms. We aim to investigate whether careful phenotyping of CLBP can reveal brain differences, particularly in the somatosensory system, between sciatica and musculoskeletal CLBP. We recruited 55 CLBP patients and 36 age- and BMI-matched healthy controls (HC) for this study. All subjects completed the Standardized Evaluation of Pain (StEP) assessment, and CLBP patients were categorized into either the musculoskeletal pain group (MSK, N=29) or sciatica group (SC, N=26). Volumetric and vertex analyses of the thalamus were performed with the FSL-FIRST toolbox using T1-weighted MRI. Groups were compared using permutation testing ($p < 0.05$). We observed significant thinning of the posterior-medial aspect of bilateral thalami (Fig 1) in the SC group compared to both MSK group and HC. The altered region lay in the pulvinar - the thalamic association nucleus. In contrast, no significant differences in morphometry were observed between the MSK group and HC, and there were no differences in total thalamic volume among the 3 groups. As the altered region is closely connected to the pre-frontal cortex (PFC), we used resting state functional MRI to compare the connectivity of the posterior thalami to the dorsal-lateral PFC (DLPFC). The SC group showed increased connection between the left posterior thalamus and the right DLPFC compared to the MSK group. These findings suggest that the posterior-medial thalamus shape change may be specific to sciatica. Moreover, the enhanced connection between this area and the DLPFC indicates functional changes in pain perception and modulation. Our study corroborates mechanistic differences between neuropathic and musculoskeletal pain and highlights the potential role of the pulvinar in sciatica. Understanding the central modulation of neuropathic CLBP may facilitate future development of targeted therapies.



Disclosures: **R. Yuan:** None. **J.D. Markman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Yellowblack Corp, Flowonix Corp. F. Consulting Fees (e.g., advisory boards); lateral Pharma, Clexio Pharma, Nectar, Editas, Pfizer, Eliem, Lilly, Biogen. **J. Gewandter:** 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.; Neurometrix. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eisana Corp. F. Consulting Fees (e.g., advisory boards); Algo Therapeutix, Eikonizo Therapeutics, GW Pharma, Neurometrix, Saluda Medical. **Z. Zhang:** None. **P. Geha:** None.

Poster

PSTR410. Central Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR410.18/AA12

Topic: D.02. Somatosensation – Pain

Title: Cellular Mechanism of 10kHz Spinal Cord Stimulation: Insights into Neuromodulation

Authors: *D. WANG, K. LEE, D. LEE, Z. KAGAN, K. BRADLEY;
Nevro Corp, Redwood City, CA

Abstract: Spinal cord stimulation (SCS) has emerged as a promising therapeutic approach for the management of chronic pain. Among various SCS modalities, 10kHz SCS has shown superior efficacy without paresthesia compared to traditional SCS techniques. However, the underlying mechanism by which 10kHz SCS exerts its analgesic effect remains incompletely understood. In this study, we aim to elucidate the mechanisms of action of 10kHz SCS through a comprehensive examination of the neural circuitry and molecular changes associated with its application.
By calcium imaging and electrophysiological recording approaches, we found that 10kHz SCS-mediated spinal cord neuron activation was eliminated by sodium channel blocker (TTX) and significantly reduced by NMDA/AMPA receptor blockers (AP5/CNQX). These data suggest that 10kHz SCS may activate the voltage-gated sodium channels and trigger the release of endogenous neurotransmitters to reduce the pain. To further identify SCS-mediated neurotransmitters, we applied GABA inhibitor (Bicuculine) during the recording and found that 10kHz SCS induced spinal neuron activation was significantly abolished by the Bicuculine. This work suggests that 10kHz SCS works primarily through enhanced GABAergic neurotransmission, thus augmenting inhibitory control over pain pathways and may potentially involve the facilitation of primary afferent depolarization (PAD) and restore the sensory information transmitting to the spinal cord dorsal horn neurons.
Our study demonstrated the neurochemical profile of 10kHz SCS on the GABA-mediated pain processing pathway. The enhanced GABAergic neurotransmission observed with 10kHz SCS may contribute to its clinically superior analgesic efficacy. These findings deepen our understanding of the specific neurochemical mechanisms underlying 10kHz SCS and pave the way for targeted modulation of GABAergic signaling in the development of novel pain management therapies.

Disclosures: **D. Wang:** A. Employment/Salary (full or part-time):; Nevro Corp. **K. Lee:** A. Employment/Salary (full or part-time):; Nevro Corp. **D. Lee:** A. Employment/Salary (full or part-time):; Nevro Corp. **Z. Kagan:** A. Employment/Salary (full or part-time):; Nevro Corp. **K. Bradley:** A. Employment/Salary (full or part-time):; Nevro Corp..

Poster

PSTR411. Barrel Cortex: Cell Types and Their Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR411.01/AA13

Topic: D.03. Somatosensation – Touch

Support: NS112612-03

Title: Changes in myelin in the somatosensory barrel cortex after unilateral whisker loss

Authors: *C. WINT[1,2], *C. WINT[3], E. PETRUS[1];
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M. Jackson Fndn. for the Advancement of Military Med., Bethesda, MD; [3]Anatomy, Physiol. & Genet., Uniformed Services Univ., Bethesda, MD

Abstract: More than one million people in the US live with limb loss; this number is expected to continue rising. Amputees can recover from their injuries by learning new motor skills to navigate daily life, but others experience maladaptive consequences such as chronic pain conditions. It is likely that changes in neural circuitry underlie both adaptive and maladaptive outcomes after injury. Myelin is a membrane that wraps around neuronal axons and enhances electrical communication between neurons. Motor learning, neuronal activity and synaptic plasticity all influence myelination in the central nervous system. After unilateral limb loss, magnetic resonance imaging (MRI) detects reduced myelin in patients and animal models, in particular in areas that connect bilateral limbs. It is unknown if this loss is maladaptive, and if so, if interventions could restore myelin density to support and enhance motor learning after injury. We used a mouse model of unilateral whisker denervation to investigate changes in myelin in the whisker processing region of the brain: primary somatosensory barrel cortex (S1BC). We hypothesized that myelin immunohistochemistry staining would be reduced in the deprived S1BC in injured mice due to the loss of the primary input (whiskers) to that brain region. Thin brain slices were stained for Myelin Basic Protein (MBP). Three groups of male and female mice were analyzed: two weeks after injury, two months after injury, and 2 months after injury combined with a whisker behavior task. Two weeks after injury a surprising increase in myelin staining was observed, followed by a decrease two months after injury. Training on a whisker task made the reduction of myelin after whisker loss less severe. Myelin may increase immediately after injury to support wiring new connections within the S1BC after unilateral whisker loss. On a longer time-scale, myelin may be reduced due to the loss of primary inputs to S1BC, but intensive whisker training may support increased neuronal activity. This increased activity may combat the myelin loss that normally occurs. Myelin formation supports motor learning, thus identifying therapies that promote myelination may be beneficial for amputee recovery.

Disclosures: **C. Wint:** None. **C. Wint:** None. **E. Petrus:** None.

Poster

PSTR411. Barrel Cortex: Cell Types and Their Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR411.02/AA14

Topic: D.03. Somatosensation – Touch

Support: NS112612-03

Title: Transcriptional Heterogeneity of Layer 4 Barrel Cortex Neurons after Unilateral Amputation

Authors: *L. IYER, E. PETRUS;
Anatomy, Physiol. and Genet., Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD

Abstract: There are nearly 2.1 million people living with limb loss in the United States. Some patients recover well after their injury by learning new motor skills, but others suffer from chronic pain conditions. Brain activity measured by functional magnetic resonance imaging (fMRI) in both subsets of patient populations has similar patterns. This makes predicting patient outcomes challenging and also impossible to determine if this neural activity is related to a beneficial or maladaptive recovery. Our research seeks to discover the neurons, synapses, and circuits that underlie these variable adaptations. Stimulation of the intact whiskers in a rodent model of unilateral whisker nerve transection (IONX) produces a robust fMRI response in bilateral primary somatosensory barrel (whisker) cortices (S1BC), similar to the activity in sensory/motor cortex of human patients. This model allows the detection of the neurons and pathways that are altered after amputation. Our hypothesis is that there are neurons in the sensory cortex that drive brain changes after injury. Previously we found that the increased intact S1BC activity is due to the stronger connection between the thalamus and Layer 4 (L4) neurons in S1BC. These L4 neurons have historically been considered a homogenous group; however, our recent single-nucleus RNA sequencing has detected at least 3 subtypes of neurons. The presence of these neuronal clusters has been identified by co-labeling them with Rorb (L4 marker) using HiPlex RNAscope. Further, genes related to synaptic plasticity such as Gria2, Homer1, and mGluR5 have increased after injury. Electrophysiology has confirmed that the changes in RNA expression have concurrent effects on synaptic function. These events likely influence downstream cortical activity in response to intact whisker use. Our work connects neural gene expression and activity patterns to behavioral phenotypes of our mouse model of amputation. This project will enhance our understanding of how the brain adapts to injury, aiming for targeted interventions to improve outcomes in amputees.

Disclosures: L. Iyer: None. **E. Petrus:** None.

Poster

PSTR411. Barrel Cortex: Cell Types and Their Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR411.03/AA15

Topic: D.03. Somatosensation – Touch

Support: NS112612-03
Intramural Program of NIH

Title: Behavioral Adaptations after Unilateral Whisker Pad Amputation in Mice

Authors: *E. PETRUS[1], E. MCCALL[2], P. WRIGHT[2], F. JOHNSON, III[1], A. KORETSKY[2];
[1]Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD; [2]NINDS, NIH, Bethesda, MD

Abstract: There are 1.6 million people living with limb loss; this number is expected to more than double by 2050. Successful recovery after amputation requires that patients learn new motor skills, such as using their residuum, intact limbs, or a prosthesis. Impediments to recovery include chronic pain conditions, anxiety/depression, or the absence of motor learning. The variety of patient outcomes may depend on the unique neural and behavioral adaptations that patients develop after injury. We have developed a rodent model of unilateral amputation by transecting the infraorbital nerve (IONX) connecting the whisker pad to the brain. Neural activity measured by functional magnetic resonance imaging (fMRI) in IONX mice closely mimics that of amputees. fMRI detects increased activity in the spared whisker barrel cortex and takeover of the injured whisker barrel cortex by the intact cortex; the synaptic plasticity mechanisms underlying these changes have begun to be assessed. This amputation model allows for the study of neural and behavioral adaptations of male and female mice after IONX injury. We hypothesized that the loss of one set of whiskers would negatively impact exploratory and whisker-task related behaviors. While IONX injury did not impact exploration of the open field, animals missing one set of whiskers did inspect novel objects more frequently with their nose. Performance on a pole localization task with the intact whisker set was equal to sham-operated mice. Finally, IONX mice had impaired performance on a gap crossing task, which requires bilateral whisker inputs. Interestingly, the injury group had variable performance on many of these tests. These results indicate that observed human variability of responses to limb loss might reflect unique neural adaptations underlying their behavioral outcomes and is not necessarily only a byproduct of the inhomogeneity of patients and/or their injuries. Further tests using chemogenetics to isolate the brain region or cell types underlying these adaptations may improve our understanding of how the brain adapts to injury and produces various behavioral outcomes. If behavioral adaptations influence beneficial or maladaptive recovery in rodents, these results may be extrapolated to inform better clinical interventions for patients after limb loss.

Disclosures: E. Petrus: None. **E. McCall:** None. **P. Wright:** None. **F. Johnson:** None. **A. Koretsky:** None.

Poster

PSTR411. Barrel Cortex: Cell Types and Their Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR411.04/AA16

Topic: D.03. Somatosensation – Touch

Support: R01-NS-065992

Title: Alterations in homeostatic plasticity in Fmr1 KO mice following unilateral whisker deprivation

Authors: *A. LAKHANI, P. WENNER;
Emory Univ., Atlanta, GA

Abstract: Fragile X Syndrome (FXS) is the most common form of inherited intellectual disability. It is caused by a loss-of-function of the FMR1 gene on the X chromosome, resulting in the absence of fragile X messenger ribonucleoprotein (FMRP). Although there are many interventional and pharmacological treatments, there is no cure for FXS. Altered cortical activity is an underlying pathology of FXS that is associated with sensory hypersensitivity and epileptic vulnerability. Normally, networks maintain activity levels within an appropriate range through a set of homeostatic plasticity mechanisms including compensatory adjustments in synaptic strength and/or intrinsic excitability. It is not clear why homeostatic plasticity cannot correct altered activity levels associated with FXS, but previous work suggests that homeostatic mechanisms are impaired in FXS models. Unilateral whisker deprivation (trimming all whiskers on one side of the snout) is a behaviorally relevant in vivo perturbation that has been shown to trigger homeostatic responses in the whisker-responsive barrel cortex. This has been expressed as an increase in whisker-evoked responses in L4 and L2/3 regular spiking (RS) excitatory neurons. In order to determine if and how homeostatic plasticity was altered in the Fmr1 KO mouse model of FXS, we trimmed whiskers every other day from postnatal day 14-21 (PD 14-21). Whiskers were deflected using a 3x3 array of piezoelectric actuators to stimulate the principal/most responsive whisker and surrounding whiskers at multiple velocities. Spiking activity was recorded using a 64-channel multi-electrode probe in the somatosensory cortex of lightly anesthetized mice. Preliminary results suggested that spiking in L5/6 RS neurons in control mice were reduced in the KO compared to WT littermates. In addition, following 7 days of whisker deprivation, the sensitivity to whisker stimulation was very different in the whisker-deprived KO compared to whisker-deprived WT littermates. Future work will focus on recording whisker-evoked spiking from fast spiking (putative inhibitory interneurons) and L4 neurons to evaluate homeostatic plasticity in FXS mice in different layers and cell types.

Disclosures: **A. Lakhani:** None. **P. Wenner:** None.

Poster

PSTR411. Barrel Cortex: Cell Types and Their Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

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Topic: D.03. Somatosensation – Touch

Support: NRTS 2199 from the American Academy of Neurology to William Zeiger
K08NS114165-01A1 from the National Institute of Neurological Disorders and Stroke to William Zeiger

Title: Investigating the role of Parvalbumin interneurons in experience-dependent plasticity of the somatosensory cortex

Authors: *B. CAMPOS[1], B. VASQUEZ[2], W. ZEIGER[3];
[1]UCLA, Covina, CA; [2]UCLA, UCLA, Los Angeles, CA; [3]Univ. of California Los Angeles, Univ. of California Los Angeles, Los Angeles, CA

Abstract: The primary somatosensory barrel cortex (S1BF) of rodents is a site of robust experience-dependent plasticity, undergoing structural and functional changes in response to peripheral sensory inputs. One of the most commonly studied paradigms for experience-dependent plasticity in the S1BF is chronic whisker trimming. In this protocol, all whiskers on one side of the face are trimmed, with the exception of one or a few spared whiskers. The cortical map representation of the spared whisker(s) increases, while the cortical map representation of the trimmed (deprived) whiskers shrinks. However, the exact microcircuit mechanisms that drive experience-dependent plasticity remain incompletely understood. In deprived barrels, changes in the intrinsic excitability of inhibitory Parvalbumin (PV) interneurons are thought to homeostatically balance the loss of feedforward excitation. Yet, the precise role of PV cells in mediating map expansion of spared whiskers is unknown. We hypothesized that PV cells serve as key regulators of experience-dependent plasticity in the S1BF. To test our hypothesis, we have been recording the activity of PV cells in Layer 2/3 (L2/3) of S1BF longitudinally using two-photon (2P) calcium imaging. We investigated responses of PV cells during chronic whisker trimming. We recorded responses of PV cells to whisker stimulation in the C1 and D1 barrels and then trimmed all the whiskers except the D1-whisker. After trimming, the number of PV cells responding to the D1 whisker increased (from 65.23% to 79.52%) in the C1 barrel, while there was no change in the number of D1 whisker responsive PV cells in the D1 barrel. Given that chronic whisker trimming shifts the responsivity of pyramidal cells in surround barrels toward the spared whisker, these results are consistent with homeostatic scaling of PV-mediated inhibition with excitation to maintain proper excitation to inhibition balance during cortical map plasticity. To define the casual role of PV cells in mediating experience dependent plasticity, we are now using chemogenetics to manipulate PV cell activity. Acute inhibition of PV cells leads to a dose-dependent reduction in whisker-evoked responses of both PV and pyramidal cells, but no change in the number of responsive cells. On the other hand, activation of PV cells reduces both the number of whisker responsive PV and pyramidal cells and whisker-evoked responses. These results are somewhat paradoxical and may reflect highly recurrent connectivity between PV and pyramidal cells in the S1BF. We are now testing the effect of chronic PV cell manipulation on cortical map plasticity induced by whisker trimming.

Disclosures: **B. Campos:** None. **B. Vasquez:** None. **W. Zeiger:** None.

Poster

PSTR411. Barrel Cortex: Cell Types and Their Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR411.06/AA18

Topic: D.03. Somatosensation – Touch

Support: STA 431/14-1

Title: A novel whisker-based texture discrimination task for freely moving mice towards defining the roles of PV and VIP neurons via chemogenetics

Authors: *A. CETIN, J. GUY, J. F. STAIGER;
Univ. Med. Ctr. Goettingen, Goettingen, Germany

Abstract: Because of the precisely defined somatotopic map, the barrel cortex (wS1) is a favorable model for the study of microcircuits and investigation of the roles of neuronal subtypes in the processing of sensory information. Physiological mechanisms of wS1 of whisker-dependent behavior have mostly been investigated on head-fixed animals to gain better control of stimulus presentation and behavioral measurement. However, there is a limited number of studies investigating whisker-based perceptual detection during natural behavioral conditions. Also, the behavioral relevance of parvalbumin (PV) and vasoactive intestinal polypeptide (VIP) expressing GABAergic neurons remains unclear. We aimed to define the contributions of PV and VIP expressing populations within wS1 towards texture discrimination in freely moving mice using chemogenetic manipulation. To this extent, we have established a textured T-maze task, which is an operant conditioning protocol for whisker-based tactile discrimination and to measure the perceptual detection threshold of freely moving mice. In this protocol, food-restricted animals were trained for a 2-choice reward task in a T-maze. Goal arms of the T-maze were cued by 2 types of texture (i.e. coarse vs smooth) blocks and the animal was expected to learn to differentiate these two textures to find the food reward in one of the arms. After reaching a 70% success level on 2 consecutive days, the contrast between these two textures was decreased to find out the discrimination threshold of the animals. Once animals' performance dropped to chance level with one of the lower contrasts, this contrast was taken as the perceptual detection threshold of the animal. We started with validating our paradigm and controlling the effect of stereotactic injection into wS1, and IP injection of Compound 21 (C21). Mice were first trained in the textured T-maze to determine baseline success, then underwent stereotactic surgery for sham injections to wS1. After recovery time, animals were retested in the T-maze for the same task, including IP injections of C21 and saline. A paired t-test on the highest 3 scores pre- and post-OP showed that surgery did not change animals' success rates in texture discrimination ($t(14) = 1.101$, $p = 0.46$). A one-way ANOVA revealed that there was no difference in animals' performance with IP injection of C21 and Saline compared to no IP injection condition ($F(2,23) = 0.142$, $p = 0.87$). Following these controls we will start with bilateral Gq-DREADD expression in both PVcre+ and VIPcre+ mice to figure out the role of PV and VIP neurons in texture discrimination via C21 administration.

Disclosures: A. Cetin: None. **J. Guy:** None. **J.F. Staiger:** None.

Poster

PSTR411. Barrel Cortex: Cell Types and Their Function

Location: WCC Halls A-C

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Program #/Poster #: PSTR411.07/AA19

Topic: D.03. Somatosensation – Touch

Support: UK Research and Innovation
Onassis Foundation

Interdisciplinary Bioscience (BBSRC Doctoral Training Partnership), University of Oxford

Title: Distinct synaptic input profiles to layer 5 somatostatin neocortical interneuron subtypes

Authors: *A. VOURVOUKELIS, C. J. AKERMAN;
Pharmacol., Univ. of Oxford, Oxford, United Kingdom

Abstract: Important advances have been made in our understanding of different subtypes of cortical somatostatin-expressing interneurons (SSTs) in terms of their morphology, electrophysiology, molecular properties, and efferent connectivity (Gouwens et al., 2020; Muñoz et al., 2017; Naka et al., 2019). However, our understanding of their afferent connectivity, considering SST subtype heterogeneity, remains limited. We examined the connectivity of intralaminarly activated afferents to layer 5 (L5) SSTs in the mouse barrel cortex, including excitatory short-term plasticity and the balance between excitatory and inhibitory input. In vitro patch-clamp recordings from acute brain slices of SST-IRESCre;Ai9 mice of both sexes (P42-P77) were combined with optogenetic control of a subcortically projecting L5 pyramidal presynaptic subpopulation, by employing a retrograde AAV system. Clustering of 12 intrinsic electrophysiological properties from 169 L5 SSTs revealed three putative subtypes. Morphological reconstructions showed layer-specific axonal profiles, supporting their alignment with the subtypes of T-shaped Martinotti (TMC), fanning-out Martinotti (FMC) and non-Martinotti (NMC) SSTs (Nigro et al., 2018). Interrogating short-term plasticity of excitatory input to the L5 SST subtypes, we found that high-frequency stimulation of the presynaptic pyramidal neuron subpopulation resulted in facilitation for Martinotti subtypes (TMC facilitation index=2.65 ± 0.3, n=12; FMC=3.5 ± 0.4, n=9), but depression for NMCs (NMCfac=0.6 ± 0.2, n=2). The facilitation index represents the mean EPSC amplitude facilitation across stimulation pulses (normalization to the initial EPSC), with reported values as mean ± SEM. We also observed distinct subtype-specific profiles in the balance of monosynaptic excitation (E) and disynaptic inhibition (I), measured as an E/(E+I) ratio. TMCs received strong monosynaptic excitation (E/(E+I)=0.72 ± 0.08), NMCs received strong disynaptic inhibition (E/(E+I)=0.03 ± 0.03), while FMCs received both (E/(E+I)=0.55 ± 0.1) (Kruskal-Wallis, $H(2)=16.49$, $p<0.001$, Dunn's multiple comparisons (Holm method) $p<0.01$ for both TMC vs. NMC, and FMC vs. NMC; n=8 per subtype - reported values as mean ± SEM). Overall, our data characterise intralaminarly activated synaptic input to L5 SST subtypes, revealing excitatory short-term plasticity dynamics and differences in the balance between excitatory and inhibitory components. We therefore provide evidence for distinct SST subnetworks, which may be differentially recruited by presynaptic partners to generate layer-specific inhibition.

Disclosures: A. Vourvoukelis: None. **C.J. Akerman:** None.

Poster

PSTR411. Barrel Cortex: Cell Types and Their Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR411.08/Web Only

Topic: D.03. Somatosensation – Touch

Support: Swiss National Science Foundation
Synapsy National Center of Competence in Research
International Foundation for Research in Paraplegia

Title: Neurogliaform cells mediate interhemispheric modulation of cortical pyramidal neuron activity

Authors: *F. MARKOPOULOS[1], R. W. CHÉREAU[1], F. BRANDALISE[1,3], V. CHIPPALKATTI[1], J. PRADOS[1,2], A. DAYER[1,2], A. HOLTMAAT[1];
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Abstract: Integration of bilateral sensory inputs requires effective communication between brain hemispheres. This interhemispheric communication is essential for sensory perception and involves reciprocal connections between homotopic sensory areas. A key role in this process is attributed to interhemispheric inhibition which, owing to its long-lasting form, is posited to operate largely through neurogliaform cells (NGCs). However, direct evidence on the role of NGCs in interhemispheric inhibition is missing. Here we show that NGCs in the mouse barrel cortex (BC) are engaged by interhemispheric callosal projections to modulate pyramidal neuron (PN) activity and sensory perception. Using optogenetics, ex vivo whole-cell recordings, and in vivo calcium imaging, we found that layer 1 and layer 2/3 (L1-3) NGCs are strongly activated by the callosal and suppressed by the thalamocortical pathway, suggesting that NGCs encode ipsilateral rather than contralateral whisker stimuli. We also found that direct stimulation of L1-3 NGCs modulates whisker-evoked activity in L2/3 and L5b PNs, and increases the perceptual threshold in a whisker deflection detection task. Furthermore, these effects were recapitulated by direct stimulation of callosal projections and deflection of the ipsilateral whiskers respectively, suggesting that the effect of the callosal pathway on sensory perception is mediated by NGCs. Our results not only prove that NGCs mediate interhemispheric inhibition, but also demonstrate their role in sensory perception via modulation of the main units involved in cortical input and output.

Disclosures: F. Markopoulos: None. **R.W. Chéreau:** None. **F. Brandalise:** None. **V. Chippalkatti:** None. **J. Prados:** None. **A. Dayer:** None. **A. Holtmaat:** None.

Poster

PSTR411. Barrel Cortex: Cell Types and Their Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR411.09/AA20

Topic: D.03. Somatosensation – Touch

Support: Swedish Research Council Distinguished Professor Program (2021-00671)

Title: Characterization of Cortical Layer 6b Ctgf Expressing Neurons in Mice

Authors: *R. KHALID, D. MASINI, A. LOCARNO, O. NETSYK, C. BROBERGER; Stockholm Univ., Stockholm, Sweden

Abstract: The cerebrocortical microcircuit has been the subject of intense investigation, but models often neglect the possible contribution of the deepest lamina, layer 6b (L6b; also known as the persistent subplate). Here, we aim to fill this gap by focusing on a subset of excitatory L6b neurons identified by the expression of connective tissue growth factor (CTGF). Using a mouse model expressing cre under the promoter of *Ctgf* (n=6) we show that L6b^{CTGF}neurons are restricted to L6b in the cortical column, expanding no further than 80µm superficial to the white matter and were not present among the white matter fibers. Next, we mapped L6b^{CTGF} projections and synaptic terminals across the brain using genetically restricted viral tracing (AAV5-hSyn1-DIO-mEGFP-Syp1-mRuby). Ctgf-cre mice were injected either in the primary somatosensory (S1-bfd; barrel field; n=3) or motor (M1; n=4) cortex. S1- L6b^{CTGF} neurons projected extensively within L6b, and local axonal projections traveled across cortical layers with prominent bouton density in L2/3 and L1. Meanwhile, M1-ctgf neurons showed less distinctive projection segregation, with dense connectivity limited to L1. We identified interregional connectivity characterized by ipsilaterally confined projections and reciprocal connectivity between S1 and M1. In S1-injected mice, projections towards M1 preferentially targeted L6a and L1. In M1-injected mice neurons innervating S1 preferentially targeted only L1. Subcortically, S1- L6b^{CTGF} neurons targeted thalamic structures that project back to the sensory-motor cortex, as well as thalamic polymodal association areas. M1- L6b^{CTGF} neurons gave rise to boutons in thalamic sensory-motor areas but avoided polymodal targets. We also performed electrophysiology of both L6b^{CTGF} and non-CTGF neurons in S1 slices. These neurons have a heterogenous firing pattern at baseline and in response to current pulses (n = 27 tdT+and n = 12 tdT-). Finally, using optogenetics, we demonstrate that CTGF+ neurons form glutamatergic monosynaptic connections in upper cortical layers targeting both pyramidal cells (n = 15/19 responsive neurons) and fast-spiking interneurons (n = 5/10 responsive neurons). Thus, L6b^{CTGF} neurons are anatomically and functionally integrated with the somatosensory-motor circuitry with long-range projections that span the local cortical column and travel intrahemispherically to target other cortical and subcortical regions.

Disclosures: R. Khalid: None. **D. Masini:** None. **A. Locarno:** None. **O. Netsyk:** None. **C. Broberger:** None.

Poster

PSTR411. Barrel Cortex: Cell Types and Their Function

Location: WCC Halls A-C

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Title: A consensus definition for layer 6b neurons in the neocortex of the adult mouse

Authors: *S.-J. KIM[1], T. A. BABOLA[2], A. C. SPIEGEL[2], M. H. LIEW[2], M. PROSKURIN[2], K. LEE[2], C. J. MATNEY[2], H. KANG[2], M. CHEVÉE[2], E. M. SCHULTEIS[2], A. E. COYE[2], J. A. KIM[2], L. A. GOFF[3,4,5], J. KIM[2,6,7], S. P. BROWN[8,4];
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Abstract: Layer 6b neurons are a population of cells in the deepest layer of the mouse cortex. These neurons are hypothesized to be equivalent to white matter neurons in the human brain, which are altered in multiple psychiatric disorders, including autism, bipolar disorder and schizophrenia. However, their functional role in the cortex remains unclear. Because previous studies have used different methods to identify L6b neurons in adult mice, it is difficult to compare their contributions to cortical function across experiments. Here, we compare commonly used methods for identifying white matter and L6b neurons to determine whether they captured the same population of neurons and a consensus definition of L6b neurons in an adult mouse. And we investigate their morphological, anatomical and electrophysical properties. We analyzed the expression of multiple molecular markers of subplate and L6b neurons using the publicly available scRNA-seq data sets and tested expression in L6b neurons retrogradely labeled from L1/2 of the cortex after early development. We confirmed that L6b neurons are identified consistently using three methods in an adult mouse: 1) expressions of Neurexophilin 4 (Nxph4) 2) expression of Connective tissue growth factor (CTGF) and 3) retrograde labeling from the L1/2 of cortex. To investigate the morphology of L6b neurons defined by the three methods, we reconstructed filled L6b neurons and imaged neurons in the Nxph4-creER mouse line. L6b neurons represent a population of spiny, multipolar excitatory neurons with homogeneous morphological properties in three cortex areas: primary motor cortex, primary sensory cortex and visual cortex. In addition, they exhibit consistent electrophysiological intrinsic properties across developmental age. We found that the primary motor cortex and primary motor cortex of L6b neurons have connections ipsilaterally. However, L6b neurons do not project to the thalamus. Furthermore, we showed that they receive synaptic input from the output neurons of the cortex, including layer 5 projection neurons and L6 corticothalamic

neurons using optogenetic experiments. Unlike subplate neurons, L6b neurons do not respond to activation of thalamic inputs. Our studies provide a consensus definition of L6b neurons and indicate that these neurons may play a role in regulating the activity level of large regions of cortex in response to ongoing cortical output, suggesting a possible mechanism whose dysregulation contributes to psychiatric disorder.

Disclosures: S. Kim: None. **T.A. Babola:** None. **A.C. Spiegel:** None. **M.H. Liew:** None. **M. Proskurin:** None. **K. Lee:** None. **C.J. Matney:** None. **H. Kang:** None. **M. Chevée:** None. **E.M. Schulteis:** None. **A.E. Coye:** None. **J.A. Kim:** None. **L.A. Goff:** None. **J. Kim:** None. **S.P. Brown:** None.

Poster

PSTR411. Barrel Cortex: Cell Types and Their Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR411.11/AA22

Topic: D.03. Somatosensation – Touch

Support: Korea Brain Research Institute (KBRI, 23-BR-02-02)
Basic science research program through the National Research Foundation of Korea (NRF, RS-2023-00248148) funded by Ministry of Science and ICT

Title: Cortical area-specific distribution pattern of persistent subplate neurons in the mouse brain

Authors: *K. LEE[1], J. KIM[2], J. KIM[1];
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Abstract: Subplate neurons (SPNs) are the first generated cortical neurons, and they contribute to formation of thalamo-cortical neural circuits in the developing brain. Early studies reported large degeneration of SPNs in the early postnatal weeks, but recent studies suggest that they might persist to adolescent and adult stage. In the present study, we investigated whether SPNs can be found in the adult mouse brain, whether they are excitatory or inhibitory, and whether they show different localization patterns throughout different cortical regions. We used connective tissue growth factor (CTGF) to identify persistent SPNs and confirmed their existence in the brains of young and mature adult mice. By using cell markers of the two most common inhibitory cortical neurons in the cortex, parvalbumin and somatostatin, we determined whether they are excitatory or inhibitory neurons. Our immunohistochemical investigation confirmed that they are neither parvalbumin nor somatostatin interneuron in both ages. Interestingly, we found that the relative layer depth of the persistent SPNs in the entorhinal cortex is significantly thicker than other motor-sensory cortices. Our results show the first comparative results about the cell identity and relative layer depth throughout different cortical

areas in the young and mature adult mouse brains, highlighting the potential roles of persistent SPNs in the normal brain and in diseases.

Disclosures: **K. Lee:** None. **J. Kim:** None. **J. Kim:** None.

Poster

PSTR411. Barrel Cortex: Cell Types and Their Function

Location: WCC Halls A-C

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Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation France
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ANR (ANR-17-EURE-0029)

Title: Sparse functional projection from barrel cortex to single SPNs in dorsal striatum and cell subtype-specific correlations with the spontaneous behaviours of mice.

Authors: *K. AMROUNE, D. ROBBE, I. BUREAU;
Aix Marseille Univ, INSERM, INMED, Marseille, France

Abstract: Animals take navigational decisions based on sensory cues. In rodents, the Barrel Cortex (BC), a region of the primary somatosensory cortex, integrates tactile information from the whiskers. Pyramidal neurons in BC project to the dorsal striatum (DS), the main input region of basal ganglia involved in decision making and motor control. Striatum has two projection-neuron populations that are in distinct pathways, direct (dSPNs) and indirect (iSPNs), which control with opposite manners a given action. Thus, the sensory corticostriatal projection onto d/iSPNs is one important determinant of basal ganglia outputs and motor control. We study the BC/DS coupling to better understand its function. Here, we used a functional mapping method, laser scanning photostimulation with glutamate uncaging, which allowed to localize the soma of presynaptic neurons in BC and to investigate the number and size of inputs received by individual SPNs. Our aim was to study the spatial organization of the corticostriatal projection and the eventual dSPN/iSPN differences. Our results demonstrate that despite diffuse and overlapping cortical axons in DS described in earlier studies, the inputs received by each SPN from BC is unique and fragmented, showing little overlap with the inputs received by nearby SPNs. Thus, it is only collectively that SPNs provide BG a full representation of whiskers. Nonetheless, projections from BC were organized topographically. Our results also confirm the dominance of the innervations from the top layer 5, although innervations from the superficial layers 2/3 strengthened with age. Variability in the extent and strength of the d/iSPN receptive field in barrel cortex was large and the differences between the dSPNs and iSPNs subtle.

Comparison between these receptive fields and the mouse spontaneous behaviours in an openfield revealed cell-subtype specific correlations.

Disclosures: **K. Amroune:** None. **D. Robbe:** None. **I. Bureau:** None.

Poster

PSTR411. Barrel Cortex: Cell Types and Their Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR411.13/AA24

Topic: D.03. Somatosensation – Touch

Support: NIH grant R35 NS127219
NIH training grant T32 GM130550

Title: Integration between barrels and cell type-specific organization in somatosensory cortex

Authors: *J. M. JUDGE[1], M. B. JACKSON[2];
[1]Neurosci., Univ. of Wisconsin, Madison, Madison, WI; [2]Neurosci., Univ. of Wisconsin-Madison, Madison, WI

Abstract: Neural circuits process complex time-sensitive information from multiple inputs by mechanisms that are poorly understood. Primary somatosensory cortex (S1) is an appealing model system of these processes due to its modular structure: it is organized into six layers with distinct cytoarchitecture and connectivity. The cortex is further organized into columns, and in S1 of mice and rats each columnar barrel processes sensory input from one main whisker. Communication between neighboring barrels are thought to depend heavily on factors such as position in whisker rows or whisker arcs, as the barrel layout changes continuously along the anterior-posterior axis. Hybrid optical voltage sensors (hVOS) allow simultaneous imaging from many cells of fast action and synaptic potentials in genetically-defined subpopulations of cells in intact cortical microcircuits. In this exploratory study, we investigated excitatory cells (pyramidal and spiny stellate) expressing an hVOS probe targeted via non-voltage-gated sodium channel Cre driver (scnn1a-tg3-Cre) in brain slices of adult mice. We observed limited signal transmission across barrels in response to extracellular stimulation with glass microelectrodes in layer 4 (L4), with 6 barrel pairs showing signal crossings and 5 showing no signal crossing (n=3 slices). In contrast, layer 2/3 (L2/3) stimulation readily activates multiple barrels in L4 whenever it activates a single barrel in L4, with 15 barrel pairs showing signal crossing and none showing no signal crossing (n=5 slices). In many of these experiments with L4 stimulation, we observed signal crossing between L4 barrels in one direction but not the other. These observations correspond with expected cortical circuit behavior based on morphological studies of L4 axons and are consistent with previous explanations of how receptive fields of multiple whiskers may transform sensory input in S1. With particular interest in timing of circuit behavior, we further hypothesize that response latency differences between neighboring barrels may depend on site of stimulation varying continuously with distance from L4 into L2/3 . Since timing can impact

circuit behavior, we hypothesize that repeating measurements after blockade of inhibitory synapses can reveal further differences relevant to circuit function. This activation of multiple barrels and their relative timing are influenced by many inputs and circuit conditions beyond the sensory information moving through S1. Characterizing the conditions and integration mechanisms involved in the selective activation of barrels may reveal underlying principles of modular organization of columnar structures.

Disclosures: J.M. Judge: None. **M.B. Jackson:** None.

Poster

PSTR411. Barrel Cortex: Cell Types and Their Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR411.14/AA25

Topic: D.03. Somatosensation – Touch

Support: NIH Intramural Research Program

Title: Distinct brain-wide presynaptic networks underlie the functional identity of individual cortical neurons

Authors: *A. INACIO, K. LAM, Y. ZHAO, F. PEREIRA, C. R. GERFEN, S. LEE; NIH-NIMH, Bethesda, MD

Abstract: Neuronal connections provide the scaffolding for neuronal function. Revealing the connectivity of functionally identified individual neurons is necessary to understand how activity patterns emerge and support behavior. Yet, the brain-wide presynaptic wiring rules that lay the foundation for the functional selectivity of individual neurons remain largely unexplored. Cortical neurons, even in primary sensory cortex, are heterogeneous in their selectivity, not only to sensory stimuli but also to multiple aspects of behavior. Here, to investigate presynaptic connectivity rules underlying the selectivity of pyramidal neurons to behavioral state in primary somatosensory cortex (S1), we used two-photon calcium imaging, neuropharmacology, single-cell based monosynaptic input tracing, and optogenetics. We show that behavioral state-dependent neuronal activity patterns are stable over time. These are not determined by neuromodulatory inputs but are instead driven by glutamatergic inputs. Analysis of brain-wide presynaptic networks of individual neurons with distinct behavioral state-dependent activity profiles revealed characteristic patterns of anatomical input. While both behavioral state-related and unrelated neurons had a similar pattern of local inputs within S1, their long-range glutamatergic inputs differed. Individual cortical neurons, irrespective of their functional properties, received converging inputs from the main S1-projecting areas. Yet, neurons that tracked behavioral state received a smaller proportion of motor cortical inputs and a larger proportion of thalamic inputs. Optogenetic suppression of thalamic inputs reduced behavioral state-dependent activity in S1, but this activity was not externally driven. Our results revealed

distinct long-range glutamatergic inputs as a substrate for preconfigured network dynamics associated with behavioral state.

Disclosures: **A. Inacio:** None. **K. Lam:** None. **Y. Zhao:** None. **F. Pereira:** None. **C.R. Gerfen:** None. **S. Lee:** None.

Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR412.01/AA26

Topic: D.05. Auditory & Vestibular Systems

Support: Wellcome Trust (WT092606AIA, WT110198/Z/15/Z, WT109148/Z/15/Z)
European Research Council (ERC CoG-MECHIDENT)
NIH (R01-DC04290)

Title: Evolutionary Prototype for Auditory Combinatorial Semantics in a Macaque

Authors: *Z. ZHANG[1], J. NACEF[1], F. POLETIEK[2], B. WILSON[3], T. D. GRIFFITHS[1], Y. KIKUCHI[1], C. I. PETKOV[1,4];
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Abstract: How human language evolved from cognitive systems shared with ancestors to living nonhuman animals remains an important open question, one with implications for the extent to which aspects of the human language system can be modelled in nonhuman animals. A key property of human language is combinatorial semantics, where information from a sequence of words is integrated to identify meaningful content. We designed a novel behavioural touchscreen task implemented with two rhesus monkeys (*Macaca mulatta*) in their home units. The task allowed us to study whether the monkeys could learn the meaning of individual nonsense speech sounds identifying colours or shapes, prior to integrating information from a sequence of two sounds identifying a specific object by its joint colour and shape properties. The paradigm was implemented in two key phases. In the first phase, the animals started by learning to associate the nonsense words with either specific colours or shapes. Learning the task was effortful and the two monkeys struggled to maintain high performance throughout the touch screen testing sessions in the colony. However, they also showed regular bouts of high performance, and they met predefined criteria for progression (i.e., majority of sessions in the testing week before progression showing above chance performance based on permutation tests). One of the monkeys recently progressed to the final phase where sequences of two sounds identified objects by both colour and shape properties. The macaque's choices were significantly greater for the objects whose colour and shape properties both matched the informative content in the two sounds, than for colour or shape foils, or foils incorrect in both colour and shape features (23

testing sessions; pairwise *t*-tests corrected for multiple comparisons: combined > foil performance, $p < 0.001$). Our next steps in this research program are to test whether the first monkey can generalize learning to probe trials of novel combinations not previously experienced and to progress the second monkey to this final phase of testing. The results provide tentative support for a primate prototype of auditory combinatorial semantics in nonhuman primates. They also demonstrate that such abstract learning is difficult for monkeys, altogether providing novel insights into language evolution.

Disclosures: Z. Zhang: None. **J. Nacef:** None. **F. Poletiek:** None. **B. Wilson:** None. **T.D. Griffiths:** None. **Y. Kikuchi:** None. **C.I. Petkov:** None.

Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR412.02/AA27

Topic: D.05. Auditory & Vestibular Systems

Support: DFG Walter Benjamin Fellowship

Title: Heartbeat influences visual and auditory perception

Authors: *E. AL[1], S. TUNE[2], S. A. SHETH[3], J. OBLESER[2], S. HAEGENS[1];
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Abstract: Our perception of the external world is not only influenced by the brain but also by the rest of the body. Here, we report two studies that investigate how body-brain interactions influence sensory perception. In the first study, we aim to answer whether cardiac and respiratory signals influence the perception of auditory signals. For this purpose, we conducted an experiment in which 19 subjects performed an auditory discriminatory and detection task. We measured respiratory and cardiac signals using a respiratory belt and ECG, and motor activity using EMG. We analyzed how (premotor) reaction times were influenced by the two phases of cardiac (i.e., systole and diastole) and respiratory activity (i.e., inhalation and exhalation) during discrimination and detection performance. During both tasks, we observed that subjects were faster to react when stimuli occurred during diastole compared to systole. No effect of the respiratory cycle was observed on reaction times. In the second study, we extended our analysis to the visual domain and asked how cardiac effects on sensory detection are influenced by distractors in the environment. We tested how the cardiac phase influences reaction times to visual stimuli in the presence and absence of distractors. Furthermore, we investigated how this interaction is reflected in broadband high-frequency activity (BHA) and visual-evoked potentials (VEP) using intracranial recordings in 18 patients with intractable epilepsy. Our results show that in the absence of distractors, subjects were slower at reacting to visual stimuli during systole compared to diastole. Furthermore, mean VEPs were observed to be attenuated in the time

windows of 98-122 ms and 258-278 ms following stimulus during systole compared to diastole in the absence of distractors. We did not observe any changes in BHA across the cardiac cycle. These effects show that the influence of heartbeat on perception depends on cognitive load.

Disclosures: E. Al: None. **S. Tune:** None. **S.A. Sheth:** None. **J. Obleser:** None. **S. Haegens:** None.

Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR412.03/AA28

Topic: D.05. Auditory & Vestibular Systems

Title: Modeling of perceived musical rhythms using intracranial electroencephalography

Authors: *M. DEXHEIMER[1], J. WALLNER[1], S. COLE[1], H. SHAH[1], C. HERFF[2], D. J. KRUSIENSKI[1];
[1]Virginia Commonwealth Univ., Richmond, VA; [2]Maastricht Univ., Maastricht, Netherlands

Abstract: The neural correlates of auditory anticipation and perception in the human brain have been explored through various neuroimaging modalities and experimental protocols. Non-invasive neuroimaging typically lacks either spatial or temporal resolution and thus makes the identification and quantification of the relevant neural processes difficult. By leveraging the spatially and temporally resolute invasive sensing modalities of electrocorticography (ECoG) and stereoelectroencephalography (sEEG), it is possible to identify relevant spatiotemporal neural features and use them to model the temporal dynamics of an auditory stimulus. We collected data from epilepsy patients (n = 8) undergoing the localization of epileptogenic zones and eloquent cortex prior to surgical resection. Six of these participants were implanted with ECoG arrays and two with sEEG. The participants were instructed to passively listen to a systematic auditory presentation whereby a series of rhythmic drumbeats were presented with varying rhythm pattern, tempo, and complexity. Embedded in the trials were random silent intervals during which the participants were to imagine the rhythm pattern continuing. The resulting ECoG and sEEG data were used to reconstruct the imagined and perceived musical rhythms using participant-specific multiple regression models trained via Lasso regression. Two variations of the models were trained, each having a different temporal context. The first is a causal, anticipatory model that uses the past 250 ms of neural data to reconstruct the current stimulus amplitude envelope. The second is a non-causal, perceptual model that uses the future 250 ms of neural data to reconstruct the stimulus envelope. Both the perceptual and anticipatory models were able to reconstruct the perceived stimulus envelope ($p < .05$) with Spearman correlations between the actual stimulus and model output reaching $r = 0.49$, while the models were not able to reliably reconstruct the surrogate stimulus envelope for the imagined intervals. For both ECoG and sEEG, it was found that the temporal cortical regions were most relevant for both the anticipatory and perceptual models. The most relevant temporal contexts for the

perceptual and anticipatory models were between 50 ms to 150 ms and -50 ms to -150 ms, respectively. While the quality of the reconstructions varied across participants, the simple linear models were trained with a very limited amount of data from electrodes in suboptimal locations for this application. It is expected that the reconstructions can be significantly improved with better electrode coverage, larger amounts of data, and more sophisticated decoding models.

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Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

Location: WCC Halls A-C

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Program #/Poster #: PSTR412.04/BB1

Topic: D.05. Auditory & Vestibular Systems

Support: Marie Sklodowska-Curie Actions (MSCA) Postdoctoral Fellowship, 101062497

Title: Characterizing generators and spatiotemporal dynamics of spontaneous tau-rhythms in human temporal cortex

Authors: *N. SCHAWORONKOW[1], P. DONHAUSER[1], D. POEPPEL[1,2];
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Abstract: Oscillations are omnipresent in human electrophysiological activity, with the most prominent rhythmic activity manifesting in the alpha-band (8-13 Hz). In the occipital and sensorimotor cortex, these rhythms have been shown to be important for excitability modulation, facilitating rapid processing of incoming stimuli, displaying a spatial organization capturing relevant dimensions of the modality-specific organization. In the auditory domain, the tau-rhythm is present in the human temporal cortex. On the premise that alpha-band rhythms are basic building blocks for processing functions that are reused across separate sensory domains, tau-rhythms are important to understand for auditory and speech processing. As the temporal cortex features a rich set of rhythmic and task-evoked activity in the theta- and delta-bands, a careful differentiated description of the spatiotemporal dynamics of tau-rhythms is needed. Therefore, we investigate generators and dynamics of tau-rhythms through the lens of high-resolution invasive electrophysiological data.
In a large iEEG dataset, encompassing both subdural grid as well as stereotactically placed depth electrodes, analysis of a diverse set of alpha-rhythm generators was performed, using segments of eyes open/eyes closed modulation as well as modulation by sound. Data-driven spatial filters were used to scan for rhythms with high alpha-band power, with an individually adjusted peak frequency. Identified alpha-band rhythms were mapped using a parcellation-based approach. Focusing on tau-rhythms in the temporal cortex, we found several independent oscillatory

sources for selected participants, with differential spatial spread across the temporal cortex and event-related desynchronization to sound. Next, we explored generative models of these rhythms using leadfield simulations, considering the possibility of traveling waves or separate oscillatory sources. The challenge to differentiate between those two cases arises because several oscillatory sources can result in apparent traveling waves due to spatial mixing. We identified wave propagation directions of prominent tau-oscillations, aligned with expectations based on different tau-generators, suggesting that the observed rhythms may arise from distinct sources within the temporal cortex.
We characterize the human tau-rhythm, identifying several oscillatory sources within temporal cortex. The findings contribute to the understanding of the family of different alpha-band rhythms and provide a necessary foundation for future investigation into functional significance of tau-rhythms in perceptual or cognitive processing.

Disclosures: **N. Schaworonkow:** None. **P. Donhauser:** None. **D. Poeppel:** None.

Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

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Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR412.05/Web Only

Topic: D.05. Auditory & Vestibular Systems

Title: Distilling neural correlates of consciousness in auditory perception using fMRI

Authors: *T. DELLERT, H. BALSTER, R. MOECK, I. SCHLOSSMACHER, M. BRUCHMANN, T. STRAUBE;
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Abstract: What are the minimum neural mechanisms sufficient for conscious percepts? In the search for neural correlates of consciousness (NCC), predominant theories disagree about the role of posterior, sensory versus widespread, fronto-parietal brain activity. Previous research has focused on vision, while other sensory modalities have been neglected. Moreover, conscious perception was often confounded with task-related post-perceptual processes, such as decision-making and report. In the present study, we aimed at isolating NCC in auditory perception from task-related activity using functional magnetic resonance imaging (fMRI) and a no-report inattentional deafness paradigm. Sixty-three human participants (37 female, 26 male; 18-32 years old) performed an auditory distractor task, while task-irrelevant speech stimuli were presented in the background. Whereas one group was informed about the speech stimuli and later reported awareness of them, another group remained uninformed and experienced inattentional deafness. After awareness was assessed, both groups were able to detect the sounds. Brain responses to the task-irrelevant speech stimuli in aware compared to unaware participants were strongly increased in the bilateral superior and middle temporal gyrus. Awareness effects in fronto-parietal areas, however, were negligible. Thus, our findings suggest a dominant role of sensory rather than widespread fronto-parietal information processing in auditory consciousness.

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Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR412.06/BB2

Topic: D.05. Auditory & Vestibular Systems

Support: NIH-NIDCD 5R01DC014279

Title: Auditory attention decoding through the classification of event-related potentials to glimpsed and masked events

Authors: *V. S. RAGHAVAN[1,2], J. O'SULLIVAN[1,2], S. BICKEL[3,4,5], A. D. MEHTA[4,3], N. MESGARANI[1,2];
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Abstract: Objective. People suffering from hearing impairments often struggle to follow a conversation in a multitalker environment. Current hearing aids can suppress background noise; however, little can be done to help a user attend to a single conversation amongst many without first knowing which speaker a user is attending to. Cognitively-controlled hearing aids have been proposed using auditory attention decoding (AAD) methods; however, these methods have not been able to meet the demands of conversational speech or handle instances of distributed attention or inattention. Here, we propose a novel framework that directly classifies auditory event-related potentials (ERPs) to glimpsed and masked speech events to determine whether the source of the event was attended. Approach. We present a system that (1) identifies auditory events using the local maxima in the envelope rate of change, (2) assesses the temporal masking of auditory events relative to competing speakers, and (3) utilizes masking-specific ERP classifiers to determine if the source of the event was attended, ultimately amplifying the source of attended events to assist the listener. Main results. Using invasive electrophysiological recordings, we showed that ERPs from recording sites in auditory cortex can effectively decode the attention of subjects. This method of AAD provides higher accuracy and shorter switch times compared with traditional CCA-based methods, permitting the quick and accurate detection of changes in a listener's attentional focus. Significance. Our framework extends the scope of AAD algorithms by introducing a linear, direct-classification method for determining a listener's attentional focus that leverages the latest research in multitalker speech perception. This work moves us closer to the development of effective and intuitive cognitively-controlled hearing assistive devices.

Disclosures: **V.S. Raghavan:** None. **J. O'Sullivan:** None. **S. Bickel:** None. **A.D. Mehta:** None. **N. Mesgarani:** None.

Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

Location: WCC Halls A-C

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Program #/Poster #: PSTR412.07/BB3

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant T32 DC010775

Title: Attention allocation and level dominance: insights from an EEG study

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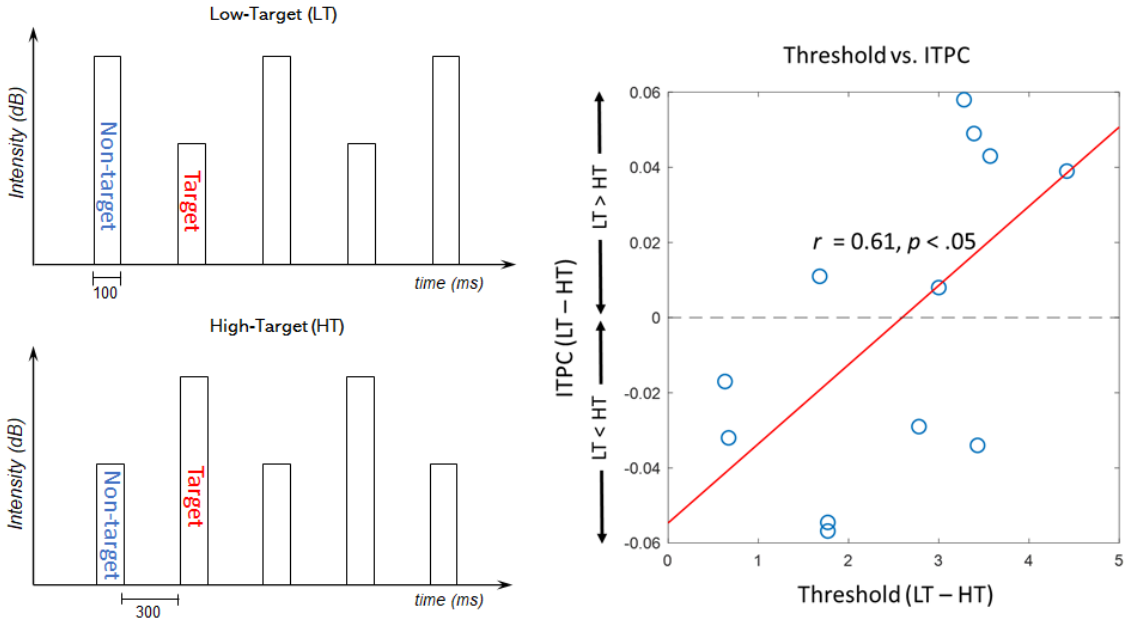
Abstract: Auditory level dominance is characterized by listeners' reliance on louder components when integrating information across a series of sounds. Past studies suggest that auditory attention is directed to the most intense elements of the sound sequence. The neural mechanisms underlying level dominance and the involvement of attention, however, remain poorly understood. This study investigated how the relative levels of neighboring sounds influence listeners' attention allocation to task-relevant sounds. In an intensity discrimination task, 12 normal-hearing listeners (18-34 years old) were presented with sequences of five sounds alternating between two intensity levels. The second and fourth sounds served as 'targets', where the increment in their levels was to be detected. During the experiment high-density EEG (128 channels) was recorded. Two conditions (figure left) were tested: 1) Low-Target condition (LT), where the 'non-targets' (first, third, and fifth) had a level of 70 dB, and the targets had a base level of 35 dB, and 2) High-Target condition (HT) was the converse of LT. Before the EEG sessions, listeners familiarized themselves with the task, and thresholds (ΔL in dB) were estimated for LT and HT conditions. Thresholds for the LT condition were significantly poorer than for the HT condition, indicating level dominance. During the EEG sessions, listeners performed the same tasks using fixed signal levels tailored to each individual. Because the two conditions share the same temporal structure (with reversed level patterns), inter-trial phase coherence (ITPC) was used to assess differences in neural phase-locking patterns between conditions. Preliminary results (figure right) suggest that listeners with lower threshold difference, or better performance, tend to exhibit *lower* ITPC in the LT than in the HT. This could be attributed to the fact they attend relatively better to the lower-level targets than those with higher thresholds, but their attention still tends to be directed to the higher-level non-targets.



Disclosures: **K. Watanabe:** None. **V.M. Richards:** None. **R. Srinivasan:** None.

Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

Location: WCC Halls A-C

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Program #/Poster #: PSTR412.08/BB4

Topic: D.05. Auditory & Vestibular Systems

Support: NIH-NIDCD

Title: Auditory attention signatures across different neural frequency bands in the human auditory cortex

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Abstract: Auditory attention decoding involves using neural signals to decode the talker on whom attention is directed in a cocktail party setting. A common method for this is stimuli reconstruction in which a representation of the attended speech is reconstructed from neural signals and correlated with the speech representations of every talker in the acoustic scene. The decoded attended talker is determined as one whose speech representation yields the highest

correlation with the neurally reconstructed attended speech representation. If spatial separation between the talkers in the acoustic scene is assumed, another approach could be estimating the location where attention is directed, i.e., the location of the attended talker. A number of non-invasive and invasive studies have investigated auditory attention using the method of stimuli reconstruction, whereas studies investigating auditory spatial attention have mostly been non-invasive. For both the methods, it is important to determine which anatomical sites and frequency bands need to be targeted for achieving the best auditory attention decoding performance. Non-invasive techniques limit both anatomical specificity and reliable measurement of high frequency (40 - 150 Hz) neural activity. Intracranial recording techniques such as electrocorticography (ECoG) and stereo-electroencephalography (sEEG) help target specific anatomical regions and can reliably measure both low and high frequency neural activity. However, invasive studies (using the method of stimuli reconstruction) have rarely looked at signatures of auditory attention beyond the high gamma band (70 - 150 Hz). It is unclear how the neural activities across various frequency bands on different sites of the auditory cortex embed signatures of auditory attention. To answer this, we recorded invasively from neurosurgical patients as they (1) listened to spatialized single-talker speech stimuli and (2) selectively attended to a single talker in a spatially-separated multi-talker speech setting. Frequency analysis of the neural activity recorded from various sites on the auditory cortex reveals location and speaker selectivity patterns consistent across different subjects. We also characterize auditory attention decoding performance across different neural frequency bands and anatomical regions of the auditory cortex. Together, these results shed light on what regions and neural frequency bands can be used for auditory attention decoding depending on the method and level of invasiveness.

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Poster

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Location: WCC Halls A-C

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Program #/Poster #: PSTR412.09/BB5

Topic: D.05. Auditory & Vestibular Systems

Support: ERC-CoG-2014-646696

Title: Neural-attentional filtering does not predict individual change in aging adults' listening behaviour

Authors: *S. TUNE, J. OBLESER;
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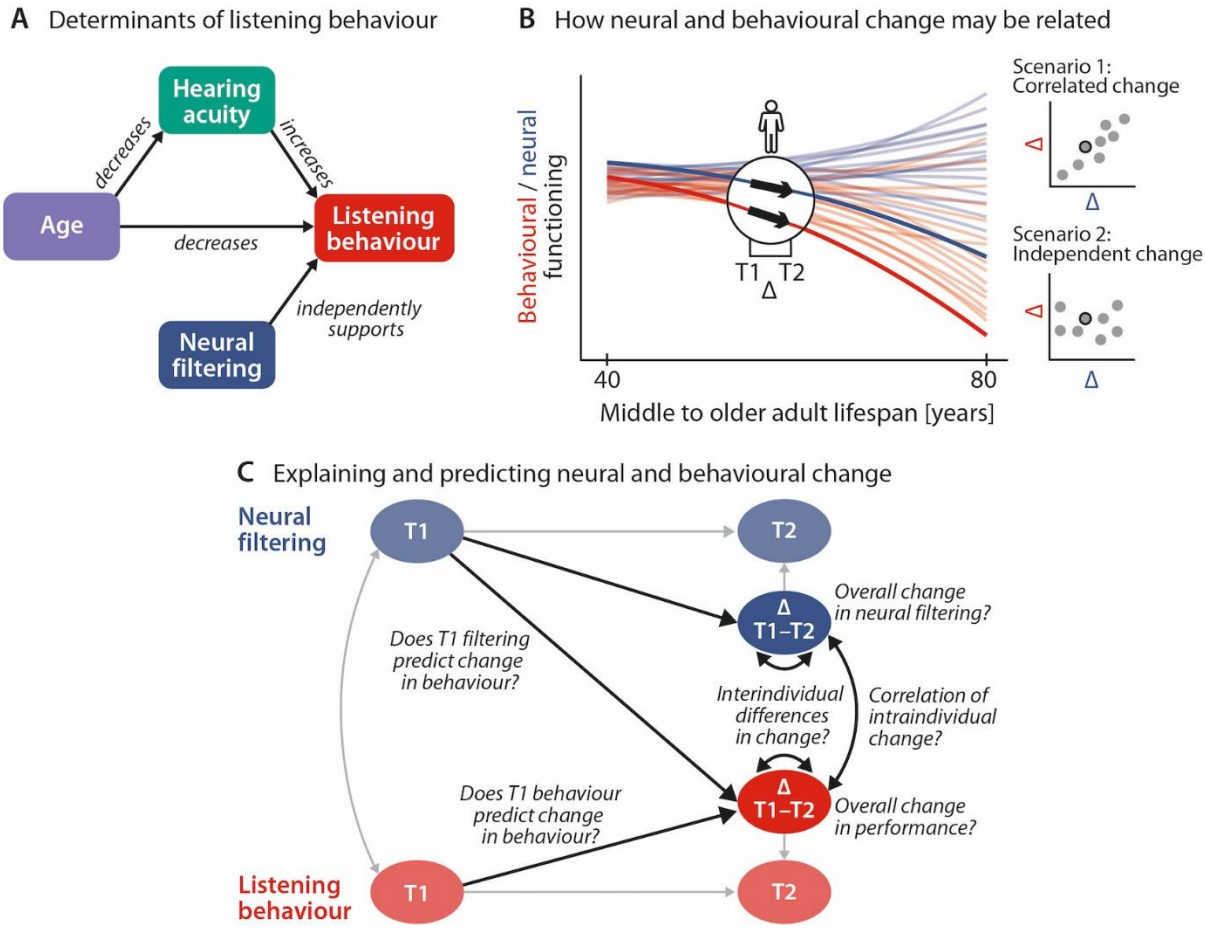
Abstract: Preserved communication abilities promote social well-being and healthy aging. While sensory acuity deteriorates, an age-independent support mechanism for communication

arises when attention-guided neural filtering of relevant sensory information in auditory cortex is preserved. Yet, how longitudinally stable is such a compensatory brain-behaviour link? More generally, has neural filtering any potency in predicting inter-individual differences in future changes in behavioural functioning? We here tracked N=105 individuals neurally and behaviourally over approximately two years (age-varying cohort of 39-82 yrs). First, despite the expected decline in sensory acuity, listening-task performance proved remarkably stable. Second, when looking into each measurement time point separately (T1, T2), neural and behavioural metrics were correlated with each other. However, neither neural filtering at T1 nor its T1-T2 change were predictive of individuals' two-year change in listening behaviour, under a combination of modelling strategies. Our results cast doubt on the translational potential of attention-guided neural filtering metrics as predictors of longitudinal change in listening behaviour over middle to older adulthood. Our data support the conjecture that audiology-typical listening behaviour and neural filtering ability follow largely independent developmental trajectories associated with significant inter-individual variability.



Disclosures: **S. Tune:** None. **J. Obleser:** None.

Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

Location: WCC Halls A-C

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Program #/Poster #: PSTR412.10/BB6

Topic: D.05. Auditory & Vestibular Systems

Support: DFG HE 7857/1-1

Title: Are pupil size and neural alpha power similarly sensitive to reward prospect under demanding listening conditions?

Authors: *F. KRAUS[1], J. OBLESER[1], B. HERRMANN[2];
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Abstract: Pupil size and neural alpha oscillatory power are often used to indicate cognitive demand, but it is unclear how much these metrics covary with an individual's motivational state. Here we tested whether pupillometry and alpha power are sensitive to both listening demand and motivational state. Participants performed an auditory gap-detection task while pupil size or magnetoencephalogram (MEG) were recorded. Task difficulty and a listener's motivational state were orthogonally manipulated through changes in gap duration and monetary-reward prospect, respectively. While participants' performance decreased with task difficulty, reward prospect enhanced performance under hard listening conditions. Pupil size increased with both task difficulty and higher reward prospect. Importantly the reward-prospect effect was largest under difficult listening condition. Moreover, larger pre-gap pupil size was associated with faster response times on a within-participant level. In contrast, neural alpha power showed no effects of reward-prospect. Of relevance to the utility of pupillometry in audiology and translational neuroscience, pupil size indexed higher motivational state especially under demanding listening. However, we could not find a similar response of neural alpha power. These results add to the mounting evidence that pupil size and alpha power are not two interchangeable physiological indices of cognitive investment.

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Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

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Program #/Poster #: PSTR412.11/BB7

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant 1RF1MH116978-01

Title: Cortical localization of auditory predictive signals: An Intracranial EEG study

Authors: *J. SHIN[1], Y.-H. WU[2], L. FAES[3], Z. YU[4], M. A. CLOOS[5], S. DEVORE[6], W. K. DOYLE[7], P. DUGAN[8], D. FRIEDMAN[9], A. SEEDAT[9], O. DEVINSKY[9], E. S. YACOUB[10], F. DE MARTINO[11], L. MELLONI[12];
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Abstract: The predictive coding theory suggests that the brain swiftly detects any deviations or discrepancies by comparing incoming sensory signals with internally generated predictions based on prior knowledge and contextual cues. When these predictions are unexpectedly contradicted, local cortical circuits generate prediction error signals, leading to the updating of predictions. Despite its significance, the precise localization and nature of the prediction error and prediction signals during auditory sequence processing remains elusive. In this study, we utilized intracranial EEG (iEEG) recordings with a high spatiotemporal resolution to elucidate cortical regions generating auditory predictive signals and to characterize their relationships. In order to simultaneously examine neural responses to unexpected deviant sounds (prediction error) and omissions (prediction), we adapted an auditory oddball paradigm where syllables were repeated four times in a sequence. Occasionally, we replaced the last repetition with either a deviant syllable (20%) or omissions (20%). We evaluated the high-gamma responses, thought to reflect multi-unit activity, to the standard syllables, deviants and omissions. We found that electrodes in the superior temporal gyrus (STG), inferior frontal gyrus (IFG), and sensorimotor areas exhibited both oddball and omission responses, with their amplitudes positively correlated. Critically, these responses were not solely driven by motor preparation, as they were also observed in a passive listening condition without any motor response requirement. Next, to explore the predictive nature of omission signals, we trained a support vector machine (SVM) to decode the syllable identity. SVMs trained with sensory-evoked high frequency broadband (HFB) activity and event-related potentials (ERPs) successfully decoded the syllable identity. However, SVMs trained with HFB or ERP responses to omissions failed to decode above chance level. In summary, our results suggest a common neural substrate for oddball and omission responses, while highlighting that top-down driven prediction signals do not convey stimulus-specific information. These findings contribute to our understanding of auditory processing and the mechanisms underlying predictive coding in the human brain.

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Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR412.12/BB8

Topic: D.05. Auditory & Vestibular Systems

Title: Modeling Audiovisual Interference in Absolute Pitch

Authors: *D. ROSENZWEIG[1], E. NING[3], D. POEPPEL[2], C. PELOFI[2];
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Abstract: How neuronal systems support invariant object classification and labeling is a fundamental problem in neuroscience. Absolute pitch (AP) is the rare ability to identify and label tones without access to external reference, and as such represents an example of auditory object recognition. A simple model to account for this might consist of a classification task for a neural network retrieving labels corresponding to fundamental frequency, but it remains unknown how this computation is implemented in the brain. Altogether, AP serves as an identifiable behavioral marker for studying architecture in the left dorsal stream contributing to rapid identification and labeling of fundamental frequency.
In this study, we examine audiovisual interference in musicians with AP and determine whether retrieval processes occur automatically and without instruction. We recruited a group of trained musicians and identified AP participants through accuracy performance on a pitch labeling task. In a behavioral audiovisual Stroop task, we found that AP musicians demonstrated interference as measured by increased RT on mismatch as compared to match trials of the task. In contrast, non-AP musicians did not demonstrate this audiovisual incongruence effect, as reflected in similar RTs across match and mismatch conditions. These behavioral findings support the hypothesis that invariant pitch classification and labeling occurs automatically and without instruction in musicians with AP. In a follow-up MEG study, we investigate the hypothesis of automatic activation for phonetic representations when AP musicians perceive tones. We then analyze the time course of this interference process to gain insight into the computational underpinnings and neural architecture supporting AP pitch labeling.

Disclosures: **D. Rosenzweig:** None. **E. Ning:** None. **D. Poeppel:** None. **C. Pelofi:** None.

Poster

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Topic: D.05. Auditory & Vestibular Systems

Support: NIH grant P50 DC015817
Lauer Tinnitus Research Center

Title: Brain state predictors of three canonical perceptual listening errors

Authors: *S. S. SMITH, J. A. SUGAI, K. E. HANCOCK, D. B. POLLEY, PhD;
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Abstract: Our perception of sound is related to - but not determined by - the acoustic waveform entering the ear. The same fixed sound can be misheard as another (error of confusion), not heard (error of omission), or heard when no sound was presented (error of commission). An observer can produce each of these error classes despite static auditory evidence and task context. In animals, this variability has been causally linked to fluctuations in global brain state - endogenous patterns of neuromodulator activity linked to arousal and attention. Here, we hypothesized that neural and physiological indices of brain state are predictive of canonical forms of auditory perceptual errors in human subjects. To investigate, we developed a sustained vigilance task in which subjects were asked to monitor a stream of tone clouds for strings of repeating tones. The task was configured such that participants could misclassify the length of strings (error of confusion), fail to report detection of a string (error of omission), and/or report a string where there was none (error of commission). Simultaneously, 64-channel EEG, pupil diameter, eye gaze, and blinks were recorded. We derived the string-aligned profiles of these neurophysiological measures for each of the behavioral outcomes, comparing against correct trials. Measurements were made in a cohort of 41 normal hearing, neurotypical, young/middle-aged adults (≤50 years, 11 male). The occurrence of a target response string elicited a stereotyped neuroelectric, oculomotor, and autonomic pupillary response. These responses were suppressed for errors of omission, yet partially present for errors of commission. Misclassifications of string length (i.e., errors of confusion) were characterized by sustained power at the counting frequency (8 Hz), which was instead reduced during correct trials. Importantly, neuroelectric and autonomic signatures for particular forms of listening errors were identifiable seconds before the string initiation. For errors of confusion, pupil diameter and global EEG activity were reduced in size several seconds before target onset ($p<0.01$). This reduction was largest during later blocks when task performance was highest. Overall, these results show that, in neurotypical human subjects, auditory errors can be accounted for by dynamic neural and physiological indices of brain state. These findings may highlight biomarkers in neurodivergent populations who remain fixed in states of heightened commission errors (e.g., auditory hallucinations and tinnitus), omission errors (e.g., catatonia), or classification errors (e.g., dementia).

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Poster

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Location: WCC Halls A-C

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Program #/Poster #: PSTR412.14/BB10

Topic: D.05. Auditory & Vestibular Systems

Support: MRC Grant MR-T032553-1
NIH Grant DC004290

Title: Neural correlates of auditory working memory precision: an intracranial EEG study

Authors: *J. I. BERGER[1], A. J. BILLIG[2], P. E. GANDER[1], M. LAD[3], S. KUMAR[1], K. V. NOURSKI[1], C. K. KOVACH[1], A. E. RHONE[1], C. M. GARCIA[1], H. KAWASAKI[1], B. J. DLOUHY[1], M. A. HOWARD, III[1], T. D. GRIFFITHS[4];
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Abstract: Working memory is the capacity to hold and manipulate behaviorally relevant information in mind. Previous work (Kumar et al., Neuropsychologia 2021 150:107691) examined auditory working memory (AWM) during maintenance of a tone using intracranial EEG and described oscillatory local field potential (LFP) correlates of AWM in auditory, frontal cortices, and the hippocampus. The present study sought to identify correlates of precision of working memory, reflecting cognitive resources available in models (Joseph et al., Brain Res 2016 1640:183-92). Neural correlates of precision were hypothesized to emerge in the hippocampus based on both LFPs and single units during maintenance and retrieval. Behavioral responses to the task and LFPs from the hippocampus (HC) were recorded in four adult neurosurgical patients undergoing invasive monitoring for presurgical localization of epileptic foci [wherein the hippocampus was subsequently found to be not a seizure focus]. In three patients, single units were also recorded in the HC and Heschl's gyrus (HG). For the AWM task, participants were presented with short target tones, each followed by a 3 s retention period. The task was to adjust a test tone to the target within 5 s. Working memory precision was calculated over all trials based on the reciprocal of the standard deviation of the response error. LFP data were analyzed using time-frequency analysis based on wavelet transforms, and single units were isolated with an automated spike-sorting procedure and examined with trial raster plots and peri-stimulus time histograms. Participants performed the task with similar precision to previously studied healthy controls (Lad et al., Sci Rep 2020 10:13997). Low-frequency LFP activity (<8 Hz) across all HC contacts persisted throughout the retention period. Low frequency activity was pronounced at the onset and following the offset of the tuning period, concurrent with high gamma (70-150 Hz) suppression. Desynchronization of theta (4-8 Hz) and alpha (8-15Hz) HC activity occurred at the end of the trial. In two participants with HG and HC electrode coverage, there was an increase in theta-band phase locking between these regions at the onset of the retention period and the offset of the retrieval period. Increases in single unit firing were evident during encoding, retention and retrieval in HG and HC. For some neurons, responses during retrieval correlated with behavioral performance. Overall, the data highlight neural correlates of precision of AWM and implicate the hippocampus in both maintenance and retrieval of non-verbal AWM.

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Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

Location: WCC Halls A-C

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Program #/Poster #: PSTR412.15/BB11

Topic: D.05. Auditory & Vestibular Systems

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ONRG-NICOP-N62909-19-1-2029
CEU-ITI

Title: Modality-independent temporal segmentation principles

Authors: *L. GARAMI[1,2], C. ANGELONI[3], M. N. GEFFEN[2], J. FISER[4];
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Abstract: Sensory systems extract patterns at varying complexity from the input. Such extraction of meaningful patterns (chunking) is key to fast and efficient coding of environmental information, but it also biases sensitivity to changes and accuracy in recognition. Despite being a common feature across different modalities, organizing principles of chunking are usually tested modality-specifically preventing the detection of the modality-independence of chunking principles and their behavioral consequence. We hypothesized that certain chunking principles and the resulting perceptual biases are similar across modalities, and their neural correlates are already present in the primary cortical areas.To test our hypothesis, we focused on a well-known auditory chunking principle called the Iambic-trochaic law. In language processing, longer syllables have the tendency to signal word ends and similarly, longer duration of a tone in a sequence without any linguistic content also tends to be interpreted by both adults and six-month-old babies as the end of a chunk. Specifically, people tend to interpret the stream of an auditory stimulus train consisting of short (S) and long (L) tones separated by silence (… S S L S S L S S L …), as a repeating pattern of SSL rather than any other alternatives (e.g., SLS). Importantly, this chunking resells in a decreased detection accuracy of randomly inserted gaps at a perceived chunk's border compared to inside of the chunk.To test the universality of this chunking principle, we tested if bias in human performance in temporal change detection is comparable in the visual modality. We implemented the SSL stream segregation go/no-go paradigm for human participants identical in vision and audition. Human participants had a lower d-prime if an unexpected gap was inserted after the long object (tone or square) in both modalities.We recorded neuronal activity in the auditory cortex (AC) of awake, head-fixed mice passively listening to acoustic stimuli. We tested whether and how neurons in the AC detect gap insertion in a repeating pattern of a similar continuous stream. We found that activity in the AC was significantly higher in response to stimuli with unexpected gap insertion when the gap was

inserted prior to the long tone than when it was inserted prior to one of the short tones.We used identical paradigms across modalities (aud/vis) and models (human/mouse). Our results support the idea of domain-general non-linguistic grouping principles and raise well-testable further questions that have the capacity to lead to a domain-independent model of sensory processing.

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Poster

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NSF SMA 1734892
NSF OISE 2020624
NSF CCF 1552946

Title: Changes in Cortical Directional Connectivity during Difficult Listening in Younger and Older Adults

Authors: B. SOLEIMANI[1], I. M. D. KARUNATHILAKE[1], P. DAS[3], S. E. KUCHINSKY[4], B. BABADI[2], ***J. Z. SIMON**[5];
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Abstract: Fully understanding the neural underpinnings of speech comprehension in difficult listening conditions requires understanding the concurrent cortical connectivity. Granger causality is a useful measure of connectivity, typically employed in functional magnetic resonance imaging (fMRI) studies, but the limited temporal resolution of fMRI restricts the capture of higher frequency neural interactions crucial for complex speech processing. On the other hand, although magnetoencephalography (MEG) can capture neural interactions at the millisecond scale, its limited spatial resolution poses challenges in conventional connectivity analyses. A recently proposed cortical connectivity analysis methodology, network localized Granger causality (NLGC), can extract Granger causal interactions in MEG data without the need for any intermediate source-localization step. This one-shot approach also effectively addresses challenges related to false alarms and localization errors, providing a robust assessment of cortical connectivity. In this study, NLGC is applied to MEG recordings from younger and older adults while performing a speech listening task with varying background noise conditions. The analysis focuses on directional cortical connectivity patterns within and

between the frontal, temporal, and parietal lobes, specifically in the delta and theta frequency bands. The results demonstrate significant age- and condition-related connectivity differences, particularly in the theta band. In younger adults, increasing background noise leads to a shift from predominantly temporal-to-frontal (bottom-up) connections for clean speech to dominantly frontal-to-temporal (top-down) connections in noisy conditions. In contrast, older adults exhibit bidirectional information flow between frontal and temporal cortices regardless of the background noise. Furthermore, NLGC allows classification of connections as either excitatory or inhibitory based on their temporal relationships, enabling a more nuanced understanding of the neural mechanisms involved in speech perception. While delta band connection types show no significant age-related changes, theta band connection types exhibit substantial changes in excitation/inhibition balance across age and condition. Supported by the National Institutes of Health (P01-AG055365, R01-DC019394) and the (National Science Foundation (SMA 1734892, OISE 2020624, CCF 1552946).

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Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR412.17/BB13

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant R01 DC016834

Title: Recall of spoken narratives by adult users of cochlear implants and normal-hearing adults with noise-band vocoding: strategic pauses alleviate pupillometry-indexed cognitive effort while listening to time-compressed speech

Authors: R. M. O'LEARY[1], J. NEUKAM[3], T. A. HANSEN[1], A. J. KINNEY[1], N. CAPACH[3], M. SVIRSKY[3], *A. WINGFIELD[2];
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Abstract: Although current cochlear implants (CI) contain as many as 22 intra-cochlear electrodes, CI users may not have spectral resolution (perceptually separated bands of frequency information) beyond the equivalent of 4 to 8 frequency channels due to factors such as current spread and neural survival. It is thus not surprising that CI users find the perception of time-compressed speech especially challenging due to the combined effects of a sharply degraded CI signal, and the reduced acoustic richness of time-compressed speech. At the cognitive level, the rapidity of time-compressed speech can deprive the listener of the ordinarily available processing time present when speech is delivered at a normal speech rate. Two experiments are reported. Experiment 1, conducted with 27 young adults with normal hearing, used noise-band vocoding

to simulate the spectrally limited sound of the CI signal. Results showed that inserting silent pauses after sentence and clause boundaries within a time-compressed narrative ("time-restoration") improved recall accuracy to the level similar to that for speech heard a normal speech rate for clear speech, and speech heard with 10-channel vocoding, and to a significant but lesser extent with 6-channel vocoding. The measurement of task-related changes in pupil diameter as an index of cognitive effort, showed the insertion of pauses in the rapid speech to reduce processing effort to a level similar to that for speech heard at a normal speech rate. In Experiment 2, 15 adult CI users were tested using the same materials. Unlike the listeners with normal hearing, meaningful improvement in recall accuracy with time-restoration was limited to a subgroup of CI users defined by better scores on a test of working memory resources, as well as higher scores on tests of word and sentence recognition. These findings are interpreted in the context of sensory-cognitive interactions within data-limited and resource-limited processes among adult cochlear implant users.

Disclosures: R.M. O'Leary: None. **J. Neukam:** None. **T.A. Hansen:** None. **A.J. Kinney:** None. **N. Capach:** None. **M. Svirsky:** None. **A. Wingfield:** None.

Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

Location: WCC Halls A-C

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Program #/Poster #: PSTR412.18/BB14

Topic: D.05. Auditory & Vestibular Systems

Support: Charles Lafitte Foundation

Title: Eeg as an indicator for perceptual difficulties in noise?

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Abstract: This study aimed to investigate whether EEG could capture the internal noise associated with cochlear synaptopathy in humans and whether it correlates with hearing difficulties. Cochlear synaptopathy has been the subject of numerous studies in the past decade, but the reaction of the auditory cortex to it remains unclear. A recent mouse study suggested that with 90% synapse loss, the auditory cortex exhibits hyper-synchronized neuronal activity (‘internal noise’) only in missed tone detection trials in noise, which could contribute to degraded behavioral performance in noisy listening conditions (Resnik & Polley, 2021). In this study, 30 participants with near-normal hearing performed a monaural tone detection task in either quiet or noise while their EEG was recorded. They also underwent tasks that have been suggested to reveal cochlear synaptopathy. The analysis aimed to determine whether single-trial EEG could predict behavior (hit vs miss) and whether such EEG prediction correlated with other indicators of cochlear synaptopathy. Ongoing EEG analyses suggest that pre-stimulus EEG activity does not predict behavioral outcomes. In contrast, significant prediction performance of

post-stimulus EEG likely reflects the presence of the P300 component for hit trials, but not earlier auditory processing stages. This prediction performance was correlated with the Speech, Spatial and Quality of Hearing questionnaire, but not with any of the other measures, such as speech perception thresholds or extended-high-frequency audiometric thresholds. More data is needed to determine the effect robustly.

Disclosures: **J. Liu:** None. **J. Stohl:** None. **T. Overath:** None.

Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

Location: WCC Halls A-C

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Support: DOD PRMRP PR170921
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Title: Tinnitus percept is associated with magnetoencephalography-derived measures of resting state connectivity between temporal and frontal cortices

Authors: *C. L. DALE[1], L. B. N. HINKLEY[1], J. HENDERSON SABES[2,4], C. DAVIS[5], A. S. BHUTADA[1,6], L. OUYANG[1,7], A. P. WALTHER[3], W. ZHANG[5], M. E. ADAMS[8], S. S. NAGARAJAN[1,9,2], S. W. CHEUNG[2,10];
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Abstract: Subjective tinnitus refers to conscious perception of sound for which no external auditory stimulus is identified. Models of tinnitus postulate increased connectivity between auditory cortex and basal ganglia, limbic areas, or frontal areas. Brain imaging may objectively determine presence and magnitude of percept and assist in understanding underlying physiology. At one of 2 study sites, 380 adults with (N = 185) and without (N = 195) tinnitus underwent 5 minutes of task-free eyes-closed magnetoencephalography (MEG), structural imaging (MRI), audiometry, and tinnitus assessment (TFI). A subset (N=44) repeated the study. Source imaging localized 1 to 4 minutes of MEG activity within brain anatomy. Voxel-based activity, parsed into

6 frequency bands and spatially-normalized, was mapped to 246 Brainnetome atlas regions. Imaginary coherence (ImCoh) and directional phase transfer entropy (dnPTE) were calculated between each unique pair of regions. Analysis of variance (rmANOVA), repeated across frequency with group, age, and site, provided F-ratios and FDR-corrected p-values for pairwise connectivity, as well as relation to TFI. Binomial linear regression queried contribution of band to group effects. Linear discriminant analysis with 10-fold cross validation assessed potential for classification. Groups differed at 4 pairs out of 30,012 queried. Using ImCoh, tinnitus exhibited increased slow oscillatory connectivity between regions in left temporal and right frontal cortex (LPHG-RIFC: $F=9.00$, $p<.01$) and (LITC-ROFC: $F=9.04$, $p<.01$), driven by delta (1 - 3 Hz, LPHG-RIFG: $B = 25.8$, $t=4.21$; LITC-ROFC: $B = 15.6$, $t=3.14$, both $p<.01$). For dnPTE, tinnitus showed decreases in intrahemispheric high gamma (63 - 117 Hz) connectivity between portions of cingulate and parietal cortex (Left: $F=12.4$, $B = -1.16$, $t=-4.67$, $p<.01$; Right: $F=12.31$, $B = -1.16$, $t=-3.97$, $p<.01$). Using connectivity of these pairs in identified bands as classifiers produced acceptable levels of diagnostic performance (AUC =.74 [.68 - .78]). TFI scores changed from Session 1 to 2 (paired $t=2.13$, $p=.045$), but with considerable distribution overlap (K-S test, $K=.27$, $p=.33$). Tinnitus-related differences in connectivity can adequately classify tinnitus and non-tinnitus participants. Commonalities among these 4 regions may further our understanding of the underlying physiology of tinnitus. Paradoxically, group connectivity differences did not relate to subjective scores used to diagnose tinnitus. However, TFI appeared to change from session-to-session, while connectivity largely did not, indicating that neural measures of tinnitus may be more stable than subjective measures.

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Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR412.20/Web Only

Topic: D.05. Auditory & Vestibular Systems

Title: Analysis of Psychophysiological Interactions (PPI) with auditory stimuli in patients with unilateral severe hearing loss

Authors: *K. YAMAMOTO, T. TSUTSUMI;
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Abstract: Previously, unilateral hearing loss was not considered a significant disability. However, recent discussions have acknowledged the impairments and psychological burdens associated with this condition. Yet, in clinical practice, there are no tests to visualize the influence of psychological factors associated with different types of auditory inputs. Therefore, we investigated the impact of inherent mental processes in auditory tasks on the functional

connectivity in unilateral severe hearing-loss patients with PPI.The subjects included 20 healthy individuals, 9 left and 12 right hearing-loss patients. They underwent 3 dimentional high-resolution anatomical MRI in a 3 Tesla MRI scanner following hearing 2 monosyllables and 1 pure tone of both ears. After imaging, we analyzed the task specific psychological effects using Matlab and SPM12 software packages. During the tasks, activity was observed in both the auditory cortex and auditory association areas for all groups. In the healthy group during the monosyllables' task, the primary auditory cortex in the left temporal area demonstrated the most significant activity and we set it as the seed. For the right hearing-loss group, there was an increased connectivity with the left superior temporal gyrus. While for the left hearing loss group, there was an enhanced connectivity with the both mid-cingulate cortex. Upon examining the correlation between these connectivity and age, the right hearing loss group showed a strong positive correlation, while the left hearing loss group demonstrated a negative correlation. The Positive correlation may suggest that monosyllable-stimuli from the left ear intensify the connectivity within the auditory region. Conversely the negative correlation may suggest that monosyllables entering from the right ear might strengthen the connection between the monosyllable stimuli and the activity of the cingulate gyrus by modulating the primary auditory cortex's activity.PPI in the both hearing loss patients showed that the psychological effects obtained from auditory information activate different neural pathways depending on the side of ear. Furthermore, the negative correlation between the cingulate gyrus and age suggests that connectivity occurs in the early onset of hearing loss, but weakens over time. Conversely the positive correlation between the auditory related areas and age suggests that not only psychological factors, but also the amount of auditory information, can strengthen the connectivity. These findings may indicate that the area connected with the auditory cortex varies depending on the side of input and the type of information.

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Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

Location: WCC Halls A-C

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Program #/Poster #: PSTR412.21/BB17

Topic: D.05. Auditory & Vestibular Systems

Support: STI2030-Major Project 2021ZD0204104
NSFC 31925020
NSFC 32171052

Title: The idiosyncratic nature of neuroplasticity: investigating the impact of congenital deafness on individual brain organization

Authors: *L. AMARAL[1], X. WANG[2], Y. BI[2,3], E. STRIEM-AMIT[1];
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Abstract: The principle of "all different, all equal" presents a challenge in cognitive neuroscience, where studies have consistently demonstrated a universal organization of the human brain. However, these investigations have been limited in capturing individual variations in neural organization. Individual differences play a crucial role in shaping certain neural patterns, with environmental factors and personal experiences contributing significantly to observed variability. In this study, we aimed to examine the role of sensory experience in functional connectivity (FC) patterns, specifically focusing on FC from the deprived primary auditory cortex of congenitally deaf individuals (N = 39) compared to a hearing control group (N = 33). Our findings demonstrate that the absence of shared auditory experience leads to increased variability in FC patterns across deaf individuals, in contrast to the more consistent patterns observed in the hearing group. To investigate the specific effects of sign language deprivation and determine whether the variations in brain connectivity are influenced by auditory deprivation or language deprivation, common among deaf children of hearing parents, we also examined the FC patterns focusing on a subset of the deaf group consisting of individuals who are native signers. Our analysis revealed comparable patterns of individual variability in FC within this subgroup, when compared to a broader analysis that encompassed both native and nonnative signers. This finding provides evidence that the individual differences in FC of the auditory cortex in deafness primarily arise from auditory deprivation. Collectively, these results highlight the interplay between brain plasticity and individual variability, showcasing the varied manifestation of reorganization across individuals. Furthermore, our study elucidates the influence of sensory experiences in establishing consistency in brain organization. These findings have important implications for the development of individualized medicine for hearing loss, such as the design of sensory aids and the utilization of restoration techniques.
*L. Amaral & X. Wang contributed equally to this work.

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Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

Location: WCC Halls A-C

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Topic: D.05. Auditory & Vestibular Systems

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UCL Graduate and Overseas Research Scholarship awarded to LC

Title: Functional neuroanatomy of musical familiarity and temporal structure in frontotemporal dementia

Authors: *L. CORE, J. D. WARREN, J. L. AGUSTUS;
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Abstract: Using music as an index of brain function and for intervention in dementia has received great attention. However, the neural correlates of music perception in different dementias remain poorly defined. In the frontotemporal dementia (FTD) spectrum, individuals with semantic variant primary progressive aphasia (svPPA) and behavioral variant frontotemporal dementia (bvFTD) often exhibit striking changes in musical function and/or retained musical skills, suggesting FTD may be an instructive disease model in which to assess brain mechanisms of music processing in neurodegenerative pathologies. We addressed this issue using functional magnetic resonance imaging (fMRI) in patients with FTD. Nineteen patients (10 svPPA, nine bvFTD) and 26 healthy age-matched controls underwent 3-Tesla 'sparse' fMRI where they passively listened to musical melodies. In a 2x2 factorial design, stimulus conditions were manipulated to assess two key dimensions of musical processing: semantic memory (familiarity: familiar vs. novel melodies) and perceptual features (temporal structure: isochronous vs. anisochronous melodies). Post-scan behavioral testing assessed participants' ability to discriminate melodies under each of the two manipulated stimulus dimensions. Information about participant demographics and musical background was also collected. Both groups exhibited a wide bi-hemispheric network of activation centered on primary auditory cortex in response to general auditory stimulation. The healthy older control group showed separable profiles of activation in anterior temporal and inferior frontal cortices for processing musical familiarity and in posterior superior temporal cortex for processing musical temporal structure. An interaction effect was also observed in left medial planum temporale whereby anisochrony had a greater impact on the processing of unfamiliar melodies than familiar melodies. Compared to healthy older listeners, the patient groups showed differential patterns of engagement of these distributed neural networks. In post-scan behavioral testing, patients with bvFTD were significantly impaired on musical familiarity judgments relative to healthy controls. This study illustrates that FTD syndromes show distinct functional neuroanatomical signatures of music perception. Future work should explore fMRI of music processing as a probe of neural function in particular molecular pathologies and a candidate marker of therapeutic potential in dementia.

Disclosures: L. Core: None. **J.D. Warren:** None. **J.L. Agustus:** None.

Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

Location: WCC Halls A-C

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Program #/Poster #: PSTR412.23/BB19

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant R01-DC019394
NSF Grant SMA 1734892

Title: Neural Tracking Measures of Speech Intelligibility: Manipulating Intelligibility while Keeping Acoustics Unchanged

Authors: *I. KARUNATHILAKE[1], J. P. KULASINGHAM[2], J. Z. SIMON[3];
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Abstract: Neural speech tracking has advanced our understanding of how our brains rapidly map an acoustic speech signal onto linguistic representations and ultimately meaning. However, it remains unclear which aspects of the corresponding neural responses correspond to speech intelligibility, which is only loosely coupled to the acoustics. Intelligibility related neuro-markers derived from such neural responses would play a crucial role in advancing our understanding of the neurophysiology of the speech understanding, evaluation of auditory function across diverse clinical populations, and hearing device evaluation. Many studies addressing this question vary the level of intelligibility by manipulating the acoustic waveform, making it difficult to cleanly distinguish effects of intelligibility from the underlying acoustical confounds. In this study, speech intelligibility is manipulated while keeping the acoustical structure unchanged, using degraded speech plus a priming paradigm. Acoustically identical three-band noise vocoded (degraded) speech segments (~20 s duration) are presented twice, but the second presentation is preceded by the original (non-degraded) version of the same speech segment. This priming, which generates a 'pop-out' percept, substantially improves the intelligibility of the second presentation of the degraded speech passage while keeping the acoustics identical. We recorded magnetoencephalography (MEG) data from 25 younger adults and investigated how intelligibility affects auditory and linguistic neural tracking measures using multivariate Temporal Response Functions (mTRFs). As expected, behavioral results confirmed that perceived speech clarity is improved by priming. mTRF analysis revealed that auditory (speech envelope and envelope onset) and phoneme onset neural responses are influenced only by the acoustics of the sensory input (bottom-up driven mechanisms). Critically, our key findings suggest that neural measures associated with the segmentation of sounds into words emerges first with better speech intelligibility, especially those time-locked at N400-like latencies in prefrontal cortex (PFC), in line with engagement of top-down mechanisms associated with priming. Taken together, our results suggest that time locked neural responses associated with lexical segmentation may serve as novel objective measures of speech intelligibility.

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Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

Location: WCC Halls A-C

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Topic: D.05. Auditory & Vestibular Systems

Support: FWO fellowship 1S89622N
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FWO fellowship 1S49823N
FWO fellowship 1290821N

Title: Neural tracking of linguistic features at different speech rates using a deep neural network

Authors: *C. PUFFAY, J. VANTHORNHOUT, M. GILLIS, B. ACCOU, H. VAN HAMME, T. FRANCART;
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Abstract: The extent to which the brain tracks a natural continuous speech stimulus can be measured by modeling the relationship between the stimulus features and the corresponding EEG. Typically acoustic features are used, but the neural tracking of lexical and linguistic features has also been shown. Lexical features (i.e., word and phoneme onsets) carry information about the prosody, while linguistic features (i.e., word surprisal, word frequency, phoneme surprisal, and cohort entropy) carry information about the value of a word or a phoneme considering the semantical context. Such information can be used as a marker of speech understanding. Nonlinear deep learning models have recently been used to assess the neural tracking of lexical and linguistic speech features. We here evaluate these models on a dataset with various speech rates to manipulate speech understanding and investigate how speech rate affects the neural tracking of linguistic features. We use the EEG of 18 participants who listened to stories at various speech rates. We developed a deep neural network, trained on a match-mismatch task to measure the contribution of linguistic features to neural tracking on top of the contribution of lexical features. In this task, the model must choose whether a segment of brain signal matches the auditory stimulus that evoked it (matched) or another arbitrary segment (mismatched). Without re-training, we evaluate this model on different speech rates. To assess whether neural tracking is related to speech understanding, we compare the model performance with behavioral measures. We hypothesize that neural tracking of linguistic features is affected by the speech rate, which provides an objective measure for speech understanding. As opposed to linear subject-specific models, our deep learning model can model nonlinearities in the brain response and does not require training on new subjects to perform the speech understanding assessment.

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Poster

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Program #/Poster #: PSTR412.25/BB21

Topic: D.05. Auditory & Vestibular Systems

Support: William Demant Foundation Grant 21-2912

Title: Predicting intelligibility of continuous speech using scalp- and ear-EEG

Authors: *H. BORGES[1,2], J. ZAAR[2,3], E. ALICKOVIC[2,4], C. CHRISTENSEN[1], P. KIDMOSE[1];

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Abstract: In the clinic, speech intelligibility is measured behaviorally by determining the speech reception threshold (SRT). The SRT is the signal-to-noise (SNR) at which 50% of the presented speech is repeated correctly by the participant. This process requires the attention of the listener, depends on the experience of the examiner, and is very labor-intensive. As an alternative, studies have shown that the SRT of matrix sentences can be predicted from electroencephalographic (EEG) measurements. The present study aims to investigate whether the SRT can be predicted from EEG collected with a continuous speech stimulus to improve the ecological validity of the paradigm. An additional objective is to investigate whether the SRT can be predicted from ear-EEG recordings. Ear-EEG is a measuring technique where EEG is measured from electrodes placed in the ear with the potential to enable discrete recordings of EEG outside the lab in daily-life situations. Twenty-two subjects (19 female), 18-29 years old (mean age 24), were recruited. All subjects were normal hearing (thresholds≤20 dB HL) at audiological frequencies from 125-8000 Hz as determined by behavioral audiometry and an average threshold of maximum 10 dB HL. Sentences from the Danish Hearing-In-Noise Test (HINT) were used to determine the behavioral SRT via an adaptive procedure. To predict the SRT from EEG, scalp and ear-EEG was recorded while the subjects were listening to 96 audiobook clips, each lasting approximately 1 minute. The subjects were instructed to listen to the speech and answer a two-choice content-related question after each clip. The audiobook clips were presented at six different SNR levels: SRT, SRT±2dB and SRT±4 and clean speech. 16 clips per SNR were presented in a randomized order. To approximate the SRT from scalp- and ear-EEG, an auditory attention decoding model that reconstructs the envelope of the presented speech from the EEG data was used. Pearson's correlation between the reconstructed envelope and the actual envelope of the stimulus was used as a measure of reconstruction accuracy. A sigmoid function was fitted to the reconstruction accuracy-vs-SNR data, and the SNR corresponding to the midpoint of the function was taken as an estimate of the SRT. The results will be discussed with focus on the relationship between the behaviorally determined SRT and the SRT predicted from scalp- and ear-EEG.

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Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

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Program #/Poster #: PSTR412.26/BB22

Topic: D.05. Auditory & Vestibular Systems

Support: MRC(UK) Grant MR/T032553/1
NIH Grant 5P50DC000242-33

Title: Prediction of speech-in-noise ability using a dynamic auditory figure-ground stimulus

Authors: X. GUO[1], E. BENZAQUEN[1], I. BRÜHL[2], M. LAD[1], ***T. D. GRIFFITHS**[3];
[1]Biosci. Inst., Newcastle Univ., Newcastle-upon-Tyne, United Kingdom; [2]Univ. of Koeln, Koeln, Germany; [3]Biosci. Inst., Newcastle upon Tyne, United Kingdom

Abstract: Auditory figure-ground stimuli have been developed as measures of central sound grouping in complex auditory scenes. Previous studies have shown that extracting a static figure comprising multiple fixed frequencies from a tone cloud correlates with speech-in-noise listening independently of peripheral hearing measured by the pure-tone audiogram (PMID: 31728002). In this study we used a dynamic figure in which the frequency components were harmonically related and followed the pitch contour of natural speech over sentences. We used this measure to predict speech-in-noise ability at the word and sentence levels, using hierarchal regression and structural equation models (SEM) that also incorporated age, peripheral hearing (pure-tone audiogram) and static figure-ground performance. We studied over 100 participants with varying age and hearing sensitivity.
Hierarchal regression showed improved predictive value of the dynamic figure-ground task (standardised coefficient: -0.28) compared to the static one (non-significant). SEM demonstrated that a latent variable based on both dynamic and static figure ground stimuli was an important predictor of speech-in-noise perception (effect size 0.52). Effect size for the audiogram was 0.32. Overall, this study has shown how peripheral hearing sensitivity, central sound grouping (of both static and dynamic sounds), and age interact with each other and their respective contribution to speech-in-noise perception.

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Poster

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Topic: D.05. Auditory & Vestibular Systems

Support: NIH R01DC020097

Title: Mid-level auditory computations predict human speech recognition in natural environmental noise

Authors: *A. CLONAN[1,2], X. ZHAI[4,1], I. STEVENSON[3,1], M. A. ESCABI[1,2,3];
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Abstract: Recognizing speech in noisy environments is a critical task of the human auditory system. While spectrum and modulation statistics both influence speech recognition in noise,

current modeling approaches are unable to predict human recognition sensitivity in distinct, real-world backgrounds. Here we assess how the spectrum and modulation statistics of natural sounds mask the recognition of spoken digits (0 to 9). We enrolled participants in a psychoacoustic study where digits were presented in various natural background sounds (e.g., water, construction noise, speaker babble; tested for SNR=-18 to 0 dB) and their perturbed variants. We perturbed the backgrounds by either 1) phase randomizing (PR) the sound spectrum or 2) spectrum equalizing (SE). PR retains the power spectrum but distorts the modulation statistics while SE distorts the power spectrum and retains modulation statistics. Even at a constant noise level, the ability to recognize foreground digits was substantially helped or harmed by these background perturbations, depending on the original background sound. To explore this interference, we used texture synthesis (McDermott & Simoncelli 2011) to manipulate individual modulation statistics from the backgrounds. We found that adding texture statistics decreased accuracy for background speech babble. Interestingly, however, adding statistics increased accuracy in a construction noise background with strong comodulations. We next developed a physiologically inspired model of the auditory system model to predict perceptual trends. Sounds were decomposed through a cochlear filter bank (peripheral stage) and a subsequent set of spectrotemporal receptive fields that model modulation selectivity in auditory midbrain (mid-level stage). Logistic regression was performed on these features to estimate perceptual transfer functions and predict human accuracy. The peripheral model (with spectrum cues alone) accounted for 67% of the perceptual variance, while the mid-level model (spectrum and modulation cues) accounted for 91%. Perceptual transfer functions allow us to identify spectral and modulation cues critical to recognition in noise. Stimulus SNR has a substantial (log-linear) influence on recognition accuracy but appears to act independently from background statistics. These findings show how the diverse spectrum and modulation content of environmental background sounds has complex effects that can either help or harm speech recognition. However, an interpretable model of mid-level auditory computations predicts perceptual sensitivity and identifies the specific acoustic cues contributing to listening in noise.

Disclosures: **A. Clonan:** None. **X. Zhai:** None. **I. Stevenson:** None. **M.A. Escabi:** None.

Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR412.28/BB24

Topic: D.05. Auditory & Vestibular Systems

Title: Alterations of microstructural properties of white matter and structural brain connectivity in moderate hearing loss

Authors: *R. QUDDUS[1], A. ZINCHENKO[2], M. AL AMIN[3];
[1]Natl. Univ. Bangladesh, Gazipur, Bangladesh; [2]Psychology, Ludwig Maximilians Univ., Munich, Germany; [3]Indiana Univ., Stark Neurosciences Res. Inst., Indianapolis, IN

Abstract: Hearing loss affects almost 30% of elderly people (> 60 years of age) and has been identified as a risk factor for cognitive decline. Hearing impairment starts with loss of hearing sensitivity in the higher frequencies and advanced over time to the mild and lower frequencies. Previous studies showed an association between hearing loss and structural change in the brain. To date, there is limited knowledge of the structural connectivity of white matter and brain network topology in moderate hearing loss (>30 dB). To examine the integrity of white matter, identify vulnerable structural connectivity, and to assess network topology, we investigated the major white matter tracts of elderly subjects having moderate hearing loss (n = 22, mean age 70.5) and compared them with age-matched healthy controls (n = 26, mean age 68.4). We have analyzed diffusion and structural MRI data from Leipzig Study for Mind-Body-Emotion Interactions" (LEMON) dataset. Diffusion MRI data was acquired at TE 80 ms, 67 diffusion direction with 1000 b values (60 volumes) and b = 0 images (7 volumes), voxel size 1.71 x 1.71 x 1.7 mm. T_1-weighted images were acquired at a voxel size of 0.99 x 1 x 1 mm. Whole brain structural connectivity was also analyzed to identify vulnerable connections in the brain. We finally analyzed network topology to measure the properties of structural networks. We observed that the fractional anisotropy of the right corticospinal tract is increased in the subjects with moderate hearing loss. Along the tract, analysis showed that the last quarter of the superior part of the right corticospinal tract had a higher fractional anisotropy. We also identified a left putamen-centered disrupted brain network in moderate hearing loss. A total of eight brain regions had disrupted structural connectivity. Further network topology analysis showed a reduced betweenness centrality and small-world network in moderate hearing loss. Surprisingly, forceps major tract was negatively correlated with good hearing while positively correlated with moderate hearing loss individuals. In summary, our results first identified the putamen-centered disruption in structural connectivity in moderate hearing loss. Increased fractional anisotropy of the right corticospinal tract could play a compensatory role since it projects fiber to the right putamen. Reduced network topology suggests that the information flow is interrupted in moderate hearing loss individuals.
Taken together, the results of impaired white matter tracts in moderate hearing loss provide valuable clues to design novel therapeutics to prevent the progression of hearing loss.

Disclosures: R. Quddus: None. **A. Zinchenko:** None. **M. Al amin:** None.

Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR412.29/BB25

Topic: D.05. Auditory & Vestibular Systems

Title: Can you tell the difference between time-reversed syllable pairs? Unraveling the question through behavioral and MEG responses

Authors: *Y. MENG, H. FAN, L. ZHANG, L. LIU;
Beijing Language and Culture Univ., Beijing, China

Abstract: Imagine a time-reversed speech even repeat a time-reversed speech, which would sound like a mission impossible. This is due to the fact that speech is essentially a continuous temporal signal. Time-reversed speech jeopardize the temporal sequence information in a continuous temporal signal, which holds the most valuable information of speech, including the sequences of words, phonemics, or acoustic features. Current research predominantly focuses on the impact of time-reversed sentences on speech intelligibility. The greatest challenge in understanding time-reversed sentences is to segment a continuous temporal signal into a sequence of discrete linguistic units, i.e, words or syllables. However, it remains unclear whether perceiving time-reversed syllables still poses a significant challenge, considering that they are already discrete units on the syllable level. Here, we used time-reversed syllable pairs as speech perception material combined with Magnetoencephalography (MEG) analysis to explore the differences in behavior and brain response to the time-reversed and normal (non-time-reversed) syllables, in order to further our understanding of the temporal sequence information encoding at the level of syllables.
During the experiment, participants were instructed to discern whether the presented syllable pairs were the same or different within two blocks. The first block is time-reversed syllable pairs, while the second block is normal syllable pairs. We created sixty-four pairs of syllables (i.e, bi1 vs bu4) that had different consonants, vowels and tones to assess the accuracy and the elicited representations of varying phonetic category information. Surprisingly, the behavioral results revealed that participants could distinguish almost all the time-reversed syllables pairs (above 90% accuracy), except for syllable pairs with different consonants (below 20% accuracy). Although their response to normal syllable pairs (mean RT=120ms) was significantly faster than time-reversed syllable pairs (mean RT=240ms) in all types, syllable pairs with different consonants exhibited notably longer reaction time compared to other types. Furthermore, the machine-learning based classification analysis of MEG response to perceived syllables revealed that the brain showed a more similar response to the time-reversed syllables and normal syllablea when it drew bottom-up attention (on the first syllable of compared syllable pairs), while it showed much different response when the syllable drew more top-down attention (on the second syllable of compared syllable pairs).

Disclosures: **Y. Meng:** None. **H. Fan:** None. **L. Zhang:** None. **L. Liu:** None.

Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR412.30/CC1

Topic: D.05. Auditory & Vestibular Systems

Title: Active representation of phonemic features in alignment with speech perception requirements

Authors: *H. FAN, L. ZHANG, Y. MENG, L. LIU;
Beijing Language and Culture Univ., Beijing, China

Abstract: How can humans effortlessly differentiate between similar-sounding words such as "bubble" and "double" based on their initial consonants? The ability to distinguish between phonemic features (i.e. consonants) from complex and transient acoustic signal, is a challenging component of speech perception. Although there has been extensive research on how the brain processes information from the syllable to the lexical level, the initial step in speech perception, i.e., the flexible and efficient processing of phonemic information, remains unclear. Here, we used Magnetoencephalography (MEG) combined with multi-voxel pattern analysis method to explore how phonemic information is represented in human brain during participants perform a speech perception task. During the speech perception task, participants were instructed to determine whether the tones (tone task, subject n=30) or consonants (consonants task, subject n=19) of the two preceding and following syllables were the same. To evaluate the specificity and precision of the elicited representations of varying phonetic category information, we constructed sixty- four pairs of syllables (eight by eight) that vary in their phonetic features of consonants, vowels, and tones. First, we used the machine-learning based classification analysis to explore the phonemic representation at the phonemic level. We found that consonants are represented left-lateralized, whereas vowels and tones are represented on both hemispheres. More interestingly, by comparing the tone discrimination task and consonant discrimination task, we uncovered that phonemic representations are adapted in response to the speech perception task at hand. Specifically, when perceiving the second syllable, the task-related phonemic features of the first syllable, such as the tone feature in the tone task and the consonant feature in the consonant task, will be automatically reactivated. Furthermore, we used representational similarity analysis (RSA) combined with multidimensional scaling analysis to explore the phonemic representation at the syllable level. Our results revealed that people actively align the second syllable to the phonemic features of the first syllable that relevant to the task, with the second syllable being reassembled to match the tone/consonant features of the first syllable for the tone/consonant task. Overall, our results indicate that the human brain is capable of effectively and flexibly handling phonetic information through active representation of phonetic features to match with speech perception task at hand.

Disclosures: **H. Fan:** None. **L. Zhang:** None. **Y. Meng:** None. **L. Liu:** None.

Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR412.31/CC2

Topic: D.05. Auditory & Vestibular Systems

Support: NIH R01 DC004290

Title: Sensory processing of native and non-native phonotactic patterns in the alpha and beta frequency bands

Authors: *M. WAGNER[1], M. RUSINIAK[2], E. HIGBY[3], K. NOURSKI[4];
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Abstract: The phonotactic patterns of one's native language are established within cortical network processing during development. Sensory processing of native language phonotactic patterns established in memory may be modulated by top-down signals within the alpha and beta frequency bands. To explore sensory processing of phonotactic patterns in the alpha and beta frequency bands, electroencephalograms (EEGs) were recorded from native Polish and native English-speaking adults (48 participants, 36 females) as they listened to spoken nonwords within same and different nonword pairs. The nonwords contained phonological sequence onsets that occur in both the Polish and English languages except for /pt/, which occurs in Polish but not in English onsets. High density 64-channel EEGs (96 data sets) were analyzed in response to the first nonword in the pairs within the context of counterbalanced listening-task conditions, which were presented on separate testing days. During one listening-task condition, participants performed a syllable identification task to the second nonword in the nonword pairs (with-task condition). For the alternate condition, participants were instructed to only listen to the stimuli (without-task condition). Sensory processing was compared in the listening-task conditions because unmasking native language patterns of speech perception has been shown to be influenced by listening and task condition. Spectral power in the low frequencies (2-29 Hz) was analyzed to the first nonword in the pairs from five dipole source-level channels and twelve regional sources, obtained through source localization modeling. Source localization modeling, using all 64-channels of EEG data, increased power for statistical analyses. The results revealed that sensory processing to native language phonotactic patterns was modulated by listening-task condition within the beta frequency band. Specifically, the with-task condition elicited beta suppression in response to native language patterns of speech perception. However, in response to the non-native language phonotactic pattern, the with-task condition found suppressed spectral power values for an expanded frequency range that included the alpha and beta frequency bands. These findings suggest that linguistic experience with phonotactic patterns modulates perceptual processing through feedback within the alpha and beta frequency bands.

Disclosures: M. Wagner: None. **M. Rusiniak:** Other; BESA, GMbH. **E. Higby:** None. **K. Nourski:** None.

Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

Location: WCC Halls A-C

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Program #/Poster #: PSTR412.32/CC3

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant R01DC020717
NIH Grant R00DC013828

Title: Visual Speech Differently Restores Temporal and Spectral Speech Information in the Auditory Cortex

Authors: *C. CAO[1], K. GANESAN[1], Q. NGUYEN[2], A. JAHN[2], J. BRISSENDEN[2], D. BRANG[2];
[1]Psychology; MICDE, [2]Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: Seeing a speaker's face facilitates accurate speech perception. Previously, research has shown that listeners make use of lipreading to restore degraded auditory speech information, but these influences fail to account for the full benefits imparted by visual speech. Prior work has hypothesized that visual speech can restore information from two additional features of speech: spectral (relative pitch) and temporal information (sound changes over time). For example, listeners can recover spectral information using speakers' mouth width and the speaker's lip closure helps listeners parse the temporal boundary between words. However, it remains poorly understood whether, and how, spectral and temporal information is restored in the auditory cortex. In the current study, we asked two questions: first, is visual speech integration regionally specific, where auditory temporal and spectral information is restored in separate areas, or regionally nonspecific, where the same region restores both kinds of information? Second, how does visual speech alter the spatial pattern of auditory system activity to improve audibility of speech? We hypothesized that visual speech restores the spatial pattern of auditory activity evoked by degraded auditory speech, making it more similar to the pattern of activity evoked by clear speech. We collected functional magnetic resonance imaging (fMRI) data from 64 subjects who listened to 200 sentences presented across five conditions-- auditory-alone original, auditory-alone temporally smeared, auditory-alone spectrally smeared, audio-visual temporally degraded, and audio-visual spectrally degraded. Univariate contrasts reveal that the same visual signal has different effects on auditory processing depending on the degraded auditory feature. Visual speech increased BOLD in the STG for both types of degraded speech, but differed across conditions in other regions: AV spectral recovery increased BOLD in Heschl's gyrus while AV temporal recovery increased BOLD in anterior STG. Second, using Representational Similarity Analysis, our preliminary results suggest that visual restoration of speech uses distinctly different mechanisms from auditory speech perception. To verify this finding, we plan to build a single-trial-based GLM regressor to examine if representational distance is closer between audiovisual filtered speech and original auditory speech than that of audio-alone filtered speech. Together, we show that auditory cortex uses visual speech signals to selectively recover features of the auditory signal that have been degraded.

Disclosures: C. Cao: None. **K. Ganesan:** None. **Q. Nguyen:** None. **A. Jahn:** None. **J. Brissenden:** None. **D. Brang:** None.

Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR412.33/CC4

Topic: D.05. Auditory & Vestibular Systems

Title: Motor synchronization to auditory and visual rhythmic input is supported by shared rhythmic-related neural mechanisms

Authors: *S. SONG, H. BIAN, L. LIU;
Beijing Language and Culture Univ., Beijing, China

Abstract: Synchronization between motor, auditory, and visual processing is essential for effective communication, both verbal and written. Studies have found that the brain's neural entrainments enable the connection between auditory processing and the production of synchronized movements, which is known as sensorimotor synchronization. Nevertheless, little is known about whether or not cortical activity that supports sensorimotor integration characterized by internal representation of rhythmic beat is similar in both modalities (audio-motor and visuo-motor). In the current study, the magnetoencephalogram (MEG) was recorded while participants performed three tasks in each modality. For the passive task, participants passively listened to an auditory metronome beat (audio rhythmic task) or watched a ball flashing within beat (visual rhythmic task) that was both delivered in 2.4Hz. For the hand tapping tasks, either by right or left hand, participants had to tap their fingers to every second visual (visuo-motor rhythmic task) or audio (audio-motor rhythmic task) pulse in attempt to disentangle the movement- and beat-related neural responses. During the passive audio/visual rhythmic tasks, MEG-derived cortical response spectrum reveals cortical tracking to audio and visual rhythms at 2.4Hz as well as at second (4.8HZ) and third (7.2HZ) harmonic frequency. During the visual-motor/audio-motor rhythmic tasks, MEG-derived cortical response spectrum reveals that there was a strong 2.4Hz beat-related entrainment and a 1.2Hz movement-related entrainment for both modalities as well as their harmonic frequencies. Most importantly, we observed sensorimotor coupling in the form of an additional 3.6Hz response during both auditory and visual tasks. Taken together, the data suggest that internal representation of rhythmic input and sensorimotor integration are supported by shared neural mechanisms that underlie time-related processing - regardless of modality. The data is relevant to growing evidence suggesting that rhythm perception and general time-related skills such as parsing may be the core deficit underlying speech and language processing deficits.

Disclosures: S. Song: None. **H. Bian:** None. **L. Liu:** None.

Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR412.34/CC5

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant R01 DC019507
NIH Grant R21 DC016086

NIH Grant R21 DC015884
NIH Grant R01 NS090874
NIH Grant R01 NS109487

Title: Identifying responses to auditory and audiovisual speech during movie viewing using high density diffuse optical tomography

Authors: *J. E. PEELLE[1], A. SHERAFATI[2], E. MILARACHI[3], M. S. JONES[4], A. BAJRACHARYA[5], A. T. EGGEBRECHT[7], T. HERSHEY[6], J. CULVER[8];
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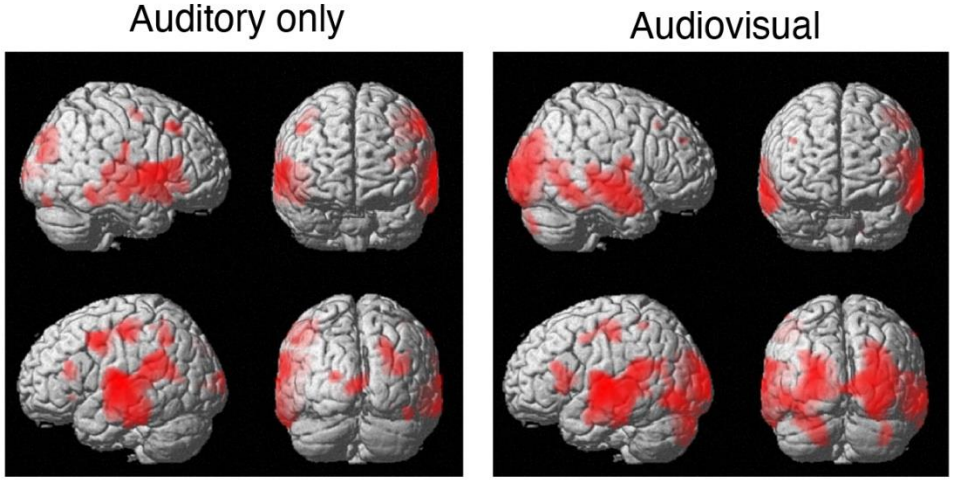
Abstract: Visual speech information aids understanding, particularly under challenging listening conditions. However, the brain mechanisms underlying audiovisual speech processing remain poorly understood. Compounding this issue is the challenge that everyday communication situations typically involve rich linguistic and environmental context not present in the majority of laboratory tests. To advance our understanding of audiovisual speech in everyday life we presented participants (n=50 adults) with a movie clip from The Good, the Bad, and the Ugly (1966). We manually identified speech events and classified these as either auditory-only or audiovisual based on the amount of the speaker's mouth that was visible. We then convolved these onset times with a canonical hemodynamic response function to provide predictors for modeling the measured response. We measured brain activity using high-density diffuse optical tomography (HD-DOT), a form of functional near infrared spectroscopy (fNIRS). HD-DOT provides even sensitivity and high spatial accuracy over a large portion of the superficial cortical surface, including large portions of the occipital, temporal, and frontal lobes, and permits localizing measurements to atlas space. Using this approach, we were able to identify responses in the temporal lobes related to auditory-only speech. Finally, we found that audiovisual speech recruited both auditory and visual cortices, as well as posterior superior temporal sulcus. Together these findings establish the feasibility of using movie-based paradigms for studying both auditory and audiovisual speech understanding.



Disclosures: **J.E. Peelle:** None. **A. Sherafati:** None. **E. Milarachi:** None. **M.S. Jones:** None. **A. Bajracharya:** None. **A.T. Eggebrecht:** None. **T. Hershey:** None. **J. Culver:** None.

Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR412.35/CC6

Topic: D.05. Auditory & Vestibular Systems

Support: Polish National Agency for Academic Exchange, Bekker Programme (BPN/BEK/2021/1/00358)
Danish National Research Foundation (DNRF 117)

Title: Differences in mismatch responses for harmonic and inharmonic sounds

Authors: *K. BASINSKI[1], A. CELMA - MIRALLES[2], D. QUIROGA-MARTINEZ[3], P. VUUST[2];
[1]Div. of Quality of Life Res., Med. Univ. of Gdansk, Gdansk, Poland; [2]Dept. of Clin. Medicine, Aarhus Univ. and Royal Acad. of Music Aarhus/Aalborg, Aarhus, Denmark; [3]UC Berkeley, Berkeley, CA

Abstract: Predictive processing accounts of perception suggest that the brain uses generative models to reliably predict incoming sensory stimuli. If these predictions are incorrect, a prediction error response is propagated that changes the generative model. A crucial tenet of predictive processing accounts is that prediction errors are precision-weighted. This means that only errors with high precision influence the generative model. Precision depends on the information content of the predicted stimuli and can be expressed as inverse variance or information entropy. Harmonic sounds are auditory signals that are composed of frequencies that are integer multiples of a fundamental frequency (F0). Inharmonic sounds can have spectra with unrelated frequencies. Importantly, the information content of harmonic sounds is smaller than inharmonic sounds, as their spectra can be described only with the F0. Thus, any prediction errors associated with inharmonic sounds should be down-weighted. In this study, we aimed to test the hypothesis that violations of predictions for harmonic sounds would elicit a stronger response (as measured with mismatch negativity, MMN) than inharmonic sounds, due to higher precision-weighting of prediction errors in harmonic sounds. Participants (N = 36) listened passively to artificially generated complex tones with spectra manipulated to be either harmonic, inharmonic (with a random pattern of jitters applied to frequencies above f0), or inharmonic-changing (with jittering patterns randomized between consecutive sounds). The tones were presented in a roving oddball paradigm with varying fundamental frequencies. We recorded electroencephalography with a 32-channel active system. We computed difference waves (deviant - standard) for harmonic, inharmonic, and inharmonic-changing conditions. We then contrasted these difference waves between conditions using a non-parametric, cluster-based permutations approach. Consistent with our hypothesis, the results revealed significantly stronger

MMN in the harmonic condition compared to the inharmonic-changing condition (cluster range 0.083 ms - 0.189 ms after stimulus onset, $p = 0.004$) but, surprisingly, not when compared to the inharmonic condition ($p > 0.05$). We have also found differences in later, P300 positivity, with weaker responses in the harmonic condition in comparison to the inharmonic condition (cluster range 0.215 ms - 0.359 ms, $p = 0.005$). These results offer only partial support to our hypothesis and reveal unexpected differences in P300 responses. Overall, this study provides new insight into precision-weighting of prediction errors in the auditory domain.

Disclosures: K. Basinski: None. **A. Celma - Miralles:** None. **D. Quiroga-Martinez:** None. **P. Vuust:** None.

Poster

PSTR412. Auditory Processing: Perception, Cognition, and Action II

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Title: Decoding maintenance and replay activity in the human auditory cortical mnemonic system

Authors: *R. M. CALMUS[1,2], Z. KOCSIS[3,1], Y. KIKUCHI[2], H. KAWASAKI[1], T. D. GRIFFITHS[2], M. A. HOWARD, III[1], C. I. PETKOV[1,2];
[1]Dept. of Neurosurg., Univ. of Iowa, Iowa City, IA; [2]Biosci. Inst., Newcastle Univ., Newcastle upon Tyne, United Kingdom; [3]Neurosci. Inst., Carnegie-Mellon Univ., Pittsburgh, PA

Abstract: There is substantial scientific interest in identifying neural signals that carry traces of the sensory past or an expectancy about the future. To date, memory traces of sensory sequences containing regularities have been decoded in auditory cortex and the hippocampus in animal models, or in humans using non-invasive neuroimaging studies. However, outside of research with animal models there is a dearth of insights on how site-specific neurophysiological signals from the auditory cortical mnemonic system may carry information reflecting maintenance activity to sounds over delays, and on the signals that may characterize the retrospective replay of regularities in a sensory sequence. We conducted an auditory statistical learning task containing non-adjacent dependencies with 12 neurosurgery patients during presurgical intracranial monitoring of refractory epilepsy. The patients listened to sequences of three nonsense words drawn from sets (X, A and B), with regularities between relevant pairs of sounds (A-B) in the sequence often separated in time by uninformative (X) words. We first analyzed the site-specific local field potentials from auditory cortex, hippocampus and frontal cortex using

traditional methods, demonstrating engagement of the fronto-temporal network in the processing of the sequence regularities. We also present the output of a time-resolved multivariate decoding analysis that reveals the latencies and timescales of sequence item representations in regions across the network. The results represent to our knowledge the first human intracranial electrophysiological evidence of hippocampal replay of stimuli during an auditory task, showing that time-compressed replay occurs before and after key sounds in the sequence. Furthermore, the results also revealed that the maintenance of sound representations within non-primary (superior temporal gyrus) auditory cortex appears to exceed that of primary (core) auditory cortex in duration. Finally, in further support of a previously presented computational model of sequence processing and binding (VS-BIND; Calmus et al., 2019), prefrontal areas including precentral gyrus appear to maintain an ordered buffer of the auditory item representations. These results elucidate critical roles for the auditory sequential mnemonic system in transforming individual sensory events into mental structures, providing insight into the specific mechanistic contributions of medial temporal, prefrontal and superior temporal regions in the maintenance, prediction and ordinal manipulation of sequential information.

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Poster

PSTR413. Visual Cognition: Decision-Making

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR413.01/CC8

Topic: D.06. Vision

Support: Intellegence Community Post-Doctoral Fellowship in Assessing Collective Intellegence

Title: Space-time mixing in neural representation

Authors: *M. LEVY;
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Abstract: Generally we think of neural computation as being a stack of filters which respond in meaningful ways to the external world. This framework underlies the success of artificial neural networks. However, we know that, while these filters capture something important about how information is brought into the brain, they don't tell us how information is created, decisions made, or really carefully characterize the response properties in response to perturbations. It is well understood that neurons adapt, which mostly falls outside of this paradigm, that the neural and perceptual responses vary predictably to both spatial and temporal modulation, and that these representations, though stable, tend to drift over time. We also know that the molecules at the synapses, where the memory is supposedly stored, have a much faster turn over rate than the memory itself, and that synapses tend to maintain their electrical properties and firing patterns in

the face of this turn over, not necessarily with the same type of proteins. Somehow there are emergent precepts which can last a lifetime despite this sea of variability and change. While we know that animals can have repetitive control over the actions of single neurons, we don't know how one-to-one this mapping is or what local computations underlie the read out. To better understand the properties of these neurons, we need to think of computation at a scale beyond that of a single cell. A phonon is an vibration that travels through a media: think of knocking on a table. The sound is carried through the table not by any single atom but by the coherent vibrations thereof. We think that in studying perception and neural computation we need to better grapple with the computational properties of the brain as a distributed computational system.

Disclosures: M. Levy: None.

Poster

PSTR413. Visual Cognition: Decision-Making

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR413.02/CC9

Topic: D.06. Vision

Support: Whitehall Foundation
E. Matilda Ziegler Foundation for the Blind
France-Merrick Foundation

Title: Concurrent Decision and Confidence Signals in area LIP

Authors: *M. VIVAR-LAZO, C. FETSCH;
Johns Hopkins Univ., Baltimore, MD

Abstract: Decision confidence plays a key role in cognitive behavior, but exactly when and how it arises in the brain remains unclear. Bounded evidence accumulation models provide a candidate mechanism by exploiting an association between the amount of accumulated evidence—discounted by elapsed time—and the probability of making a correct choice. Consistent with this idea, neurons in macaque area LIP that represent the accumulated evidence (known as a decision variable, DV) also predict the monkey's confidence reports. However, the question still looms as to whether the updating and termination of a DV for choice and confidence can occur in parallel, as opposed to sequentially, and whether LIP activity can explain choice, reaction time (RT), and confidence when measured concurrently on the same trials.We designed a peri-decision wagering (Peri-DW) assay in which monkeys simultaneously reported choice and confidence in a random-dot motion task. Both components of the decision were reported when the subject initiated a saccade to one of four possible targets (left + high bet, left + low bet, right + high, or right + low), thereby isolating the temporal window that could directly inform the choice and wager. Quantification and modeling of behavior suggests that a bounded accumulation process can explain the animal's choices, reaction times, and confidence.

Specifically, psychophysical kernels indicate a coinciding stimulus epoch that informs choice and confidence, and predictions from the model agree with empirical findings: greater accuracy and faster reaction times for high- compared to low-confidence decisions. Analyses of 54 LIP neurons from one monkey reveal signatures attributable to an accumulation-to-bound process, including ramp-like firing rates graded by motion coherence, convergence to a common firing rate before the saccade, and expected patterns of variance and autocorrelation. Importantly, these signatures do not differ in their timing when the neurons are grouped by their preferred saccadic target (i.e., high vs. low wager), which argues against the possibility that confidence is purely post-decisional. To gain further insight into the LIP responses, we simulated a 4D accumulation process (anticorrelated race) mapped onto four populations in LIP, each having response fields overlapping one of the four choice targets. Early simulation results resemble the choice- and confidence-dependent modulation of LIP responses seen in the empirical data. Follow-up analyses will apply generalized linear models (GLM) to further elucidate the neural dynamics underlying concurrent evaluation of a decision and its associated degree of confidence.

Disclosures: M. Vivar-Lazo: None. **C. Fetsch:** None.

Poster

PSTR413. Visual Cognition: Decision-Making

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR413.03/CC10

Topic: D.06. Vision

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Title: Pulvinar-prefrontal inputs facilitate adaptive updating with sensory history during visual decision-making

Authors: *Y. LEOW[1], A. BARLOWE[1], C. T. LUO[1], Y. OSAKO[1], M. JAZAYERI[3], M. SUR[2]; [2]Dept. of Brain and Cognitive Sci., [1]MIT, Cambridge, MA; [3]Massachusetts Inst. of Technol. Dept. of Brain and Cognitive Sci., Cambridge, MA

Abstract: Processing sensory information to generate decisions and action is a central component of learned, goal-directed behavior. Our sensory landscape is constantly filtered through our prior expectations and ongoing goals. Under greater perceptual uncertainty, perceptual processing becomes more susceptible to the influence of prior history, which can induce biases that compromise decision-making. However, trial history can also be used adaptively to guide decisions when perceived similarity across stimuli is used to guide switching

or repeating actions. We trained mice on a two-choice random dot motion discrimination task, varying sensory certainty with the proportion of coherent target dot directions. We found that mice adopted a strategy of comparing the similarity of stimulus directions across trials to make switch/stay decisions. The Anterior Cingulate Cortex (ACC) is a frontal region that integrates uncertainty for switch/stay decisions from diverse inputs including the pulvinar/lateral posterior (LP) nucleus (LP, rodent homolog), a higher-order visual thalamic nucleus. The pulvinar serves as an integrative hub with reciprocal connectivity with the ACC and many other visual and associative cortices. Pulvinar, specifically its interactions with the frontal cortex, has been implicated in effective visual decision-making and predictive processing, but the information conveyed by this pathway has been challenging to resolve given the broadly divergent projections of pulvinar neurons. Leveraging genetic tools available in rodent models, we performed projection-specific two-photon calcium imaging and optogenetic manipulations of LP-ACC axons in mice performing the visual discrimination task. We show that LP-ACC visual stimulus representations were gated by task engagement, and encoded multiple task variables scaled by stimulus uncertainty. LP-ACC responses were also highly modulated by previous trial information, which was also graded by the uncertainty associated with the previous trial. Critically, we found that estimated similarity in stimulus directions across trials also modulated LP-ACC activity. Optogenetic activation of LP-ACC axons during stimulus evaluation impaired perceptual discrimination and reduced psychometric slopes, due to altered interactions between current and previous trial information. Our findings demonstrate that the LP-ACC inputs support decision-making by providing a read-out of ongoing uncertainty, integrated over time with behavioral history, to adaptively tune neuronal responses and guide goal-directed behavior on a trial-to-trial basis.

Disclosures: **Y. Leow:** None. **A. Barlowe:** None. **C.T. Luo:** None. **Y. Osako:** None. **M. Jazayeri:** None. **M. Sur:** None.

Poster

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Topic: D.06. Vision

Support: NIH Grant F30EY035113
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Title: Computation of context in the midbrain reticular nucleus during perceptual decision-making

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Abstract: Perceptual decision-making requires the integration of dynamic sensory evidence from the environment with internal state variables (e.g. context, priors, value) to choose an appropriate action plan. Classical models of perceptual decision-making ascribe decision formation to forebrain sensorimotor regions, with the midbrain existing at the end of a feedforward pathway to relay motor commands. However, there is substantial evidence that the midbrain, especially the superior colliculus (SC), participates in the visual decision process. Recent work has suggested that an additional midbrain region - the midbrain reticular nucleus (MRN) - may be part of the perceptual decision network, as it was one of the few regions that encoded visual and action selection signals in a brain-wide survey of 2-alternative forced choice (2AFC) task-related activity in mice (Steinmetz et al, 2019).
Previous studies have noted motor functions of the MRN, including roles in locomotion (Roseberry et al, 2016) and saccades (Waitzman et al, 2002), while more recent work has described a role in interacting with forebrain regions to guide cue-triggered actions (Inagaki et al, 2022). We sought to characterize whether the wide variety of motor and sensory signals found in MRN are topographically organized. Thus, we conducted Neuropixels 2.0 recordings throughout MRN while both task-naive and trained mice passively viewed task stimuli. We found topographic organization of sensory/motor coding in MRN and increased visual and auditory responses to task stimuli after task training.
Additionally, we sought to assess whether the MRN, in addition to containing action selection signals, contains internal state signals required to compute an abstract decision. We designed a novel visual discrimination wheel-turning task that allows for the distinguishing of visual, motor, and contextual signals by including a condition in which the same stimulus requires different motor responses depending on the task Block (i.e. context). In Neuropixels 2.0 recordings during task performance, we found contextual modulation of visual and action-related responses of single neurons in canonical decision-making regions including secondary motor cortex, caudoputamen, and SC as well as in the MRN. Analyzing the population activity structure in these regions, we find evidence for a computation in which context alters the integration of evidence by shifting the attractor landscape.
In sum, our results suggest that the MRN is not a passive motor command structure, but rather, a spatially organized and temporally dynamic player in distributed brain circuits for perception, cognition, and behavior.

Disclosures: **J.R. Shaker:** None. **D. Birman:** None. **N.A. Steinmetz:** None.

Poster

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Topic: D.06. Vision

Support: Dutch Research Council (NWO)
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Wellcome Trust
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Title: Mice adapt to temporal regularities in perceptual decisions

Authors: *M. FRITSCHE[1], A. MAJUMDAR[1], L. STRICKLAND[1], S. LIEBANA GARCIA[1], R. BOGACZ[2], A. LAK[1];
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Abstract: Perceptual decisions not only depend on current sensory information, but are also influenced by recent choices and their outcomes. Such choice history biases are ubiquitous in psychophysical datasets and have been observed in mice, rats, monkeys, and humans. While maladaptive in standard randomized experiments, these biases are hypothesized to reflect advantageous strategies to exploit temporal regularities of natural environments. Crucially though, natural environments exhibit a multitude of temporal regularities whose exploitation requires an adaptive use of different history biases that match the prevailing temporal structure. However, it is unclear whether and how observers could adapt their choice history biases to different temporal regularities. To address this, we trained mice in a visual decision-making task. In each trial, we presented a grating patch on the left or right side of a computer screen and mice indicated the grating location by steering a wheel with their forepaws, receiving water reward for correct responses. After mice reached expert proficiency on random stimulus sequences, we systematically manipulated the temporal regularity of visual input by changing the trial-by-trial transition probabilities between successive stimuli across different days. In addition to neutral stimulus sequences in which stimulus location was chosen at random [p("Repeat") = 0.5], we exposed mice to a repeating environment in which stimulus locations were likely repeated across successive trials [p("Repeat") = 0.8], and an alternating environment in which stimulus locations likely switched from the previous trial [p("Repeat") = 0.2]. We show that mice can adapt their choice history biases to these different temporal regularities in the sequence of visual stimuli to facilitate successful visually-guided decisions. This adaptation was slow, evolving over hundreds of trials across several days. It occurred alongside a fast non-adaptive choice history bias, limited to a few trials. We show that these fast and slow trial history effects are well captured by a normative reinforcement learning algorithm with multi-trial belief states, comprising both current trial sensory and previous trial memory states. Our results thus reveal the adaptive nature of choice history biases and cast these biases as the result of a continual learning process to facilitate decisions in an uncertain world.

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Poster

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Program #/Poster #: PSTR413.06/CC13

Topic: D.06. Vision

Title: Investigating the neural basis of cue combination in large cohorts of human subjects

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Abstract: Everyday life requires people to make decisions based on many sources of information. In the perceptual realm, this is called cue combination (combining information from multiple sensory cues, or information sources). The ability to effectively combine information sources is thought to be limited by trial-to-trial variability in neural responses. We and others have shown that response variability can be changed by cognitive processes such as attention or by drugs, like stimulants, used to repair cognition or treat patients with disorders of attention. These observations lead to the hypotheses that 1) cognitive processes and cue combination share neural mechanisms and 2) processes that affect cognition, like healthy aging, brain disorders, or drug use, might also affect cue combination. We therefore hypothesize that on a person-by-person basis, there will be a link between the way people combine information within a modality (e.g. two forms of visual information) and across modalities (combining visual and auditory information), and that these abilities will depend on age, self-reported use of stimulants (e.g. coffee or methylphenidate [Ritalin]), and other health or demographic information. We tested these hypotheses by measuring the ability of large cohorts of human subjects to combine information within and across sensory modalities in online psychophysical experiments. We designed a psychophysical task that enhances our ability to identify subtle changes and trial to trial variability in cue combination behavior by asking participants to make analog rather than discrete decisions. On each trial, participants observed between one and three sensory cues (motion direction, spatial arrangement of visual stimuli, and changes in pitch) and indicated a combined judgment by clicking on an appropriate target location on a circle. We assessed cue combination by comparing performance judging each individual cue alone, pairwise cues, and all three cues together. We analyzed participant data within and between groups using a combination of frequentist statistics and Bayesian inference.

Disclosures: **K.S. Allen:** None. **M.R. Cohen:** None.

Poster

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Program #/Poster #: PSTR413.07/CC14

Topic: D.06. Vision

Support: National Science and Technology Innovation 2030 Major Program (grant 2021ZD0203703)

Title: Reduced sensory processing efficiency after switching relevant sensory dimensions in flexible perceptual decision making

Authors: *T. LUO, M. XU, Z. ZHENG, G. OKAZAWA;
Ctr. for Excellence in Brain Sci. and Intelligence Technology, Chinese Acad. of Sci., Shanghai, China

Abstract: Humans can flexibly switch rules to categorize sensory stimuli, but categorization performance is known to degrade immediately after a rule switch. This switch cost has been extensively studied in a variety of experimental tasks, but the mechanisms responsible for this phenomenon remain controversial. Here, we examined what aspects of decision-making processes are affected at the moment of rule switch, based on a framework we built previously to quantitatively model face categorization behavior (Okazawa et al., Cell 2021; J Neurosci 2021). In the task, subjects switched between two categorization rules for the same face stimuli every 2-6 trials. Face stimuli were randomly chosen from a 2D face stimulus space, whose axes corresponded to the two categorization rules. Each axis was generated by continuously morphing two prototype faces, forming a range of task difficulty. During the stimulus presentation, the morph levels rapidly (~100 ms) fluctuated at a subliminal level; this enabled us to test how subjects weighted sensory evidence to make a decision. We found that, immediately after switching the categorization rule, subjects' choice accuracy remained unchanged while reaction times increased substantially for any stimulus difficulty level. Notably, psychophysical reverse correlation showed that this switch cost was accompanied by an initial reduction in sensory weighting aligned to stimulus onset. This reduction occurred regardless of the preparation time given after a rule switch was informed but recovered 200-300 ms after stimulus presentation. Fit to evidence accumulation models confirmed that the initial reduction in sensory weight accurately account for the observed patterns of choice accuracy, reaction times, and psychophysical kernels after task switch. These results suggest that, after switching relevant sensory dimensions, decision-making circuits must undergo fine tuning to optimize the efficiency of information flow based on the incoming sensory information.

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Poster

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Program #/Poster #: PSTR413.08/CC15

Topic: D.06. Vision

Title: Double dissociation of spontaneous alpha-band activity and pupil-linked arousal on additive and multiplicative perceptual gain

Authors: *A. PILIPENKO[1], S. AFRAKHTEH[2], A. FEGHHI[2], A. GERGEN[2], N. GUPTA[2], J. SAMAHA[1];
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Abstract: Conscious visual perception of a weak stimulus is a probabilistic process governed not only by external stimulus properties but also by internal states of the observer. However, which internal states influence perception and via what mechanisms remain debated. Here, we studied the influence that spontaneous changes in alpha-band activity and spontaneous fluctuations in pupil size have on stimulus detection across a range of contrast values spanning each observer's contrast response function (CRF). We found that states of weak pre-stimulus alpha power induced an "additive" shift in the CRF, whereby stimuli were reported present more frequently at all contrast levels, including contrast of zero (i.e., false alarms). In direct contrast, pupil size measured during the same pre-stimulus window had a "multiplicative" effect on subsequent detection such that stimuli occurring during large pupil states (putatively corresponding to higher arousal) were perceived more frequently the higher the contrast level, without a corresponding boost in false alarms. In other words, alpha power impacted detection criteria equally across the CRF but not detection sensitivity (d') whereas changes in pupil-linked arousal impacted sensitivity, particularly for higher contrasts. Interestingly, spontaneous changes in pupil size and alpha power were positively correlated across trials, which could imply that some of the effect of alpha on detection may be mediated by pupil fluctuations. However, when we examined the effect of alpha power residualized for pupil size, we still observed the same additive effect of alpha on the CRF, corresponding to criterion shift. Our data imply that strong alpha power inhibits perceptual detection by an additive suppression factor, rather than by a divisive scaling of contrast responses, a profile which better described the effect of pupil-linked arousal. We suggest that fluctuations in alpha-band power do not correspond to changes in arousal as the two have dissociable effects on behavior. Rather, alpha power seems to reflect the baseline level of excitability in the visual system, independent of arousal.

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Poster

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Program #/Poster #: PSTR413.09/CC16

Topic: D.06. Vision

Title: Towards proper visual psychophysics by and for marmosets

Authors: D. TROTTER[1], P. CHEN[1], L. DEFELICE[1], A. LAUDANO[1], D. P. ROWLEY[2], ***A. HUK**[3];
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Abstract: Introduction.
Although the common marmoset has gained significant popularity as a model organism for systems neuroscience, reasonable concerns persist regarding their proclivity to perform conventional psychophysical tasks. If they could perform such tasks well, connections to work in

other NHP and rodent species would be greatly strengthened. Here, we sought to test the idea that the primary weakness in their task performance was species-inappropriate training approaches and task details (as implemented by human experimenters), rather than a general inability of this model organism to do psychophysics.
Methods.
Instead of adopting approaches from macaques, we started with a psychophysical paradigm recently developed to generate useful visual behavior in mice (e.g., International Brain Laboratory, eLife, 2021). This task involves presentation of a visual stimulus (sinusoidal grating) to the left or right of a computer monitor; the subject receives reward for using a steering wheel to move the stimulus to the center of the screen. If the subject can see the stimulus, they can do this easily and intuitively; if they cannot, the direction of their wheel-turns will be random. We developed a marmoset-centric steering wheel apparatus and attempted to train marmosets on this task, starting with high contrasts and gradually weaving in lower ones to make visibility challenging.
Results.
Marmosets learn the sensory-motor mapping in the first few sessions, clearly turning the wheel to make responses, and exhibiting clear indications of reward expectation. Within approximately 2 weeks of training, a test subject was capable of performing several hundred trials within an hour, even with threshold-level contrasts present. Contrast sensitivity fell within the expected range (< 10% contrast at ~5 deg eccentricity). Lapse rates were small. Incorporation of eyetracking is a promising extension for both confirming task engagement and constraining visual stimulus presentation.
Discussion.
Marmosets appear capable of performing relatively conventional psychophysics, so long as the apparatus and sensory-motor mapping are comfortable and intuitive for this species. Marmosets are likely neither stupid nor lazy, and the rapid success of task training we observed suggests this framework could be generalized to a variety of discrimination and detection tasks.

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Poster

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Topic: D.06. Vision

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Title: Estimating and integrating the uncertainty of naturalistic stimuli

Authors: *C. R. PLATE[1], Z. M. BOUNDY-SINGER[2], C. M. ZIEMBA[2];
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Abstract: When our perception is uncertain, we make inconsistent perceptual decisions. We have the ability to reflect on the reliability of our decisions in the form of confidence. To usefully guide behavior, this sense of confidence should integrate all sources of decision-relevant uncertainty into a single decision reliability estimate. However, this metacognitive ability is not perfect, and can vary across individuals and contexts. Confidence is often studied in experiments where decision reliability is varied through changing a single dimension of stimulus strength (e.g. only varying the orientation of a simple visual pattern in an orientation discrimination task), or not varied at all. Here, we sought to test the limits of a subject's ability to appropriately integrate the uncertainty of complex, naturalistic stimuli into their perceptual confidence reports. We created a set of synthetic texture stimuli matched to the statistics of natural images and asked participants to judge in which direction their dominant orientation was rotated from vertical. Subjects reported their decision and binary confidence level with a single button press. Both accuracy and confidence reports were incentivized by awarding a large number of points for high confidence correct responses, but a substantial loss of points for high confidence errors. Points were added to a running total across trials, and paid out at the end of the experiment. To control decision reliability, we varied stimulus features known to increase orientation uncertainty as well as neural signatures of uncertainty in the primary visual cortex. These include orientation rotation strength, the dispersion of oriented image energy, and contrast. However, we additionally manipulated the presence of higher-order, naturalistic pixel correlations, which are known to decrease neural signatures of uncertainty in visual cortical regions downstream of V1 without affecting the orientation content of the stimuli. We fit the responses of subjects with the CASANDRE model for confidence which allows us to assess both the precision of uncertainty representation and the extent to which different sources of uncertainty are appropriately integrated into a single confidence signal. Our results indicate that subjects generally demonstrate the ability to represent and integrate naturalistic uncertainty into their perceptual confidence reports, but with some striking and idiosyncratic failures as well.

Disclosures: C.R. Plate: None. **Z.M. Boundy-Singer:** None. **C.M. Ziemba:** None.

Poster

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Topic: D.06. Vision

Support: Whitehall Foundation
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Title: Effects of recent sensory experience on cue weighting during multisensory integration

Authors: Y. R. HAILE[1], S. J. JERJIAN[2], *C. R. FETSCH[3];
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Abstract: Multisensory integration is fundamental to our rich perception of the world yet is not fully understood mechanistically. Studies across many different pairings of sensory modalities suggest that cues are combined in a linear weighted fashion, where the weights are proportional to a cue's relative reliability. This has been termed reliability-based cue weighting and is a signature of statistically optimal or near-optimal integration. The conventional interpretation is that reliability is estimated in a pure "bottom-up" fashion, i.e. exclusively from the stimulus-driven response of sensory neurons. However, recent findings in humans show that cue weights are influenced by explicit knowledge the task relevance of a cue, and by estimates of cue reliability from the recent past. Here we test for the first time in non-human subjects whether and how recent sensory experience influences cue-weighting behavior, with the long-term goal of understanding the neural basis of top-down influences on multisensory integration.Two rhesus monkeys performed a 2AFC heading discrimination task using visual and vestibular self-motion cues. On a given trial, the stimulus could be delivered as vestibular-only (inertial motion), visual-only (optic flow), or combined (both presented synchronously). Monkeys used a saccadic eye movement to report whether their perceived heading was to the left or right of straight ahead. The relative reliability of the cues was controlled by varying the optic flow coherence, and the weights were estimated from cue-conflict trials in which the heading specified by visual and vestibular cues were separated by a small conflict angle, Δ (3°). The effect of recent experience was examined by analysis of cue weights, conditioned on whether the previous N consecutive trials were (a) vestibular-only vs. visual-only, or (b) high coherence vs. low coherence.We found that as few as 2 consecutive trials of a given type had a substantial effect on the weights assigned to cues on the next trial. When cue-conflict trials are preceded by two vestibular-only trials, the vestibular weight is observed to elevate significantly. Similarly, consecutive visual-only stimuli alter animals cue weighting behavior, but in a manner dependent on their coherence. The results support the notion that cue weights are modulated by recent sensory experience rather than being solely driven by momentary estimates of cue reliability. Preliminary behavioral analyses of a post-decision wagering version of the task suggest that subjective confidence in the heading judgment plays a role in gating this top-down process, the neural substrate of which is under ongoing investigation.

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Poster

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Topic: D.06. Vision

Support: DFG Grant 3038041019
Wellcome Trust

Title: Distinct decision processes for 3D and motion visual stimuli in humans and macaques

Authors: *R. RANGOTIS[1], S. NOWAKOWSKA[1,3], E. KAKAEI[2], A. AKANDE[1], K. KRUG[1,4];
[1]Sensory Physiol., [2]Cognitive Biol., Otto-von-Guericke Univ., Magdeburg, Germany; [3]Inst. for Auditory Neurosci., Univ. Med. Ctr., Göttingen, Germany; [4]Dept. of Physiology, Anat. and Genet., Univ. of Oxford, Oxford, United Kingdom

Abstract: Neurophysiology localised perceptual decision signals for binocular 3D depth and motion stimuli to visual area V5/MT. Drift diffusion modelling (DDM) is widely used to investigate the underlying decision-making processes by simulating decisions as noisy evidence-accumulation which terminates once a threshold is crossed. Two key questions remain unexplored: 1. To what extent are modelled decision processes affected by the visual stimulus (here 3D depth vs. motion) and by the effector for the response (hand vs. saccades)? 2. Are the modelling parameters comparable between monkeys and humans? To answer these questions, we tested 2 male monkeys (*Macaca mulatta*) and 20 humans (13 females, 7 males, aged 18-40, mean age 29.6, range 18-40 years) on different stimulus/effector combinations in a 2-alternative forced-choice task. Stimuli were a 3D structure-from-motion cylinder or a random dot kinematogram (RDK), requiring perceptual decisions about binocular depth or direction of motion, respectively. Effectors comprised hand and saccadic eye movements. Monkeys only performed the cylinder task with saccadic responses and the RDK task with hand movements, whereas humans performed all combinations in a counterbalanced order. Psychophysical performance and reaction times were comparable between species on the same task. The parameter distributions of a hierarchical DDM model (distance to bound, non-decision time, drift-rate slope, drift-rate intercept, bias) indicated a difference in distance to bound specific to the visual stimulus in both species. Using dimensionality reduction, linear discriminant analysis (LDA) revealed a strong separation by stimulus type but not for the effector for the human data ($p<0.001$, ROC analysis). Nearly identical clustering was observed for the monkey data when it was projected onto the same space with almost complete overlap. We conclude that the DDM reveals distinct brain processes for perceptual decisions about visual motion and binocular 3D stimuli in humans and macaques, although perceptual signals for both have been localised to the same brain area. We found no distinction between hand and eye movement responses in either species.

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Poster

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National Natural Science Foundation of China 32171034
National Natural Science Foundation of China 32061143003
Israel Science Foundation 3318/20

Title: Dynamics of population neuron response to heading perception in different multisensory cortical areas

Authors: *F. ZENG[1,2], A. ZAIDEL[2], A. CHEN[1];
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Abstract: Neurons in multisensory cortices involved in heading perception, respond to multiple task-relevant variables, for example heading direction and choice. However, that makes it difficult to distinguish the functional properties of these neuronal populations. In our study, we recorded neuronal activity from the ventral intraparietal cortex (VIP), dorsal medial superior temporal cortex (MSTd), and parietoinsular vestibular cortex (PIVC) in 6 adult male rhesus macaques during a heading discrimination task. We applied a targeted dimensional reduction analysis by projecting the activity of each neuronal population onto a low-dimensional subspace, defined by dynamic motion parameters. We found VIP has a strong choice component, acceleration is not so clear in visual condition, but present in vestibular condition. MSTd has strong velocity component in visual condition, but also has the other two components in vestibular condition. PIVC has weak visual response, but a strong acceleration component for vestibular condition. Our results show that dynamic motion variables can be decoded from the different cortical populations, in accordance with the prevalence of the signals in each area.

Disclosures: F. Zeng: None. **A. Zaidel:** None. **A. Chen:** None.

Poster

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Topic: D.06. Vision

Support: National Institute of Neurological Disorders and Stroke (NIH Grant R21NS125372)

Title: Effects of focused-ultrasound disruption of the blood-brain barrier on parenchymal inflammation, behavior, and functional connectivity in the marmoset monkey

Authors: *I. ZIMMERMANN ROLLIN[1], T. PARKS[2,3], D. SZUZUPAK[2], S. CHOI[2], D. J. SCHAEFFER[2];
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Abstract: The common marmoset monkey (*Callithrix jacchus*) is a species of rising prominence in the neurosciences due to their small size, ease of handling, fast breeding, and their shared functional and structural brain characteristics with Old World primates. With increasing attention on modeling human brain diseases in marmosets, understanding how to deliver therapeutic or neurotropic agents to the marmoset brain non-invasively is of great preclinical importance. In other species, including humans, transcranial focused ultrasound (tFUS) aided by intravenously injected microbubbles has proven to be a transient, reliable, and safe method for disrupting the blood-brain barrier (BBB), allowing for the focal passage of therapeutic agents that do not otherwise readily traverse the tight endothelial junctions of the BBB. The critical gap that we address here is to document parameters to disrupt the BBB reliably and safely in marmosets using tFUS. By integrating our marmoset brain atlases and the use of a marmoset-specific stereotactic targeting system, we conducted a series of systematic transcranial sonication experiments in nine marmosets. We demonstrate the effects of center frequency, acoustic pressure, burst period and duration, establish a minimum microbubble dose, estimate microbubble clearance time, and estimate the duration that the BBB remained open to passage. Successful BBB disruption was reported in vivo with MRI-based contrast agents, as well as Evans blue staining assessed ex vivo. Histology (Hematoxylin and Eosin staining) and immunohistochemistry (Iba1, TUNEL, CD68 and CD206) indicated that the BBB can be safely opened with the parameters derived from these experiments. Next, an in vivo longitudinal experiment was conducted in four adult marmosets to determine the effect of opening the BBB in the forelimb area of primary motor cortex (area 4ab) on both reaching behavior (as indexed by the Valley Task) and functional connectivity, as assessed by resting state fMRI (rsfMRI) acquired at 9.4 T MRI. Both the behavior assessments and rsfMRI were conducted longitudinally, before and after the BBB was perturbed. Performance on the Valley Task was recorded and scored for accuracy and the rsfMRI data was preprocessed via the Marmoset Brain Connectome seed-based functional connectivity pipeline. Increased variance in both contralateral arm reaching behavior and functional connectivity were observed following sonication. Together, we give a comprehensive account of the parameters necessary to open the BBB and concomitant effects on inflammation, behavior, and functional connectivity.

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Poster

PSTR413. Visual Cognition: Decision-Making

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR413.15/DD2

Topic: D.06. Vision

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Bundesministerium für Bildung & Forschung (BMBF): 01GQ1907
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Title: Representation of a continuous decision variable in human extrastriate visual cortex

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Abstract: Subjects often infer the state of the environment by accumulating noisy sensory input over time. Neurophysiological studies of this process have focused on categorical decisions (e.g., upward vs. downward motion). Yet, many real-life decisions require inferring continuous environmental variables and translating them into continuous motor outputs. We aimed to identify the neural dynamics underlying visual evidence accumulation towards a continuous decision in the human cortex. We further studied the interplay between categorical and continuous formats of representation of a decision-relevant environmental variable. To this end, we employed a task combining a categorical judgment and continuous estimation, reported at different times of an evidence stream. 34 participants performed the task while we monitored their neural activity using Magnetoencephalography (MEG). On each trial, they viewed a sequence of 12 checkerboard patches ('samples') drawn from a Gaussian distribution with a fixed standard deviation (20°) and a mean that varied continuously from trial to trial (-14° to 14° relative to a reference line on the midline). After the sample sequence, they reported an estimate of the underlying mean by moving a cursor with a joystick. After half the sequence, they were either prompted to report a binary decision (mean left or right from reference line) or received a visual cue that indicated the true category with 75% validity. We tracked stimulus-selective neural processing via the lateralization (left vs. right hemisphere) of MEG signals. We analyzed the time courses of this lateralization for a range of frequencies and in 180 regions covering the cerebral cortex. We used encoding models and information theoretic measures to relate single-trial modulations of power lateralization to the mean sample positions and/or the behavioral estimation reports. In both conditions with intermittent choice and cue, the mean of the stimulus sample positions were encoded in the alpha (8-12 Hz) power lateralization in visual cortical areas, most prominently in the intraparietal sulcus. This encoding was sustained during a delay following the stimulus sequence and preceding the estimation report, but also present during the previous stimulus processing. Information-theoretic analysis indicated that subjects used this neural signal for generating their behavioral estimation reports. The strength of sample mean encoding predicted the individual estimation precision. We conclude that alpha-band activity in extrastriate visual cortex encodes the accumulated sensory evidence guiding a continuous visual decision.

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Poster

PSTR413. Visual Cognition: Decision-Making

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Title: Consistency with previous judgment biases the readout, but not the encoding of sensory evidence for subsequent decisions

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Abstract: Many perceptual decisions are formed by accumulating streams of sensory evidence across time. Recent psychophysical work has shown that human decisions are more strongly governed by evidence that is consistent compared to inconsistent with their previous categorical judgments, a form of confirmation bias. Here, we asked whether this biased processing results from a selective encoding or selective readout of consistent sensory evidence in the brain.34 healthy human participants performed a continuous visual decision-making task during magnetoencephalography (MEG) recordings. After viewing a sequence of checkerboard patches (samples; angular positions drawn from a Gaussian), they were prompted to report the underlying mean by moving a cursor on the screen with a joystick. After half of the sequence, they were also asked to report a binary decision about the category of the mean up to this point (left vs. right from a reference line). We reconstructed the fine-grained spatial patterns of MEG responses to stimulus sample for each of a large number of areas covering the complete cortical surface. For each area, we used mutual information to quantify the encoding of individual stimulus samples and subjects' estimations of the underlying mean in the local response patterns. Importantly, we also quantified the intersection information between sample, neural response, and estimation, as the statistical signature of sensory information representation which is also used for decisions. Participants' estimations of the mean were more strongly influenced by later samples that were consistent, compared to inconsistent, with their previous categorical choice. By contrast, there was no difference in the encoding of samples (mutual information) between consistent and inconsistent samples in visual cortex or any other cortical area. Yet, intersection information in dorsal and early visual cortex was larger for samples consistent than inconsistent with the categorical choice, and the individual strength of this neural readout effect correlated with the effect of consistency on behavioral evidence weighting.We conclude that the consistency of sensory evidence samples with previous categorical judgments selectively modulates their readout (not encoding) for the behavioral decision. In our task, these

computations entail the routing of the sample information to circuits involved in evidence accumulation for the estimation report and the updating of the decision variable resulting from this accumulation.

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Poster

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Title: Shaping perceptual decision formation by GABA-A and NMDA receptor manipulation

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Abstract: Theoretical work has implicated two neurotransmitter receptors in the accumulation of evidence for decision formation: N-methyl-aspartate glutamate (NMDA) receptors for the slow, recurrent excitation and gamma-amino-butyric acid (GABA)-A receptors for rapid inhibition. Here, we quantified the impact of GABA-A and NMDA receptors on perceptual decision-making under uncertainty.
We combined placebo-controlled, systemic pharmacological interventions with quantitative behavioral analysis in healthy human participants. 20 participants performed a visual decision-making task under NMDA receptor blockade (memantine, 15 mg), GABA-A receptor boost (lorazepam, 1 mg), or a glucose placebo (all oral administration, double-blind and cross-over design). Participants viewed circular drifting gratings in the left and right hemifield, each with independently fluctuating contrasts (10 contrast samples of 100 ms) and were subsequently prompted to report the side with the larger average contrast across the sample sequence, followed by auditory feedback. The sequence of stimulus categories (left or right stronger) across trials changed between repetition probabilities of 0.5 or 0.8 (blocks of 120 trials). This design promotes accumulation of information within trials (across contrast samples) and across trials. We quantified the impact of contrast fluctuations at each sample position on choice. The

resulting psychophysical kernels exhibited primacy, stronger weighting of samples occurring early compared to late in the sequence. This decay of evidence weighting was amplified under lorazepam. Trial history biased participants' choices dependent on the context, with significant repetition of previous-trial stimulus categories in repetitive but not random blocks. This adaptive history bias was reduced under memantine.
We conclude that GABA-A and NMDA receptors predominantly shape the formation of decisions at distinct timescales: GABA-A receptors affect the within-trial weighting of sensory evidence, while NMDA-receptors affect the across-trial accumulation of information into history-dependent biases. Reduction of the latter under NMDA-blockade may be due to interference with synaptic short-term plasticity. We currently use simultaneously collected magnetoencephalography (MEG) data to analyse the encoding of sensory samples in visual cortex and the build-up of a decision variable in premotor cortex. Drug-induced changes in these cortical decision dynamics will help constrain mechanistic interpretations of our behavioral findings.

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Poster

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Title: Past shifts present in a repulsive-followed-by-attractive two-stage process in human brains

Authors: *M. LUO, H. ZHANG, H. LUO;
Peking Univ., Beijing, China

Abstract: Past shifts present in a repulsive-followed-by-attractive two-stage process in human brains Authors Minghao Luo, Huihui Zhang, Huan Luo School of Psychological and Cognitive Sciences, IDG/McGovern Institute for Brain Science Peking University, China **Disclosures** M.L: None. H.Z: None. H.L: None. **Abstract** Serial bias refers to the automatic shifting of perception in current trial by preceding trials. The effect occurs on a wide range of features and in different directions, i.e., repulsive or attractive. Meanwhile, the underlying neural mechanism and how attention modulates the process remain unknown. Here we employed electroencephalography (EEG) and magnetoencephalography (MEG) recordings in a 2-D continuous spatial perception task to address the question. Participants were instructed to

memorize the location of a red dot stimulus within a 2-D continuous space (encoding period) and reproduce it later (decision period). In accordance with previous behavioral findings, location perception is attracted to that in the preceding trial. Importantly, time-resolved multivariate decoding reveals the co-occurrence of past and current trial information during both encoding and decision periods. Crucially, by examining the representational angle between the neural representation of past-trial reactivation and present-trial information, we demonstrate a two-stage past-present shifting neural profile - flipped and aligned relationships for early encoding and late decision periods, respectively. Moreover, only the late attractive neural shift is modulated by attention and correlates with serial bias behavior. Overall, our study provides novel neural evidence supporting that serial bias involves a repulsive-followed-by-attractive two-stage process, wherein past information first inhibits the present in a task-irrelevant way and is then integrated with the present based on attentional modulation.

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Poster

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Title: Neural basis of visual information integration and decision-making

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Abstract: Decision-making is a long-studied topic in neuroscience. We have an increasingly good mechanistic understanding of the neural circuits that allow animals to temporarily integrate specific decision variables. However, it remains unclear how these circuits combine, often conflicting, information from multiple sensory channels to form a single decision. Recently, we have described how the larval zebrafish anterior hindbrain integrates visual motion to decide about swimming direction. Other studies, focusing on different sensory stimuli, have identified the same brain area as a central processing structure for sensory-motor control. This raises the hypothesis that the anterior hindbrain forms a general integration hub for decision-making. Here, we employ a combination of behavioral experiments, computer simulations, and two-photon functional imaging to algorithmically and mechanistically describe how larval zebrafish integrate motion and luminance cues. Our behavior experiments and computational simulations argue for

a parallel arrangement, in which separate modules temporally integrate information from distinct visual processing streams. Our imaging experiments support these findings, revealing distinct and partially overlapping activation patterns with slow temporal dynamics. These results allow us to build detailed neural networks whose predictions we test using newly established circuit dissection tools. Together, this means we can describe in mechanistic detail how brains combine and evaluate information extracted from multiple visual features.

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Poster

PSTR413. Visual Cognition: Decision-Making

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Program #/Poster #: PSTR413.20/DD7

Topic: D.06. Vision

Title: Choice probability as movement planning

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Abstract: Perceptual decisions involve the detection of sensory information, the accumulation of evidence, and the execution of motor responses. Although different brain regions specialize in individual functions, signals related to multiple aspects of decision-making are often found in the responses of individual neurons. For example, sensory neurons often exhibit fluctuations in firing rates that predict perceptual decisions. These fluctuations can be characterized with a metric called choice Probability (CP). CP might reflect a feedforward propagation of random variability in responses to sensory stimuli to higher-order decision-making structures. Or it might capture a top-down signal that relays the outcomes of perceptual decisions back to sensory neurons. Here we suggest a third possibility, based on the fact that most CP studies make use of a saccadic eye movement as the behavioral readout. This creates a possible link between CP and the phenomenon of movement selection that has often been reported in the oculomotor system. Specifically, firing rates are often higher in response to receptive field (RF) stimuli when the animal intends to make a saccade toward them and lower when saccades are planned in the opposite saccade direction. As a result, fluctuations in single-neuron activity can depend on oculomotor plans, and indeed, movement selection effects have frequently been reported in visual cortical areas. This led us to wonder whether previous observations of choice probability in the visual cortex could to some degree reflect the effects of movement selection for saccades. To investigate this, we trained monkeys on a standard visual motion discrimination task and recorded neural activity from the middle temporal (MT) area, a region with neurons encoding motion direction and exhibiting CP. We found that CP values were higher for neurons whose receptive fields were closer to the direction of an impending saccade. This suggests that fluctuations in MT firing carried information about oculomotor selection, and we found that this

encoding emerged through training. Furthermore, pharmacological inactivation of MT neurons led to a behavioral bias to make saccades away from the inactivated RFs, similar to the effects of inactivation in the oculomotor system. These results suggest that training on a task with fixed sensorimotor contingencies introduces movement-related activity in brain regions that are usually considered sensory, and that this plasticity can shape CP in individual neurons.

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Poster

PSTR413. Visual Cognition: Decision-Making

Location: WCC Halls A-C

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Program #/Poster #: PSTR413.21/DD8

Topic: D.06. Vision

Title: Metacognitive Introspection Influences The Dynamics of Perceptual Evidence Accumulation

Authors: *W. DOU[1], S. AFRAKHTEH[2], D. ALONZO[2], K. CALLWOOD[2], J. SAMAHA[3]; [1]Univ. of California Santa Cruz, Santa Cruz, CA; [3]Dept. of Psychology, [2]Univ. of California, Santa Cruz, Santa Cruz, CA

Abstract: Metacognitive introspection refers to the ability to introspect upon the effectiveness of one's performance and is often studied as confidence in the outcome of a choice. Current models propose that confidence can be computed in an evidence accumulation framework where confidence is governed by the amount of pre- or post-decision evidence accumulated over time. However, these models make the untested assumption that sensory evidence is first accumulated, and only then evaluated for confidence. Here, we tested whether the act of introspecting about confidence alters sensory evidence accumulation as indexed by the Central Parietal Positivity (CPP) EEG component, a neural signature of the decision variable. Thirty-two participants perform a dot-motion discrimination task while electrical brain activity (via EEG) is recorded. On half of the trials, participants only reported the decision about the direction of the motion. On the other half of the trials, after participants reported the decision, they rated their confidence in the decision. The presentation of the two types of trials was counterbalanced across participants and motion coherence varied across three levels, providing various strengths of sensory evidence. The results showed that the CPP built up faster on the confidence-rating trials than on the decision-only trials, suggesting that introspection boosted evidence accumulation. Moreover, confidence signals were evident in preparatory motor activity prior to the first decision being made, which indicates that confidence evidence does not wait until the first-order decision threshold has been reached. Our results challenge the traditional accounts of confidence computation assuming that evidence accumulation and confidence rating occur sequentially.

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Poster

PSTR414. Cortical Planning and Execution of Reaching and Grasp Movements

Location: WCC Halls A-C

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Topic: E.04. Voluntary Movements

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Title: Neural substrate for the engagement of the ventral visual stream in motor control in the macaque monkey

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Med. and Surgery, Univ. of Parma, Parma, Italy

Abstract: Previous studies from our lab (Borra et al Neurosci Biobehav Rev. 75:65-90, 2017) showed that two interconnected hand-related areas involved in the "lateral grasping network", the ventrolateral prefrontal (VLPF) area 12r and the intraparietal area AIP, are targets of projections from an intermediate sector of the inferotemporal (IT) area TEa/m, located in the lower bank of the superior temporal sulcus. Furthermore, this same IT sector is a source of projections also to two areas involved in the oculomotor network, the caudal VLPF area 45B and the intraparietal area LIP (Borra and Luppino Neurosci Biobehav Rev. 126:43-56, 2021). The present study aimed to obtain a more comprehensive view of the possible involvement of this IT sector, located at the highest hierarchical levels of the ventral visual stream, in different aspects of motor control. To this purpose, the neural tracer Wheat Germ Agglutinin was injected in this intermediate part of TEa/m in five macaque monkeys at different AP levels. The results showed extensive connections to all subdivisions of areas TEa and TEp, extending rostrally in the temporal pole and caudally in areas TEO and FST, especially after more caudal injections. In the medial temporal lobe, rich connections were observed with the rostral area 36 and parahippocampal areas TF and TH. Extratemporal connections of TEa/m were rather selective. In the parietal cortex, there were connections at a variable extent across cases, involving the caudal part of the hand-related area AIP and the rostral part of the oculomotor area LIP, and, in the parietal operculum, the hand field of area SII. In the frontal lobe, the labeling tended to involve, in the VLPF, at a variable extent across cases, the hand-related area 12r, the oculomotor area 45B, and a caudal sector straddling over caudal area 46v and dorsal area 45A, which are part of the frontal oculomotor domain, and in the orbitofrontal cortex, areas 12o and 12m. A weak connection was also observed with the hand-related ventral premotor area F5a or the adjacent Granular Frontal Opercular area. Finally, in all cases, there were connections with a sector of the dysgranular insula connected with several parietal and frontal hand-related areas.

The present data strongly suggest that the inferotemporal area TEa/m is an integral part of the large-scale functionally specialized networks for controlling hand actions and oculomotor behavior. Accordingly, the ventral visual stream would not be involved just in perception per se but could play a fundamental role in guiding skeletomotor and oculomotor behavior. Furthermore, motor signals broadcasted to ventral visual stream areas could be crucial for perceptual processes.

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Poster

PSTR414. Cortical Planning and Execution of Reaching and Grasp Movements

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Topic: E.04. Voluntary Movements

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Title: Input-shaped neural dynamics enhances robustness to microstimulation in motor cortex

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Abstract: Although accumulating evidence suggests that the motor cortex serves as a dynamical machine that generates motor commands by autonomous temporal evolution, it remains unclear how inputs contribute to neural dynamics for motor generation. Here, we trained two monkeys (*Macaca* mulatta, Gd and Lp) to perform delayed (200 or 900 ms) reaches toward visual targets, either static (center-out) or moving circularly (Li et al., 2018). Moving targets generally required longer preparation time and higher hand velocity. During the delay, moving targets evoked higher neuronal firing rates and less tangled neural trajectories. Demixed PCA and SVM decoding results revealed continuous target location coding and progressive sensorimotor transformation in moving-target condition. To further examine underlying neural dynamics, we delivered subthreshold intracortical microstimulation (ICMS) (100 ms @300 Hz) via single microelectrodes around the GO and simultaneously recorded neural activities using 64-ch S-probe in M1 or PMd. For static-target condition, ICMS prolonged reaction times (RTs, ΔRTs = +9.4 ms* for Gd; +13.6 ms*** for Lp), but had little effect on RTs for moving target (ΔRTs = -3.6 ms for Gd, n.s.; -6.5 ms for Lp, n.s.), especially for long delays. Accordingly, neural states evolved more slowly after ICMS for static versus moving-target conditions. The optimal

subspace hypothesis (Churchland and Shenoy, 2007) proposes that movement is delayed until errors in preparation are resolved. Hence, continuous target input for moving target may enhance motor cortical dynamic, enabling faster error resolution. To test this hypothesis, we adapted an E/I neural network model of optimal control (Kao et al., 2021) to simulate the tasks. Specifically, we made three empirical assumptions of interception: (1) a feedforward target location, (2) sequentially updating of optimal states organized in a circle, and (3) larger feedback input. In the model, moving target inputs drove richer preparatory activities throughout the delay period. Input was added to a random subset of neurons as simulated ICMS. The perturbation transiently increased potential motor error, resolving faster for moving- versus static-target condition, producing less error at movement onset. This suggests that target input shapes neural population geometry that facilitate efficient computations. Combined with continuous feedback input, neural dynamics in motor cortex becomes more resistant to unexpected perturbations.

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Poster

PSTR414. Cortical Planning and Execution of Reaching and Grasp Movements

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Program #/Poster #: PSTR414.03/DD11

Topic: E.04. Voluntary Movements

Title: Joint specification of action in neocortex and striatum

Authors: *J. PARK[1], P. POLIDORO[2], C. FORTUNATO[3], J. ARNOLD[2], B. MENSH[2], J. A. GALLEGO[3], J. DUDMAN[4];
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Abstract: The interaction between two major forebrain structures, the cortex and subcortical striatum, plays a critical role in flexible, goal-directed action. Traditionally, it has been proposed that striatum is critical for selecting what type of action is initiated while the primary motor cortex is involved in the online control of movement execution. However, recent data suggests that the striatum may also play a crucial role in specifying movement execution. These alternative hypotheses about striatal function have been challenging to reconcile because they make indistinguishable predictions when comparing very different actions. To overcome this challenge, we developed quantitative models that led to a somewhat paradoxical insight: only by comparing neural activity during similar actions could we make strongly distinguishing predictions. To test this idea, we designed a novel reach-to-pull task that required mice to reliably select between two similar but distinct reach targets and pull forces. By recording neural activity simultaneously in both the cortex and subcortical striatum during the task, we found that the activity was uniquely consistent with a model in which both the cortex and striatum jointly specify movement execution. Overall, our findings provide new insights into the complex

interplay between the cortex and subcortical striatum in guiding goal-directed actions, and demonstrate the importance of using quantitative models and carefully designed experimental tasks to uncover novel insights into brain function.

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Poster

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Title: Similar encoding but unique timing of movement-specific signals across mouse forelimb sensorimotor cortex

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Abstract: Flexible and adaptive forelimb movements require close coordination between motor commands and sensory feedback. The primary motor (M1) and somatosensory (S1) cortices are richly interconnected, yet how they interact to coordinate these signals is not well understood. To characterize M1-S1 interactions, we developed a forelimb reach-to-grasp task where head-fixed mice made sound-cued reaches to grasp water rewards from two distinct spout targets (based on Galinanes et al. 2018). During this behavior, we recorded layer 2/3 excitatory cells of contralateral forelimb M1 and S1 using 31 Hz two-photon calcium imaging in GCaMP6f transgenic mice. We identified M1 neurons sending projections to S1 using AAVretro-tdTomato injections in ipsilateral forelimb S1. We recorded simultaneous activity of unlabeled M1 cells (M1-unl, 430-782 cells/dataset) and M1-S1 projection cells (125-135) in two mice, with forelimb S1 cells recorded in separate sessions (572-990). To characterize target-specific information, we decoded the cued spout target from single-trial population activity with a cross-validated linear classifier. Spout target was decoded with high accuracy from all three populations following the sound cue (92-97% M1-unl, 90% M1-S1, 85-90% S1). However, performance peaked first in

M1-unl cells, followed by M1-S1 cells, with S1 cells peaking last (43-75 ms, 63-94 ms, 118-119 ms). Further, we found that population activity in the classifier dimension was highly consistent across trials for M1-unl cells, whereas M1-S1 and S1 varied substantially. To quantify forelimb kinematics, we extracted 25 angular degrees of freedom across the forelimb from 3D markerless tracking data (DeepLabCut). We then decoded these joint angles from population activity using cross-validated ridge regression. Proximal and distal joint angles were best decoded from M1-unl cells (45-65% variance explained), with S1 decoding similarly well (43-52%). However, joint angle information was decoded less well from M1-S1 projection cells than the other two populations (25-42%). These results show that detailed movement signals are present across M1 and S1, with target-specific signals arising earliest in M1, yet suggest that the direct layer 2/3 pathway from M1 to S1 is not the sole source of this shared activity.

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Poster

PSTR414. Cortical Planning and Execution of Reaching and Grasp Movements

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR414.05/Web Only

Topic: E.04. Voluntary Movements

Support: NIH New Innovator Award: 1DP2NS111817-01

Title: Lfp independent components as markers for internal neural processes

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Abstract: To understand how signals generated by a single or ensemble of neurons relate to behavior it is necessary to link externally measured real-world events (external markers) to neural activity patterns, operationally defined here as trial alignment. An external marker is usually a time point that represents a notable event, such as the time of a stimulus or a behavioral response. Even when the timing of external events can be precisely recorded, the local timing of the related neural activity may vary depending on internal states (e.g. motivation, attention, reaction time) which can not readily be observed. These factors can lead to misalignment of neural responses, which can hinder the analysis of the informational content of spiking patterns (especially information reflected by precise spike timing patterns). Thus, it would be ideal to align neural data to internal signals representing internal cognitive and perceptual events. The present work evaluates a novel technique to extract signals from local field potentials (LFPs) which may reflect more precisely local cortical processing. We analyzed neural activity recorded using chronically implanted microelectrode arrays in primary motor cortex (MI), ventral

premotor cortex (PMv), and dorsal premotor cortex (PMd) of macaque monkeys performing a cued grasping task with instructed delay. Considering that local field potentials (LFPs) represent composite signals that receive contributions from multiple neural sources (synaptic currents and spikes), we applied a blind source separation approach to isolate their constituent signals. We found independent components (ICs) in the low frequency band of the LFP (0.1-3Hz) associated with every phase of the behavioral task. Initial results show that aligning neural activity using LFP ICs as internal markers produces more consistent spiking patterns leading to discrete classification of object and grip conditions matching or exceeding external task events. Classification was consistently improved (5-20% range) across all pre-movement events. Our results suggest that using internal markers to set temporal reference frames for spike train analysis could potentially improve decoding for BCI applications where external events such as the intention to initiate a movement are not associated with behavioral events that can be readily observed.

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Poster

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Topic: E.04. Voluntary Movements

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(MIS) 5048512

Title: Mirror neurons encode action kinematics

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Abstract: The relationship between the neuronal activity of mirror neurons (MirNs) and the kinematics of actions remains unexplored, despite reports of low-level movement features influencing MirNs' activity. This study aims to investigate whether MirNs encode observed action kinematics using multiple linear regression (MLR) to model kinematic parameters based on neural activity. MirNs were recorded from the ventral (n=120) and dorsal (n=140) premotor cortex of macaque brains during the execution and observation of grasping actions. We collected twenty (20) kinematic parameters, including 3D positions and velocities of the wrist, index finger tip, thumb tip, and the distance and its rate of change between the index and thumb. MLR was applied, using ten principal components, to estimate trial-averaged kinematic parameters from trial-averaged neural activity in each area. The results showed a strong correlation between neural activity and kinematics, with excellent fits for both areas and all kinematic parameters (R^2

values: 0.66-0.99 for PMd activity, 0.78-0.99 for PMv activity). To assess the relationship's strength and exclude overfitting, a decoding analysis was conducted. Predicted kinematics for individual trials were generated by multiplying single pseudo-population trials with previously determined regression coefficients. An SVM classifier was then trained on actual kinematic data and tested on predicted kinematic data, evaluating MLR's robustness with intertrial variability in neural and kinematic data. The decoding analysis revealed that grip information decoded from the twenty measured kinematic parameters reached 0.8 (maximum 1). When tested on predicted kinematics from both areas, the decoder showed significant performance during the epoch from the middle of the movement to the middle of object holding. Decoding performance was slightly higher for kinematics predicted by PMv activity compared to PMd activity (0.5 and 0.4, respectively; maximum 1). In both cases, the information decoded from predicted kinematics accounted for over half of the information obtained from measured kinematics. In conclusion, these findings demonstrate accurate prediction of kinematic parameters from neural activity using a simple linear model and indicate the presence of grip-related information within predicted kinematics, similar to measured kinematics. Thus, these results strongly support the population-level encoding of kinematics by MirNs.

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Poster

PSTR414. Cortical Planning and Execution of Reaching and Grasp Movements

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Title: Visual activity modulates population dynamics during the initiation of identical grasp movements.

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Abstract: An important objective in systems neuroscience is to comprehend how different movements are initiated and controlled in the neural network of the brain. In recent years, it was proposed that the neural population response at the onset of movement determines the subsequent movement-related neural dynamics independent of time and context, known as the optimal subspace hypothesis. In contrast, two studies have shown that the neural response at movement onset of the same movement can vary considerably between immediate and delayed movements. Furthermore, there is increasing evidence that neural activity in motor areas is not only modulated by movement preparation and execution, but also by active vision. We hypothesize that the differences in the neural response at movement onset for the same

movement can be explained by the presence of different amounts of visual processing. To investigate the visual, preparatory and movement related dynamics in the fronto-parietal grasping network, we recorded neural data from AIP, F5 and M1 of two monkeys performing a mixed immediate and withheld grasping task. By utilizing a targeted dimensionality reduction, we identified different orthogonal population subspaces for visual, movement preparatory and movement execution related activity. Neural subspaces were identified only based on the delayed trials, for which visual, preparatory and execution related activity is temporally separated by task design. Yet, the identified subspaces captured equal amounts of variance also for immediate movements, confirming the validity of the subspace decomposition. For delayed trials, visual-related processing of the cue signal was already completed at the time of movement onset, whereas for immediate trials, visual activity was also present at the time of movement onset. Interestingly, preparatory subspace occupancy at movement onset was similar for immediate and withheld movements, irrespective of the amount of visual activity. These results suggest that only the preparatory response at the time of movement onset determines the subsequent movement, but not the entire population response. Consequently, these findings indicate that visual activity can only be transformed into preparatory activity, and preparatory activity only into execution activity, even if visual activity is still present. Our results therefore refine the optimal subspace hypothesis for the presence of different population responses at movement onset.

Disclosures: **N. Zdun:** None. **H. Scherberger:** None. **B. Dann:** None.

Poster

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Topic: E.04. Voluntary Movements

Support: SFB 1528

Title: Using Neuropixels to compare neuronal population activity across the macaque fronto-parietal grasping network

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Abstract: In everyday life, primates perform a wide variety of grasping movements. The kinematics of grasping in primates depend on object characteristics such as fragility, size of contact surfaces, texture, and weight. As a result, specific grip types are used for each object and intended action. The frontoparietal grasp network, in particular the anterior intraparietal area (AIP) and the ventral premotor cortex (F5) is involved in the planning and execution of grasping

movements. Many studies have investigated various aspects of the visuo-motor transformation within and between these areas. However, our understanding of how population dynamics are distributed within these areas is limited due to methodological constraints. The recently developed Neuropixels probes allow to simultaneous record from 384 channels, and therefore from a large neuronal population, in a single electrode penetration. To explore intra-areal differences in neural population activity, we trained a female Macaca mulatta to perform a visually instructed delayed grasping task. Using Neuropixels probes, we simultaneously recorded from 384 channels per day at various locations in AIP and F5, and then investigated differences in the population dynamics at various locations within and across AIP and F5. Analysis of the peristimulus time histograms (PSTHs) suggest that there exist two distinct groups of neurons at the anterior pole of AIP. Furthermore, a comparison of responses under condition-dependent modulation across sites using the Euclidean distance measure or the population variance in the neuronal space indicated the presence of a gradient across the sulcal extension of AIP. More importantly, we conducted a decoding analysis based on single-trial activity. Since it is unlikely to find an exact correspondence between neurons across different recording sessions, we employed Procrustes analysis to align a 10-dimensional subspace of latent variables between individual recording sessions. Our aim was to achieve the best possible match between the sessions. Decoding analysis with separated decoder training and testing across recording locations showed similar decoding performances in AIP and F5, i.e., showing no apparent gradient, neither within nor across the two cortical areas. Future analysis will further investigate the population dynamics of the fronto-parietal grasping network.

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Poster

PSTR414. Cortical Planning and Execution of Reaching and Grasp Movements

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Title: Synchronous and isolated beta burst dynamics following recovery from stroke in primates

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Abstract: Dexterous movements rely on coordinated cortical and subcortical networks, but how these networks support recovery of movement after stroke is poorly understood. Many studies

have focused on the role of perilesional motor cortex (pMoCtx) in vicariation, but relatively little is known about how pMoCtx regains coordination with perilesional subcortical networks (pSubCtx). This process is likely critical as recent work across hundreds of patients demonstrates that atrophy to subcortical gray matter structures is strongly associated with poor sensorimotor outcomes (Liew et al., 2021). Further, work in rodents has also indicated that increased cortical-subcortical coupling is predictive of recovery (Guo et. al. 2021). Uncovering how cortical-subcortical interactions re-emerge following recovery from stroke may lay the foundation for novel approaches to multi-area neuromodulation to improve movement control after stroke.
To investigate how cortical-subcortical interactions are re-established in pMoCtx and pSubCtx to support hand control following stroke, we trained rhesus macaques to perform a reach-to-grasp 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 ventral premotor cortex (pMoCtx) and a chronic linear microelectrode probe was implanted subcortically targeting motor thalamus (pSubCtx). Here, we report that pMoCtx beta bursts can be divided into two types: 1) synchronous, high amplitude bursts involving most pMoCtx channels and 2) isolated, lower amplitude bursts involving only a few pMoCtx channels. Synchronous cortical bursts are frequently coordinated with pSubCtx beta bursts whereas isolated bursts are not.
We find that immediately following the lesion, synchronous bursts are prevalent during reach-to-grasp behavior. As dexterity recovers, these synchronous bursts become rare during behavior, but common during the post-movement period. In contrast, isolated bursts increase in prevalence both during behavior and during the post-movement period.
Overall, these results may reflect subcortical-cortical interactions that are pathologically synchronized early after stroke thereby over-entraining cortical spiking activity and disrupting movement. With recovery, pMoCtx and pSubCtx can flexibly couple or uncouple depending on behavioral phase. Further, these observations support a role for low amplitude isolated beta bursts rather than high amplitude synchronous beta oscillations in healthy movement control.

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Poster

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Support: German Research Foundation (DFG) CRC-889 "Cellular mechanims of sensory processing"
RU-1874 "The Physiology of Distributed Computing Underlaying Higher Brain Functions in Non-Human Primates"

Title: Neural representation of visual feedback and target uncertainty in the fronto-parietal reach network

Authors: *L. K. AMANN[1,2], V. CASASNOVAS[1,2], E. FERREA[1,3], A. GAIL[1,2,4,5];
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Abstract: Neural systems are constantly confronted with sensory uncertainty. During reaching sensory uncertainty can affect the position of one's own effector (feedback uncertainty) and the location of the desired movement endpoint (target uncertainty). Previous findings showed that during movement planning increased visual target uncertainty leads to a broadening of reach goal representations in the dorsal premotor cortex (PMd) of macaques. Here we test whether this finding generalizes to other brain areas within the frontoparietal reach network and if similar mechanisms can be observed between target and feedback uncertainty.
Two male rhesus monkeys performed a memory-guided center-out reach task while keeping ocular fixation. The animals moved a cursor by operating a haptic manipulandum. We introduced feedback uncertainty by replacing the cursor with a cloud of five randomly moving dots, the position of which was drawn from a 2D Gaussian distribution. The width of the distribution experimentally determined the level of feedback uncertainty; its center was controlled by the animal's hand movement. To vary target uncertainty, we either revealed the actual target location or presented an array of bars drawn from a random distribution centered on the actual target location. We recorded neural activity using implanted microelectrode arrays from the primary motor cortex (M1), PMd, and the parietal reach region (PRR).
We found that high target uncertainty led to decreased hit rates and increased initial reach errors. Conversely, high feedback uncertainty resulted in increased reaction times without affecting the initial reach error. In PMd, neurons with preferred directions opposite to actual reaching direction showed higher firing rates during movement when feedback uncertainty was high compared to when it was low. Neither uncertainty type nor level modulated neural activity in M1 during planning or movement periods. SVM decoding of reach direction showed lower accuracy for both types of uncertainty during late planning in PMd. Additionally, in PRR, we noticed a trend of reduced decoding performance during movement when feedback was uncertain.
Our findings suggest that PMd relies on sensory information about both hand and target location to form an appropriate movement plan and uncertainty of either type leads to impairment. In contrast, parietal areas seem to be mostly affected once the movement has already been initiated and sensory feedback about the hand position needs to be integrated to update the current motor command.

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Poster

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Title: Sensory predictions are embedded in cortical motor activity

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Abstract: When moving through the world, such as biking down a bumpy trail, we encounter external forces that cannot be predicted by our own motor output. Since delayed sensory feedback is too slow to allow accurate state estimation, predicting expected sensory input could make rapid feedback control more stable. However, we don't know how flexibly the nervous system can use prior information to make sensory predictions, nor how these predictions are implemented in the brain. To answer this, humans and macaques performed a reaching task where visual cues indicated the probability of the direction of upcoming elbow perturbations. Humans and monkeys integrated these priors into sensory predictions on single trials, biasing their responses to perturbations. High-density neural recordings in monkeys revealed a widespread signature of sensory predictions in prefrontal, premotor, and motor cortex, but not in somatosensory cortex. Artificial neural networks trained to perform generalized reaching tasks by controlling a biomechanical model of the arm with realistic sensory delays and noise emergently generated sensory predictions, showing remarkably similar muscle and neural activity to humans and monkeys, while other models with less realistic control or impoverished training did not match empirical results. Together, these results reveal a crucial role of sensory predictions in movement control.

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Poster

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Title: Preparatory cortical activity predicts moving target location for upcoming manual interception

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Abstract: Dynamical systems perspective posits that preparatory activity is promoted toward a movement-specific optimal subspace to set initial states seeding the motor generation. However, it remains to be determined how the initial states for generating movements toward moving target are formed in accordance with target motion. To probe how motor cortex prepares for manual interception, we trained one monkey (*Macaca mulatta*) to perform a delayed reach task to a target static or moving circularly (Li et al., 2018). We recorded single-unit activity from the primary motor cortex (M1, ~80 units), dorsal premotor cortex (PMd, ~80), primary somatosensory cortex (S1, ~50), and posterior parietal cortex (PPC, ~50) with four 8*8 Utah arrays. Each dataset consisted of a recording session encompassing ~260 single-unit and multi-unit recordings. To analyze preparatory population activity, we identified the two-dimensional 'preparatory subspace' via principal component analysis (PCA), based on M1 and PMd neurons' activity in both static- and moving-target conditions. In the static-target condition, subspace activity depended on reach direction and seemed stable over the delay period, consistent with Elsayed et al. 2016. However, in moving-target conditions, preparatory activity appeared to change gradually from target onset to the GO cue, suggesting tuning was time-locked to target motion, rather than final motor goal. Furthermore, the neural state angle between the first two PCs (35.1% explained variance) of the preparatory subspace predicted future target locations (lead time ~ 460 ms) in each moving-target condition. Our results show that motor cortex predicts moving target trajectories to initiate interception. Preparatory activity in motor cortex may play an important role in coordinating actions that demand dynamic spatiotemporal transformation.

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Poster

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Topic: E.04. Voluntary Movements

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Title: Local network connectivity underlying goal-directed neural activity in the motor cortex

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Abstract: Brain functions are an emergent property of network connectivity. Previous studies linking local network connectivity to neural coding in the mammalian brain focused mainly on the sensory cortex, without reference to behavior. Little is known about the relationship between connectivity and behavior-related activity within higher-order cortical areas. Here we focused on the motor cortex, using naturalistic behavior in which mice gathered rewards with multidirectional tongue reaching. This behavior, which does not require training, allowed us to study the motor cortex before its activity is shaped by learning a specific task. Motor cortex neurons exhibited conjunctive tuning to reward position and reward outcome. We used an all-optical method for *rapid* large-scale mapping of causal functional connectivity *in vivo*. Mapping connectivity between ~25,000,000 excitatory neuronal pairs revealed fine-scale columnar architecture in layer 2/3 of the motor cortex. Specifically, nearby neurons displayed a 'Mexican hat' spatial profile of excitation and inhibition, with local like-to-like connectivity according to reward-position tuning. The local connectivity patterns were non-uniform, with a preponderance of weakly coupled neurons and sparse strongly coupled neurons that function as network hubs. Hub neurons had weak reward-position and reward-outcome tuning but strongly influenced neighboring neurons. This network of neurons, encoding position and outcome of movements to different motor goals, may be a general substrate for reinforcement learning of complex, goal-directed behaviors.

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Poster

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Title: Visual feedback uncertainty during BCI movements reflects in fronto-parietal neural population activity in rhesus macaques

Authors: *V. CASASNOVAS[1,2], L. K. AMANN[1,2], E. FERREA[1,3], A. GAIL[1,2,4,5];
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Abstract: Sensory feedback guides movement but can also be uncertain. For example, visual feedback about hand position might be less accurate when reaching in low light conditions. Here we asked whether and how visual feedback uncertainty affects frontal and parietal sensorimotor areas in the neurophysiology of movement planning and execution. We trained two rhesus macaques to perform memory-guided center-out reaches controlling either a cursor (low uncertainty) or a cloud (high uncertainty). The cloud consisted of five randomly moving and short-lived dots, whose initial position was repeatedly drawn from a 2D Gaussian distribution. The width of the distribution determined the level of positional uncertainty about the center, which was controlled by the animal. Monkeys performed the task through a brain-computer interface (BCI) to neutralize proprioceptive feedback and to require them to rely exclusively on visual feedback. We recorded from chronic microelectrode arrays in the fronto-parietal reach network, including primary motor (M1) and dorsal premotor (PMd) areas, used as input to the BCI decoder, and the parietal reach region (PRR). Behavioral results showed that movement control was impaired with the cloud compared to the cursor and this was mostly reflected in the late stage of reaches. Analyzing neural population dynamics, we found that activity patterns corresponding to high and low uncertainty conditions, respectively, laid in separate subspaces, demonstrating an influence of feedback uncertainty across the three cortical areas. Trial-to-trial variability of population activity patterns was increased for high uncertainty in M1 and PMd during the late planning period, and for all three areas during early movement. Furthermore, SVM decoding of target direction based on neural activity performed worse in high compared to low uncertainty trials around early movement. Our results suggest that feedback uncertainty increases state exploration during planning for frontal areas, whereas parietal areas reflect feedback uncertainty once movement starts and updates of state estimate are relevant for movement control. Reduced decoding performance in high uncertainty trials suggests at least partial representation of target relative to effector position in frontal and parietal areas. Overall, our results show that frontal and parietal sensorimotor areas take into account the uncertainty of visual feedback and provide novel evidence on how this affects the representation of movement-related parameters at the neural population level.

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Poster

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Title: Dynamic modulation of cortico-cortical interactions during visuomotor behavior

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Abstract: The cortical networks involved in the control of visuomotor behaviors include various regions ranging from the parietal to the frontal lobe. It has been hypothesized that feedback connections originating from motor areas exert top-down influences on the posterior parietal cortex during visuomotor behavior.[1] A detailed description of the dynamic reciprocal flow of information within the fronto-parieto-occipital network, however, is still lacking. The current study aims to investigate the functional relevance of motor information processed in visual-parietal areas and understand its role during behavior. To this end, two rhesus macaques were trained in a visuomotor sequential reaching task. At each trial, animals reached visual targets appearing sequentially by means of a robotic exoskeleton. Neural recordings were acquired by extracellular multi-electrode Utah arrays (Blackrock Neurotech) implanted in five cortical regions along the visuomotor pathway, including the dorsal premotor (PMd) and primary motor cortex (M1), the parietal areas 7A and dorsal prelunate (DP) and the visual areas V1 and V2. Eye and hand positions were recorded by EyeLink 1000 (sr-research) and Kinarm (Bkin Technology), respectively. To assess cortico-cortical interactions during this task, we focused on the high-gamma activity (HGA, 55-150 Hz) of the local field potentials, which reflects a mesoscopic measure of neural activation correlating with multi-unit spiking activity.[2] We observed transient area-specific modulations in the power of the HGA related to relevant task events, such as stimulus onset and movement onset. To segregate independent sources of neural activation, an Independent Component Analysis was applied on the HGA. In order to unravel the network's dynamic reciprocal interactions, we performed directed functional connectivity analysis based on Transfer Entropy between the independent sources. Preliminary results reveal the co-existence of significant feedforward visuo-parietal interactions, as well as feedback fronto-parietal interactions. They are both more prominent after the initiation of the hand movement. Ongoing analyses are exploring how these interactions are modulated in relation to the behavioral performance of the monkeys in the task.

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Disclosures: **G. Bardanikas:** None. **S. Jana:** None. **F. Barthelemy:** None. **A. Riehle:** None. **S. Grün:** None. **A. Brovelli:** None. **T. Brochier:** None.

Poster

PSTR414. Cortical Planning and Execution of Reaching and Grasp Movements

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR414.16/DD23

Topic: E.04. Voluntary Movements

Support: NEI Grant EY012135
Washington University Cognitive Computational and Systems Neuroscience Fellowship

Title: Temporal eye-hand coordination may be subserved by connections between PRR and LIP

Authors: *J. KANG[1], E. MOOSHAGIAN[1,2,3], L. H. SNYDER[1];
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Abstract: The lateral intraparietal area (LIP) in macaque posterior parietal cortex has been proposed to participate in motor preparation for saccades, spatial attention, salience mapping, categorization, and evidence integration. Forelimb movement planning also influences LIP and thus LIP has been proposed to be involved in eye-hand coordination. We revisited the coding of reaching movements in LIP and the possibility of a role in eye-hand coordination. LIP population activity is increased by movements of the contralateral arm but is not affected by movements of the ipsilateral arm. Since most LIP cells have contralateral receptive fields, this finding is consistent with coding reaches that do not cross the body midline (uncrossed). Most reaches in the home cage are uncrossed. However, by separately considering cells with ipsilateral response fields, we found that activity reflects only the identity of the arm that is reaching and not whether or not the reach crosses body midline. We previously showed that the parietal reach region (PRR) encodes plans for contralateral arm movements and sends information to LIP prior to a reach. This is consistent with LIP activity being modified by a reach with the contralateral arm. We tested for a causal role for this information transfer. It is difficult to block within-hemisphere PRR to LIP connections. However, callosal connections are easily blocked by injecting lidocaine into the portion of the corpus callosum that contains crossing fibers from PRR. Blocking these fibers degraded the temporal coordination between the eye and arm for crossed reaches compared to uncrossed reaches. In contrast, inactivating LIP with muscimol had the reverse effect, degrading coordination in uncrossed compared to crossed reaches. This is consistent with interactions between LIP and PRR subserving temporal eye-hand coordination, with signals related to the contralateral arm sent from PRR to LIP in either the same or opposite hemisphere, depending on whether the reach is into the contralateral or ipsilateral field (uncrossed or crossed). Interestingly, LIP inactivation not only degraded coordination for uncrossed reaches, but also improved coordination for crossed reaches. This could reflect a

competition between left and right LIP during crossed reaching. Inactivating the LIP that would subserve the more common uncrossed reach relieves this competition and results in an improvement in crossed-reach eye-hand coordination.

Disclosures: J. Kang: None. **E. Mooshagian:** None. **L.H. Snyder:** None.

Poster

PSTR414. Cortical Planning and Execution of Reaching and Grasp Movements

Location: WCC Halls A-C

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Program #/Poster #: PSTR414.17/DD24

Topic: E.04. Voluntary Movements

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Japan Science and Technology Agency
Strategic Research Program for Brain Sciences

Title: Primary motor cortex prospectively computes future spinal reflex

Authors: *T. UMEDA[1,2,3], O. YOKOYAMA[4], M. SUZUKI[4], M. KANESHIGE[5], T. ISA[1,2,6], Y. NISHIMURA[4,2,6,7];
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Abstract: The descending motor drive and somatosensory feedback via the spinal reflex converge on spinal motor neurons for limb movements. However, it remains unknown how descending motor drive coordinates with the spinal reflex to generate adequate muscle activity for the achievement of intended limb movements. Here, we show that the primary motor cortex (M1) prospectively computes future spinal reflex to provide appropriate motor commands to spinal motor neurons. We simultaneously recorded activities in motor cortices, afferent neurons, and forelimb muscles of monkeys performing reaching movements and analyzed the sequential flow of neural information. We found that the activity of motor cortices encodes subsequent afferent activity. Multivariate information decomposition elucidated that motor cortical activity influences muscles not only through the direct descending pathway but also through the "transafferent pathway" composed of the descending plus subsequent spinal reflex pathways. Selective disruption of the afferent pathway reduced the estimated transafferent component of muscle activity, providing causal evidence for delayed activation of limb muscles through the

transafferent pathway. Furthermore, among the motor-related areas, the M1 encodes the most information about muscle activity transmitted via the direct descending and transafferent pathways. Thus, the M1 leverages spinal reflex action to drive limb muscles efficiently in voluntary movements.

Disclosures: **T. Umeda:** None. **O. Yokoyama:** None. **M. Suzuki:** None. **M. Kaneshige:** None. **T. Isa:** None. **Y. Nishimura:** None.

Poster

PSTR414. Cortical Planning and Execution of Reaching and Grasp Movements

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR414.18/DD25

Topic: E.04. Voluntary Movements

Support: NIH Grant NS102259
NIH Grant MH121009

Title: The Influence of Uncertainty on Preparatory Activity in Motor Cortex During Reaching

Authors: *T. ARAKERI[1], J. M. DILL[1], R. C. MANJAREKAR[1], K. M. GOTHARD[2], A. J. FUGLEVAND[1];
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Abstract: In our daily lives we are constantly forming and correcting motor plans to flexibly interact with the external world. Often times the information available during the process of planning is incomplete, thereby causing uncertainty about the movement goal. It is unclear how the motor system prepares movements under different levels of uncertainty, and how this uncertainty affects the ability to re-prepare movements. Using a forced reaction-time paradigm, a rhesus macaque was trained to produce center out reaching movements to one of two diametrically opposing potential targets. In this task, a timing cue flashed three times in succession (500 ms between flashes). The third flash was the go-cue and the monkey had to launch the movement coincident with it (within 100 ms). The two potential targets were displayed at the time of the first flash and then disappeared. The actual target was then displayed at varying times prior to 'forced' movement initiation at the go-cue. We manipulated uncertainty about the target location using a coloring scheme that indicated the probability (p) of where the final target would appear. For example, when both potential targets were white, $p = 0.5$, and the final target could appear at either location with equal probability. When the time between actual target display and movement onset was briefer than typical visual processing time (~ 100 ms), the monkey had to 'guess' the location of the target. In these cases, and when guessed incorrectly, the monkey corrected the movement midstream. We found that the monkey was able to re-prepare movements faster with increasing levels of uncertainty. While the monkey performed this task, we recorded 202 neurons from the dorsal pre-motor and primary motor

cortex. During the preparatory period, we observed a graded reduction in the distance between neural states for the two potential movements with increasing levels of uncertainty. Such an outcome in neural state-space could theoretically explain the faster re-preparation times under higher levels of uncertainty. Regardless, differences in preparatory states still led to highly similar population dynamics during movement execution. Furthermore, we also found a dimension in neural state-space that separated the neural states based entirely on the level of uncertainty during both movement preparation and execution. Whether this dimension overtly represents uncertainty is an open question. Overall, these results provide insight on the structure of neural population activity in the motor cortex while preparing and executing movements under conditions of uncertainty.

Disclosures: T. Arakeri: None. **J.M. Dill:** None. **R.C. Manjarekar:** None. **K.M. Gothard:** None. **A.J. Fuglevand:** None.

Poster

PSTR414. Cortical Planning and Execution of Reaching and Grasp Movements

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR414.19/DD26

Topic: E.04. Voluntary Movements

Support: Pennsylvania HRFG

Title: Neural dynamics common to reaching movements executed by the native arm and using a brain-computer interface

Authors: *H. MAO, B. A. HASSE, A. B. SCHWARTZ;
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Abstract: Neural activity in the primary motor cortex has long been associated with controlling muscle activation and movement generation. The successful operation of many brain-computer interfaces, on the other hand, relies on motor cortical activity in the absence of movement. How neural activities are similar, or different, between these two scenarios is still not well understood. To further explore neural correlates under these different control modes, we compared activity patterns in the primary motor cortex during arm- and BCI-controlled reaches. Two rhesus monkeys were trained to perform a standard center-out reaching task in a virtual-reality setup. The task requires moving a cursor in VR from the center to one of the 16 peripheral targets. All targets are located on a circle within a vertical plane facing the subjects. Movement of the cursor was controlled by either the hand's trajectory (HC condition) or via a brain-computer interface (BC condition). Subjects performed HC and BC trials in separate sessions. Neural activity in the primary motor cortex was recorded using two Utah arrays. Offline spike sorting identified 208 units (both single- and multi-unit) from monkey R and 291 from monkey N. This study analyzed 116 and 189 units from the two subjects, with firing rate modulation greater than 5 spikes/s across both time and task conditions. Trial-averaged firing rate profiles for the 16 movement

directions were calculated for each unit, in HC and BC sessions respectively. A linear dimensionality reduction method was then used to identify three neural subspaces: Qhc, Qbc and Qmix, where Qhc explains neural activity variance during HC but not BC trials, and vice versa for Qbc. Qmix accounts for variances of both HC and BC data. Each of the three subspaces is 7-dimensional and is orthogonal to the other two subspaces. Qhc and Qmix together explain 90.8% HC data variance for monkey R (89.5% for monkey N), and Qbc and Qmix capture 89.3% (87.0%) BC variance. Among these three neural subspaces, Qmix captured more data variance than the other two. For BC neural activity, Qmix explained 50.3% (44.2%) variance whereas Qbc 39.0% (42.8%). For HC data, Qmix explained 65.3% (62.8%) variance while Qhc 25.5% (26.2%). Although some firing rate variance was isolated to the subspace specific for HC or BC, these results show that the subspace assigned to both HC and BC data (Qmix) explains most of the motor cortical activity. Since only a small amount of variance was accounted for in the HC-only subspace (Qhc), and since the muscles were active only in the HC session, this result suggests that much of the neural activity in the motor cortex is independent of muscle activation.

Disclosures: H. Mao: None. **B.A. Hasse:** None. **A.B. Schwartz:** None.

Poster

PSTR414. Cortical Planning and Execution of Reaching and Grasp Movements

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR414.20/DD27

Topic: E.04. Voluntary Movements

Support: ASAP-020572
NIH Office of Research Infrastructure Programs P51-OD011132

Title: Comparative imaging study of the spontaneous and task-related activity of neurons in the primary and supplementary motor area in monkeys

Authors: *A.-C. MARTEL[1,2,3,4], D. PITTARD[2,3,4], A. DEVERGNAS[2,5,4], J. J. NASSI[6,4], J. DOWNER[6,4], W. YU[6,4], B. RISK[7,3,4], T. WICHMANN[2,3,5,4], A. GALVAN[2,3,5,4];
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Abstract: Calcium imaging through head-mounted miniature microscope has become a common technique to analyze the activity of neuronal ensembles in awake animals. However, the use of this technique in non-human primates remains limited. Here, we report the use of calcium imaging and miniature microscopes in rhesus macaques to study the activity of neurons in the primary motor cortex (M1) and the supplementary motor area (SMA), two regions essential for the planning and execution of movement. We used GCaMP6f to monitor calcium transients in

M1 and SMA neurons in three rhesus macaques. We co-injected AAV5-Thy1s-tTA and AAV5-TRE-GCaMP6f into the arm region of M1 and SMA, in the right and left hemispheres, respectively. A microendoscope gradient-index (GRIN) lens was chronically inserted in the same regions. Two to four weeks later, we performed imaging experiments with a miniature microscope (Inscopix), while animals were sitting quietly in a primate chair (spontaneous activity) then while performing an arm-reaching task to touch targets on a screen to receive a juice reward. The data was processed to reflect changes in fluorescence (Δf/f), and the calcium dynamics were quantified using spike deconvolution method (OASIS). We analyzed the relation of calcium transients to spontaneous and task-related movements in groups of M1 and SMA neurons and present preliminary results from 4 sessions (1-2 sessions per animal). Across sessions, we identified 17 ± 11 (mean ± SD) and 9 ± 6 cells in M1 and SMA. So far, Therecordings in SMA showed lower activity (0.08 ± 0.1 calcium spikes/s) and lower amplitude (3.7 ± 1.0 Δf/noise) than recordings in M1 (0.24 ± 0.3 calcium spikes/s; 5.3 ± 3.4 Δf/noise) in the spontaneous state. This calcium activity tends to decrease when the animal is involved in the reaching task compared to spontaneous state, with a decrease to 0.06 ± 0.09 calcium spikes/s in SMA and 0.2 ± 0.3 in M1 during the task. Cross-correlation analyses of the activity recorded in the spontaneous state are currently underway to identify clusters of cells with correlated activity in M1 and SMA, as are studies of movement-related activation patterns. Post-mortem histology in two animals indicated strong and extensive GCaMP expression at the site of injection, with the lens being located at the center of the GCaMP expression. Histology is still in progress for the other animals. This is the first report of calcium imaging of ensembles of M1 and SMA neurons in macaques. Our preliminary results suggest that M1 and SMA differ in the degree of spontaneous activities and support our planned use of this technique to study the activity of cortical regions in NHP models of neurodegenerative diseases.

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Poster

PSTR415. BCI and Neural Prosthetics: Technology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR415.01/DD28

Topic: E.05. Brain-Machine Interface

Support: NIH 1U01NS123668-01
1U01NS123668-02S1
DGE-1644868

Title: Design and optimization of a low-noise scalable multiplexed neural interface

Authors: *G. SHULL[1,2], T. JOCHUM[2], Y. HUANG[3], Y. SHIN[3], H. FANG[3], J. VIVENTI[2]; [2]Biomed. Engin., [1]Duke Univ., Durham, NC; [3]Dartmouth Col., Hanover, NH

Abstract: Neural interfaces are a critical part of brain computer interfaces (BCIs) and serve to bridge the gap between the brain and electronics. A tradeoff ubiquitous across neural interfaces is that of spatial coverage and spatial resolution as the number of recording channels is limited by connector sizes and backend electronics. An approach to overcome this limitation is to embed active electronics at the electrode site, which enables multiple electrodes to share the same output wire through time division multiplexing (TDM), thus increasing the number of recording channels without increasing the number of output wires. However, a drawback of TDM is increased noise due noise aliasing, and fabrication-dependent physical characteristics, which contribute to transistor noise. The goal of this work is to design a low-noise, front-end recording system small enough to fit at the electrode site using commercial complementary-metal-oxide-semiconductor (CMOS) fabrication combined with post-fabrication processing to create a flexible, low-noise, TDM neural interface. To achieve this goal, we designed a front-end recording system beneath the electrode (termed "pixel") that enables low-noise multiplexing by 1) adding filters, 2) providing modest gain, and 3) buffering the output signal while consuming low-enough power to be implanted in the brain (< 40 mW/cm^2, or < 1 μW in 50 μm x 50 μm). The electrodes are arranged in a single column with 32 channels spanning an intracortical depth of 1.6 mm, to span the cortical thickness of a rat, and a width of 70 μm to minimize tissue damage (termed "shank"). Using this shank we designed a proof-of-concept 12-shank, 384-channel intracortical multiplexed array and sent the design to a CMOS foundry for fabrication. Our design high pass filters the signal from the electrode followed by differential amplification, low pass filtering, and buffering before multiplexing. An innovation of this work is creating a tunable high pass filter which allowed the filter to also fit within the area constraints. The design achieves a 28 dB gain over a band of 0.1 Hz - 10 kHz with noise < 10 μV rms while consuming 0.7 μW/channel, which enables both local field potential (LFP) and action potential (AP) recording with 6× lower noise and 25× smaller area than previous active arrays. Future work will focus on quantifying *in vitro* and *in vivo* recording performance. This work can be applied broadly across neural interfaces to create high-channel-count arrays and ultimately improve BCIs.

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Poster

PSTR415. BCI and Neural Prosthetics: Technology

Location: WCC Halls A-C

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Program #/Poster #: PSTR415.02/EE1

Topic: E.05. Brain-Machine Interface

Support: NIH Grant UG3NS120172

Title: Detecting analog to digital conversion artifacts in electrophysiological recordings using Intan Technologies integrated circuits

Authors: *K. BARTH, C. SCHMITZ, T. JOCHUM, J. VIVENTI;
Dept. of Biomed. Engin., Duke Univ., Durham, NC

Abstract: Intan Technologies offers amplifier integrated circuits that are widely used by neurotechnology companies and research labs for acquiring neurophysiological data from many channels (up to 64 channels / chip) at high sampling rates (up to 30 kSPS). Intan Technologies RHD chips contain miniaturized front-end circuitry that perform signal amplification, filtering, and digitization, which enable compact recording systems. However, we found that the analog-to-digital converter (ADC) in the RHD2000 series amplifier chips can introduce digital artifacts in the recorded data. The digital artifacts manifest as: 1) jumps, where the ADC rapidly switches to an incorrect output code, and 2) flatlines, where the ADC maintains the same output code over time. We developed an approach for detecting these digital artifacts by recording a variety of in-vitro and in-vivo signals using Intan RHD amplifier chips. With the chips' digital-signal-processing mode disabled, we detected the occurrence of digital artifacts in a recording by examining the distribution of ADC output codes. We detected jumps at ADC output codes with successive zero occurrences and flatlines at ADC output codes with higher probability occurrences. Further, we developed an artifact-correction strategy of re-running the ADC calibration phase in the Intan RHX software based on these detections from ADC output codes. Our findings are of great relevance to many in the neuroscience community who use Intan Technologies amplifier chips for neurophysiological experiments. These results provide a methodology to detect these digital artifacts and trigger ADC recalibration to preserve neurophysiological signal integrity.

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Poster

PSTR415. BCI and Neural Prosthetics: Technology

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Program #/Poster #: PSTR415.03/EE2

Topic: E.05. Brain-Machine Interface

Support: 1U01NS123668

Title: Advantages of current sensing circuit architecture for highly multiplexed neural interfaces

Authors: *M. HILL[1], J. VIVENTI[2];
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Abstract: Interfacing with the brain at a large spatial scale with fine resolution is key to understanding and treating neurological disorders. For example, the performance of motor and speech prosthetic systems can be improved with high-density micro-electrocorticography (μECoG) recordings. As the number of electrodes increases, conventional electrode array wiring becomes unwieldy and prone to mechanical failure, as each electrode requires an individual external wire connection. Time division multiplexing using integrated flexible transistors allows

many electrodes to share a single output wire. However, as the multiplexing ratio increases, the signal to noise ratio (SNR) of the device decreases due to increases in aliased noise. Prior results show current sensing can achieve high SNRs across frequencies of interest (32 dB at 10 Hz; 42 dB at 100 Hz, and 59 dB at 1000 Hz), specifically showing large increases in SNR in the High Gamma band (70 - 150 Hz) compared to voltage sensing strategies (22 dB at 100 Hz). In this poster, we investigate the circuit architecture needed for high-speed time division multiplexing with current sensing. In addition, we compare in vitro performance of current sensing against traditional voltage sensing while varying the multiplexing ratio. This current sensing strategy (i) decreases aliased noise, (ii) reduces the footprint of each pixel, and (iii) removes the need for a voltage offset. These results demonstrate the enhanced scalability of current sensing strategies over voltage sensing when utilizing active time division multiplexing.

Disclosures: **M. Hill:** None. **J. Viventi:** None.

Poster

PSTR415. BCI and Neural Prosthetics: Technology

Location: WCC Halls A-C

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Program #/Poster #: PSTR415.04/EE3

Topic: E.05. Brain-Machine Interface

Support: NIH 1R01DC019498
NIH 1U01NS123668

Title: Open-source platform for wireless characterization of neural electrode impedance and reliability

Authors: *J. E. SMITH, I. RACHINSKIY, T. JOCHUM, J. VIVENTI;
Biomed. Engin., Duke Univ., Durham, NC

Abstract: Fully implanted neural prosthetic systems require wireless interfaces to reduce infection risk and enable long-term use. Conventional approaches to enable implanted electronics with wireless telemetry rely on hermetically-sealed packages that are large enough to perform helium leak testing. However, the ceramic feedthroughs that are typically used in these devices limit the number of electrodes that can be implemented. New approaches for encapsulating electronics are needed to enable scaling to thousands of channels. We propose a method of encapsulation wherein both an electrode array and the requisite electronics for signal acquisition and wireless communication are fully enveloped by liquid crystal polymer (LCP), which is well regarded for its biocompatibility, low water transmission, and reduced risk of delamination. While there are commercially available solutions to conduct long-term reliability studies of electrodes using wired connections, the connectors add failure modes to the test that are not representative of the failures electrodes would encounter when implanted. This work presents an open-source platform (electrical design, software) for wireless characterization of electrode impedance and, to quantify the reliability of the polymer encapsulation, the conductance of

interdigitated electrodes (IDEs) positioned within inner layers. Measurements are requested of an encapsulated microcontroller wirelessly using near field communication (NFC) and are logged automatically. Firmware can be flashed to the embedded controller over-the-air. Power is provided through an inductive link. Calibration, array multiplexing, user-defined waveforms, and service interruption notifications are supported. Key metrics of performance (impedance accuracy and precision at 1 kHz) are comparable to existing, wired solutions. This work is significant in that it: (a) inherently resolves former limitations of soak testing related to chamber and connector leakage, allowing for very long-term evaluation using a fixed volume of saline solution and (b) may serve as an open-source reference for the rapid development of related wireless characterization applications. In future work, we will use this platform to evaluate the stability of LCP encapsulation through accelerated aging to quantify the viability of chronic applications and predict expected lifetimes.

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Poster

PSTR415. BCI and Neural Prosthetics: Technology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR415.05/EE4

Topic: E.05. Brain-Machine Interface

Support: 23YB1210

Title: A fully implantable wireless system for continuous monitoring of neural activity in deep brain regions of a freely moving primate

Authors: ***J.-Y. KIM**[1], C. JE[1], J. YUN[1], Y. KANG[1], Y. LEE[2], K. LIM[3], C.-Y. JEON[2], J. WON[2], M. KIM[2], S. LEE[1];
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Abstract: Freezing of gait (FoG) is the most disabling gait disorder in Parkinson's disease (PD). Closed-loop deep brain stimulation (DBS) in the subthalamic nucleus (STN) is used for PD treatment. To provide appropriate stimulation parameters based on the clinical state of patients with PD, there is an increasing need for equipment capable of continuously monitoring neural activity in deep brain regions during unrestricted movement. However, there are few studies on long-term continuous monitoring of deep brain neural activity in freely behaving states. Therefore, we developed a fully implantable wireless system that can continuously record neural activity in the STN and amygdala regions, and we confirmed its long-term stability in a freely moving primate. The proposed fully implantable wireless system consists of a recording part that utilizes an Intan chip with an 8,192 sampling rate and implanted electrodes with 50 kohm impedance at 1 kHz for neural recording. It also includes a wireless data transmission part that

employs the Nordic Bluetooth Low Energy protocol with a data rate of 720 kbps at a range of more than 2.0 m away from the primate. Furthermore, a power management part with sleep on mode in process and a high capacity of 2,550 mAh primary battery ensure more than 120 days of operation. An adult (14-year-old) cynomolgus macaque (Macaca fascicularis) is prepared. The procedure was performed on the custom-built CT/MRI-compatible stereotaxic frame under general anesthesia. The deep brain recording electrodes were inserted vertically into the right STN and the right amygdala, and fixed to the skull using dental cement. We wirelessly recorded local field potentials (LFP) activity in the STN and amygdala regions of the primate during freely moving and fearful states. While he was walking, beta power in the STN were reduced compared to resting state. The amygdala beta and gamma power were increased when he felt scared compared to resting state. The consistent spectral changes were observed over a period of more than 4 weeks, confirming long-term stability of our fully implantable system. Our system will be particularly useful in continuous monitoring of deep brain signals in freely moving primates. As well, it will be used to study social behavior in freely moving monkeys and to investigate appropriate stimulation parameters in clinical studies.

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Poster

PSTR415. BCI and Neural Prosthetics: Technology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR415.06/EE5

Topic: E.05. Brain-Machine Interface

Support: VA MERIT 1 RX003129

Title: A Neural Interface system for an Osseointegrated Prosthetic Control

Authors: L. A. SEARS[1], A. DESHMUKH[2], N. ARMSTRONG[3], A. FERNANDO[3], D. LASEGA[3], V. GO[3], A. J. SUMINSKI[4], J. C. MORIZIO[3], S. O. POORE[1], *A. M. DINGLE[1]; [1]Surgery, [2]Biomed. Engin., Univ. of Wisconsin, Madison, Madison, WI; [3]Electrical and Computer Engin., Duke Univ., Durham, NC; [4]Dept. of Neurolog. Surgery, Univ. of Wisconsin-Madison, Madison, WI

Abstract: State-of-the-art neural prosthetic systems must be capable of high-fidelity stimulation and recording of nervous tissue, imposing stringent performance demands on the telemetry of high-bandwidth neural information. We present an ovine model for the Osseointegrated Neural Interface (ONI) for bi-directional prosthetic control, which appeases these demands via novel surgical methodology and a sophisticated neural interface system. Our surgical procedure saw one mature non-lactating female sheep undergo amputation and reaming of the thoracic limb at the distal metacarpus. Subsequently, the surgical team utilized a slap-hammer to secure an osseointegrated abutment. We followed osseointegration by coupling a stimulation electrode

with the distal end of the target nerve and transposing it into the freshly reamed medullary canal with recording electrodes attached proximally to the corticotomy. Finally, we implanted the transmitter and receiver capsule into the sheep's shoulder. Now intact, the ONI system could receive programmed electrical stimulation patterns and transmit continuous recordings of the stimulated major nerve and EMGs from nearby muscles. We generated neural recording data by remotely triggering preprogrammed stimulation patterns which were delivered to the coupled nerve. The transmission of said stimulation patterns along the nerve could then be validated by analyzing the recording output in NeuroExplorer for activity mirroring the stimulation parameters. The presence of stimulation artifacts in our recording data demonstrating the programmed frequency and pulse width of 60 Hz and 200 μs, respectively, verifies our neural interface system can remotely stimulate and continuously record from a major nerve. Our poster showcases the transmitter and receiver implantable capsule technology and surgical approach that forms the backbone of the ONI device. We will present the functional specifications, benchtop test results, and 3D printing and coating processing steps for the capsule, as well as the surgical specifications of electrode and capsule placement. In addition, we will cover electrophysiology data with various *in-vivo* stimulation patterns and post-processing data analysis used to validate the ONI.

Disclosures: **L.A. Sears:** None. **A. Deshmukh:** None. **N. Armstrong:** None. **A. Fernando:** None. **D. Lasega:** None. **V. Go:** None. **A.J. Suminski:** None. **J.C. Morizio:** None. **S.O. Poore:** None. **A.M. Dingle:** None.

Poster

PSTR415. BCI and Neural Prosthetics: Technology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR415.07/EE6

Topic: E.05. Brain-Machine Interface

Support: NIH U01 NS128612
NIH R01 EB026439
NIH U24 NS109103
NIH U01 NS108916
NIH P41 EB018783
McDonnell Center for Systems Neuroscience
Fondazione Neurone

Title: A general-purpose software platform for closed-loop neuromodulation

Authors: *W. ENGELHARDT[1,2,3], J. MELLINGER[3], A. BELSTEN[1,3], J. R. SWIFT[1,3], P. BRUNNER[1,2,3];
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Abstract: Implementing closed-loop neuromodulation therapies is a challenging and expensive endeavor. It requires developing software capable of acquiring signals from a bio-signal amplifier, analysis of these signals, and initiation of precisely timed stimulation, all of which need to be accomplished in real time with very low latency. Developing this software is difficult, as it requires a wide range of expertise ranging from interfacing with hardware to real-time signal processing. Even when successfully implementing such a system for one set of hardware, it often then only works within the laboratory that conceived it. This is because of the inherent heterogeneity in the devices that interact with the nervous system and the lack of standardized interfaces to access and control them. Collaboration thus often necessitates acquiring the same hardware (i.e., amplifier and stimulator) across all sites, which can sometimes be cost-prohibitive. Implementing software that uses these amplifiers and stimulators within a real-time acquisition and feedback software platform, such as BCI2000, would eradicate these obstacles. Multiple stimulators, amplifiers, and peripheral devices have been implemented in BCI2000 to provide a maximum amount of real-time feedback and to make the configuration effortless. The abstraction of BCI2000 experiments from the underlying hardware allows for seamless collaboration between institutions. To best configure the stimulation parameters, a stimulation configuration tool has been created to assist users in specifying their desired parameters, visualizing them, and exporting them directly into BCI2000. A cortico-cortical evoked response BCI2000 filter visualizes the time-locked stimulation response at each electrode in real time. With these improvements, BCI2000 successfully facilitates closed-loop neuromodulation experiments across multiple institutions and heterogeneous underlying hardware platforms.

Disclosures: W. Engelhardt: None. **J. Mellinger:** None. **A. Belsten:** None. **J.R. Swift:** None. **P. Brunner:** None.

Poster

PSTR415. BCI and Neural Prosthetics: Technology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR415.08/EE7

Topic: E.05. Brain-Machine Interface

Support: NINDS (UF1NS107659)
NSF (1707316)
NINDS (RF1NS128667)
NSF (2129817)
NIMH (1RF1MH120005-01)
NINDS (R01NS118606)

Title: Efficient, rapid, and minimally invasive implantation of individual non-functional motes with penetrating subcellular-diameter carbon fiber electrodes

Authors: *J. G. LETNER[1], M. BARROW[2], E. MOON[2], P. R. PATEL[1], J. M. RICHIE[1], J. L. LAM[3], J. LEE[2], A. KAMBOJ[5], B. MANI[5], Y. SUN[2], G. ATZENI[6], B. KOO[1], J. LIAO[6], D. CAI[4],

D. SYLVESTER[2], J. D. WEILAND[1], H.-S. KIM[2], T. JANG[6], J. PHILLIPS[5], D. BLAAUW[2], C. A. CHESTEK[1];
[1]Biomed. Engin., [2]Electrical Engin. & Computer Sci., [3]Dept. of Neurosurg., [4]Cell and Developmental Biol., Univ. of Michigan, Ann Arbor, MI; [5]Electrical and Computer Engin., Univ. of Delaware, Newark, DE; [6]Information Technol. and Electrical Engin., ETH Zurich, Zurich, Switzerland

Abstract: Arrays of individual wireless chips are an emerging architecture for neural interfacing. These chips could improve brain machine interfaces by removing the wired connection through the scalp, increasing biocompatibility with their submillimeter size, and improving implant targeting with their modular placement. While several approaches using this architecture are in development, little is known regarding their implantation into the brain, such as the required pitch between chips, insertion rate, and how many can be implanted at once. In particular, a minimally invasive method to swiftly insert many individual chips floating on the brain with penetrating subcellular-scale electrodes remains to be demonstrated. Our group has proposed two such electrode designs integrating a highly biocompatible (Patel, *JNE*, 2016) 8.4 µm diameter carbon fiber electrode with a wireless chip: the ReMote and StiMote. We previously validated chip components for recording electrophysiology (Lim, *ISSCC*, 2020; Lim, *VLSI*, 2021; Lim *JSSC*, 2022), stimulation (Lee, *VLSI*, 2023), optical power harvesting (Sun, *PVSC*, 2022) and wireless uplink (Atzeni, *VLSI*, 2022). To test the implantation and develop fabrication methods for the proposed designs, we made and implanted non-functional (NF) carbon fiber mote analogs. Silicon chip bases were micromachined to have dimensions (240 x 240 x 300 µm, L x W x H) similar to our proposed functional designs and with a 20-30 µm diameter hole. Fibers were manually placed into these holes, secured with epoxy, and cut to lengths targeting 0.5 or 1.0 mm. NF motes with 1 mm fiber lengths were suspended via excess fiber and encapsulated with Parylene-C and blowtorch sharpened (Welle, *TNSRE*, 2021) to enable unsupported insertion into the brain. The bases of completed NF motes were fixed to the end of a shaft with polyethylene glycol (PEG) in 3x3 or 4x4 grid patterns. As motes aggregated in molten PEG, the grid pitch was limited to the chips' own dimensions. Implantation required dissolving this PEG in saline once the silicon bases reached the brain's surface to release the devices. To validate this method *in vivo*, we inserted into rat cortex (male, Long-Evans) five 3x3 grids of NF motes with 0.5 mm fiber length in N=3 rats and two 4x4 grids with 1 mm fiber length in N=2 rats. Of the 3x3 mote grids, 37 of 45 motes inserted successfully (82%), where six of the failures were part of the second insertion trial . Of the 4x4 grids, 31 of 32 motes inserted successfully (96%). These insertions suggest that grids with a higher chip count are possible, and motivates further investigation of the biocompatibility of this insertion method during implantation and for months afterward.

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Poster

PSTR415. BCI and Neural Prosthetics: Technology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR415.09/EE8

Topic: E.05. Brain-Machine Interface

Support: 1U01NS126052-01

Title: In Vivo and In Vitro Development of Ultramicroelectrode Stimulation

Authors: *B. ROMANAUSKI[1], E. OLIVO[2], S. DEWBERRY[2], K. SINCLAIR[2], Y. WU[3], A. AZAMI[3], C. EZEH[2], B. BINEY[2], K. KUMARAVELU[5], R. VETTER[6], J. HETKE[6], D. KIPKE[7], S. F. COGAN[4], W. GRILL[5], M. E. ORAZEM[2], K. J. OTTO[2];
[1]Univ. of Florida, Gainsville, FL; [2]Univ. of Florida, Gainesville, FL; [4]Bioengineering, [3]Univ. of Texas at Dallas, Dallas, TX; [5]Duke Univ., Durham, NC; [6]NeuroNexus Technologies INC., Ann Arbor, MI; [7]NeuroNexus Technologies, Inc., Ann Arbor, MI

Abstract: Electrical interfacing with the brain is undergoing a transformation. High-density microelectrode arrays, such as the Neuropixels array, oversample action potentials from individual neurons enabling high fidelity and reliable unit recording (Jun *et al.*, 2017). Other reports show that ultra-microelectrodes arrays (UMEAs) with subcellular substrate cross-sectional dimensions lead to increased recording lifetimes (*e.g.*, Guitchounts *et al.*, 2013; Luan *et al.*, 2017). Clinical and research applications of microstimulation (µStim) would presumably benefit from these approaches; yet the technology is not optimized for usage by the community. Our goal is to optimize CNS microelectrode array (MEA) and UMEA µStim usage by 1) testing the separate hypotheses that *MEAs and UMEAs can deliver safe, effective levels of cortical electrical stimulation* and then 2) distributing optimized software and hardware to collaborators for assessment.
To achieve this goal, we are working on three projects: 1) *In Vitro* Testing, 2) Acute *In Vivo* experiments, and 3) Chronic *In Vivo* Experiments. First, our group has developed models to characterize the Electrochemical Impedance Spectroscopy of MEAs and UMEAs which have allowed us to better understand the degradation of UMEAs and their coatings over time. We have also developed finite element models (FEM) of MEA and UMEA stimulation in the rat cortex to model the effects of electrode size, electrode material, electrode spacing, anatomical location, and stimulation intensity on the neuronal activation in the cortex to inform our *in vivo* experiments. Second, acute rat experiments are being performed in which we are stimulating and recording through UMEAs and are gathering neural activation data throughout the layers of the cortex to verify the EIS and FEM models developed in the first part of this project. These experiments also provide information on safe stimulation parameters to reduce tissue damage and extend the life of the UMEAs and their coatings. Third, as this project progresses, we will continue into chronic studies with rats which will provide information on the long-term stability and effectiveness of UMEAs over time.

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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.; Qualia Oto. **W. Grill:** None. **M.E. Orazem:** None. **K.J. Otto:** None.

Poster

PSTR415. BCI and Neural Prosthetics: Technology

Location: WCC Halls A-C

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Program #/Poster #: PSTR415.10/EE9

Topic: E.05. Brain-Machine Interface

Title: Recording data from the 16-channel NeuroNexus Neurotrophic Electrode

Authors: *P. KENNEDY[1], D. FURMAN[2], J. HETKE[3], D. S. ANDREASEN[4];
[1]Neural Signals Inc, Duluth, GA; [2]Arctop Inc, San Francisco, CA; [3]Neuronexus Inc., Ann Arbor, MI; [4]Georgia Tech. Res. Inst., Smyrna, GA

Abstract: The 2 wire (single channel) Neurotrophic Electrode records about 20 identified single units that are functionally active. In an attempt to improve the harvesting of more single units, the NXNE 16-channel thin-film electrode array has been designed and implemented by NeuroneXus Inc. It has been implanted in rat vibrissa cortices. In two rats, 260 and 240 spikes have been recorded, with inter-spike interval histograms, cross correlation analyses and event-related firings suggesting the presence of 80 single units in one rat and 60 single units in the other rat. Preliminary decoding results produced by connecting NXNE data to Arctop Inc. software for real-time analysis demonstrate viability of the signal for complex pattern recognition tasks and dictionary creation. The pattern of single unit firings between different vibrissa deflections being different, for example, allows words or phrases to be associated with different firing patterns, thus allowing the audible production of different phrases by deflecting different vibrissa. Studies are ongoing.

Disclosures: **P. Kennedy:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 50%, Neural Logistics LLC. **D. Furman:** None. **J. Hetke:** None. **D.S. Andreasen:** None.

Poster

PSTR415. BCI and Neural Prosthetics: Technology

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Program #/Poster #: PSTR415.11/EE10

Topic: E.05. Brain-Machine Interface

Support: NIH Grant 5R01NS104344-05

Title: Ultrathin Amorphous Silicon Carbide Microelectrode Arrays for Chronic Recording in Rat Motor Cortex

Authors: *E. N. PAUL, J. R. ABBOTT, J. O. USORO, P. HAGHIGHI, N. GERAMIFARD, Y. WU, T. J. SMITH, B. S. STURGILL, S. PATNAIK, A. G. HERNANDEZ-REYNOSO, J. J. PANCRAZIO, S. F. COGAN;
Bioengineering, Univ. of Texas at Dallas, Richardson, TX

Abstract: Brain-machine interfaces use neural activity to control external devices like tablets and neuroprosthetics. Microelectrode arrays (MEAs) can record neural activity but signal quality tends to decline after implantation due in part to glial scarring, which leads to neuronal loss. For example, the active electrode yield (AEY), the proportion of working channels recording single units, has been shown to fall below 30% as soon as 16 weeks post-implantation and the signal-to-noise ratio (SNR) to fall from 8.6 right after implantation to 6. Previous work shows that reducing the cross-sectional area of MEAs can reduce glial scarring. Because of this, our goal here is to develop an ultra-thin MEA that can improve long-term neural recordings. We fabricated 4 shank amorphous silicon carbide (a-SiC) - a biocompatible, chemically inert, and electrically insulating material - MEAs with 4 electrode sites per shank (16 channels total) with a 160 μm^2 shank cross-sectional area. We implanted our MEAs in the left motor cortex of 7 female rats and performed weekly anesthetized neural recordings for 16 weeks. Signals were filtered using a band pass filter (300-3000 Hz) and common median reference to reduce noise and artifacts. We isolated spikes with a threshold of -4σ from the mean, then used k-means clustering followed by manual verification to identify single units. We computed the AEY and single units' average waveforms. We divided the voltage peak-to-peak (Vpp) by the root-mean square (RMS) value of the noise to compute SNR. We used linear regression followed by an F test between calculated slope and a null hypothesis of slope=0 to determine if Vpp, noise, or SNR changed significantly over 16 weeks. The day of implantation we observed an AEY over 90% which declined to 50% over 16 weeks. Vpp and SNR also declined. At the time of implantation, mean values were as follows: Vpp = 141.4±11.2 µV, noise = 8.7±0.35 µV, SNR = 15.7±0.92. Regression slopes for each value were: Vpp = -2.07 µV/week ($p<0.0001$), noise = 0.03 µV/week ($p=0.08$), SNR = -0.24/week, ($p<0.0001$). These results show a 23% decline in Vpp, 5% increase in noise, and 24% decline in SNR after 16 weeks post-implantation. The stability of noise shown in this research suggest that using ultra-thin a-SiC devices can minimize glial scarring that result in only 5% increase of noise values. This will be verified in future studies by assessing the histological response. However, preliminary neurophysiological results from a-SiC MEAs suggest that the quality of signal over time, represented by the SNR, is higher than the reported values in literature for commercial silicon MEAs, supporting the long-term use of these ultra-thin devices.

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Poster

PSTR415. BCI and Neural Prosthetics: Technology

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Program #/Poster #: PSTR415.12/EE11

Topic: E.05. Brain-Machine Interface

Support: NIH NINDS R21 NS125461-01

Title: Flexible and Hydrophylic Carbon Nanotube Fibers as Microelectrodes for Neural Stimulation and Recording

Authors: *N. ALVAREZ, C. RUHUNAGE;
Univ. of Cincinnati, Cincinnati, OH

Abstract: Implantable micro electrodes are generally employed to record and stimulate electrical activity of neurons in the nervous system. Most of the electrodes available today are made of metals and silicon that lack required flexibility resulting on a mechanical mismatch between soft tissues and rigid metals which induces faster biofouling and inconsistent performance for long-term applications. Biofouling triggered by inflammatory responses dramatically affect the performance of neural electrodes, resulting in decreased signal sensitivity and consistency over time. Thus, long-term clinical applications require electrically conducting flexible electrode materials with reduced dimensions and antibiofouling properties. These characteristics reduce the degree of inflammatory reactions and increase lifetime of neural electrodes. Carbon nanotubes (CNTs) are well known for their flexibility, electrical conductivity and chemical inertness. This talk will report the use of CNT fibers for neural stimulation and recording, and subsequent covalent functionalization of CNT fibers and films surfaces with hydrophilic, antibiofouling phosphorylcholine (PC) molecules. The electrochemical and spectroscopic characteristics, impedance properties, hydrophilicity, and in vitro antifouling nature of the functionalized CNT surfaces will be presented. The hydrophilicity of the functionalized CNT films was demonstrated by a decrease in the static contact angle from 134.4° ± 3.9° before to 15.7° ± 1.5° after one, and fully wetting after three functionalization cycles respectively. In addition, the extent of protein absorption on the functionalized CNT films were significantly lower than that on the non-functionalized CNT film. Surprisingly, the faradic charge-transfer properties and impedance of the CNT assemblies were preserved after functionalization with PC molecules. These functionalized CNT assemblies are promising for the development of low-impedance neural electrodes with higher hydrophilicity and protein-fouling resistance to inhibit inflammatory responses.

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Poster

PSTR415. BCI and Neural Prosthetics: Technology

Location: WCC Halls A-C

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Program #/Poster #: PSTR415.13/EE12

Topic: E.05. Brain-Machine Interface

Support: ERC Grant 759998 "FeelAgain"
SNSF Grant 197271 "MOVEIT"

Title: Design and fabrication of a novel neural interface for selective peripheral nerve stimulation comprising 2-photon polymerized penetrating structures integrated on a flexible polyimide-parylene substrate

Authors: *F. CIOTTI[1], A. CIMOLATO[1], P. PALOPOLI[2], J. WEICHART[3], S. RASPOPOVIC[1];
[1]Dept. of Hlth. Sci. and Technol., [3]Dept. of Mechanical and Process Engin., [2]ETH Zürich, Zürich, Switzerland

Abstract: The potential of peripheral nerve stimulation in the treatment of clinical conditions is strongly limited by the currently available electrode technology. The high selectivity necessary to obtain desired clinical outcomes with minimal adverse effects implies the use of highly invasive multielectrode arrays and complex surgeries, which restrict their viability in chronic human implants. Through highly detailed computational models of target peripheral nerves (sacral, pudendal, and vagus nerves), we developed a novel neural interface design comprising both intrafascicular and extraneural electrodes, i.e., spikes radially penetrating the nerve and flat electrodes arranged on the nerve surface. We optimized configuration of electrodes and design parameters for highest selectivity through automatic optimization methods, and compared its selectivity, repeatability, and invasivity with respect to state of the art interfaces. The promising results obtained in-silico prompted the actual realization of the device. To guarantee the conformity of the substrate to the nerve surface, the backing of the device is made of a thin polyimide layer (< 10 μm). The penetrating spikes are fabricated with a 2-photon polymerization process with a biocompatible resin. This allows for extremely high aspect ratios, 3D resolution (< 1 μm), and design flexibility. We exploited this technique to optimize the shape of the spikes for mechanical resistance. Moreover it allowed us to micro-texture their surface to increase the effective surface area and therefore the charge injection capacity, simultaneously reducing the foreign body response of the body to the implanted device. A conductive layer made of titanium and gold is then sputtered on substrate and spikes and patterned by wet etching with ultra-thick photoresist. A conformal, biocompatible insulative layer of Parylene-C is then deposited by chemical vapor deposition, and finally the surface electrode, spike tips, and connector pads are exposed by patterning the parylene with highly anisotropic ICP-RIE, simultaneously releasing the device by etching through both parylene and polyimide layers along its contours. Lastly, we evaluated its performance by electrochemical testing, showing promise for future in-vivo studies to achieve the predicted functional improvements, possibly contributing to making more advanced neurostimulation therapies clinically viable.

Disclosures: **F. Ciotti:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ETH Zürich. **A. Cimolato:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property

rights/patent holder, excluding diversified mutual funds); ETH Zürich. **P. Palopoli:** None. **J. Weichart:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ETH Zürich. **S. Raspopovic:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ETH Zürich.

Poster

PSTR415. BCI and Neural Prosthetics: Technology

Location: WCC Halls A-C

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Program #/Poster #: PSTR415.14/EE13

Topic: E.05. Brain-Machine Interface

Support: Private gift

Title: Scalable Wireless Neuromorphic Microsensor Network with Dynamic Spike Sensing for Implantable Brain-Machine Interface

Authors: *J. LEE[1], A.-H. LEE[1], V. LEUNG[2], L. LARSON[1], A. NURMIKKO[1];
[1]Brown Univ., providence, RI; [2]Baylor Univ., Waco, TX

Abstract: As the main currency of neuronal signaling, action potential events occur sparsely in brain circuits due to efficient spike encoding which allows cells to effectively utilize limited resources such as bandwidth and energy. However, recording and reporting these sparse events at fixed sampling frequency can lead to inefficient sampling and communication, especially in ultra-miniaturized wireless neural sensor implants with tightly constrained energy and RF bandwidth. To address this challenge, we have developed dynamic spike sensing (DSS) strategy for wireless "neuromorphic" microsensors in the context of Brain-machine interfaces (BMI), drawing inspiration from dynamic vision optical imaging sensors (DVS). In our system, each wireless sensor employs sophisticated spike-encoding algorithms to convert spike band neural signals into binary spikes and transmits only the encoded spike events through an RF link. Distinct from DVS cameras, however, our spike encoder incorporates an adaptive threshold to account for electrode-specific noise and encodes the magnitude of the change in spike temporal coding which enables spike sorting in subsequent analysis. The implementation of DSS is realized in the form of a silicon microchip, a "Neurograin". The chip with a size of only 400 µm × 400 µm, is equipped with a neural amplifier, analog-to-digital converter, DSS encoder, and circuitry for wireless energy harvesting and data communication operating at 915 MHz. While the microchip samples spike band signals at a nominal rate of 4.6 kHz, the DSS encoder achieves a significant compression ratio of 3.5% while preserving essential waveform features necessary for spike sorting. Through simulations, we have determined that up to a thousand wireless microsensors can collectively record spike signals within our nominal RF bandwidth of 10 MHz. The compression capability is made possible by the dynamic adjustment of the sampling rate in the DSS engine, allowing for an increase in the sampling rate in the presence of spike events. We have successfully demonstrated the fully wireless functionality of the DSS Neurograin in

benchtop and small animal experiments, showing its ability to accurately report spike events and form a network. The proposed dynamic event sensing approach is scalable to large-scale wireless neuromorphic sensor networks with broad applications.

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Poster

PSTR415. BCI and Neural Prosthetics: Technology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR415.15/EE14

Topic: E.05. Brain-Machine Interface

Support: Private gift

Title: Network of Implantable Wireless Microchip Stimulators for Spatially Distributed Intracortical Patterned Neuromodulation

Authors: *A.-H. LEE[1], J. LEE[1], V. LEUNG[2], L. LARSON[1], A. NURMIKKO[1];
[1]Brown Univ., Providence, RI; [2]Baylor Univ., Waco, TX

Abstract: While prior research suggests the viability of free-floating implanted wireless cortical microstimulators [Khalifa, A., et al. 2018, Piech, D.K., et al. 2020], scalability remains a challenge to achieve patterned stimulation with a limited number of stimulation channels achieved, typically from a single multielectrode device. What is desired is an approach to generate complex stimulation patterns such as to evoke a sensory percept or correct abnormal neural activity. Here, we present an ultra-miniaturized ASIC measuring 300 μm × 300 μm operating at 915 MHz, a silicon microchip capable of programmable intracortical stimulation, RF energy harvesting, and bidirectional data communication. Given the small volume of the ASIC, this device is developed to enable intracortical stimulation at high spatial resolution. The distinct feature of the prospect of a neuromodulation scheme where many spatially distributed stimulators can be wirelessly networked to achieve large scale neural stimulation at multiple points across the brain. The system leverages a wireless daisy chain communication scheme capable of delivering remote commands up to a thousand microchips as a program of patterned stimulation within 300 μs using a 1 MHz downlink interface. We also report on the addition of an energy storage capacitor for keeping the required incident RF power within regulatory limits, to be integrated directly on the silicon substrate of the chip. Using microfabrication techniques for post-processing, we have monolithically integrated intracortical penetrating electrodes or planar PEDOT: PSS electrodes on the microchips which were fabricated in TSMC 65 nm CMOS process. We demonstrate the stimulation and wireless networking capabilities of microchips in a conductive medium as well as in-vivo rodent experiments. In the animal experiment, fully implanted cortical microstimulator delivers focal electrical current pulses up to 100 μA to evoke neural and behavioral modulation. The results suggest the potential of this type of wireless,

spatially distributed network of microstimulator system as a tool for advanced neuromodulation applications such as patterned electrical stimulation for use in the sensory neural prosthesis.

Disclosures: **A. Lee:** None. **J. Lee:** None. **V. Leung:** None. **L. Larson:** None. **A. Nurmikko:** None.

Poster

PSTR416. Posture and Gait: Control, Learning, and Biomechanics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR416.01/EE16

Topic: E.06. Posture and Gait

Support: NSERC RGPIN-2017-04175

Title: Balance reactions to visual displacements in a virtual reality environment.

Authors: ***J. E. MISIASZEK**[1,2], R. N. CHANDRA[1], S. G. HEMAKUMARA[1], J. FORERO[1]; [1]Fac. of Rehabil. Med., [2]Neurosci. and Mental Hlth. Inst., Univ. of Alberta, Edmonton, AB, Canada

Abstract: The potency of visual feedback in the control of standing balance is highlighted by the entrainment of postural sway to the continuously oscillating motion of a visual surround in the "moving room" paradigm. We recently demonstrated that small amplitude, transient moving room displacements induced brief, balance corrective responses, indicating that visual feedback may play a role in correcting for unexpected balance disturbances. Visual motion within virtual reality (VR) is also known to induce lean and postural adjustments in relation to the perceived movement. However, it is not known if small amplitude, transient visual stimuli in VR are similarly effective in generating balance corrective responses. Here, we recreated the "moving room" paradigm with VR. We hypothesized that forward motion of the virtual surround would induce a forward sway reaction and that light touch of a stable reference would reduce the response. Additionally, we tested if seeing the motionless support surface (floor) influenced the response evoked. To test this, participants wore VR goggles that generated a virtual enclosure with either 3 walls and ceiling that moved relative to a stable floor (F), or an enclosure that included a shelf that obscured the floor (O), and either asked to touch (T) or not touch (NT) a stable reference. The virtual enclosure was programmed to create a forward linear displacement (1.25 cm, 124 mm/s peak velocity). A total of 20 naïve participants were allocated to 1 of 4 groups (ONT, OT, FNT, FT) and were unexpectedly exposed to 10 visual displacements. All ONT participants reacted by swaying forward, whereas 3/5 OT participants swayed forward but with a smaller amplitude, and the remaining 2/5 swayed backward resulting in an unpaired Hedge's g between ONT and OT of -2.31. In contrast, 2/4 FNT participants swayed forwards while 2/4 swayed backwards, with absolute amplitudes comparable to the forwards sways of the ONT group. 4/5 participants in the FT group swayed forwards, but the absolute amplitudes of the responses were smaller than the FNT group. Overall, small amplitude, forward virtual surround

displacements evoke forward balance corrections, consistent with previous natural world findings. However, seeing a stationary floor or touching a stable reference while in the moving virtual surround often resulted in an atypical backward sway response not observed previously in natural world studies, suggesting that sensory information from virtual environments can lead to conflict in standing balance control.

Disclosures: **J.E. Misiaszek:** None. **R.N. Chandra:** None. **S.G. Hemakumara:** None. **J. Forero:** None.

Poster

PSTR416. Posture and Gait: Control, Learning, and Biomechanics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR416.02/EE17

Topic: E.06. Posture and Gait

Support: DST-SERB CRG/2020/005911

Title: Control of balance under varying odds of a postural perturbation

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Abstract: Perturbations to balance such as hit or push are often experienced in everyday life. When exposed to external predictable perturbations, the central nervous system (CNS) uses anticipatory and compensatory postural adjustments (APAs and CPAs, respectively) to maintain balance. APAs are changes in muscle activity that occur prior to the onset of an expected perturbation and are followed by CPAs (corrective changes post-perturbation). CNS uses several key features of a perturbation to make predictions about the upcoming impact for generation of optimal APAs and CPAs. While the effect of predictability on APAs has been studied in terms of varying perturbation magnitudes (low/high) and timing (self-paced/reaction time); in all such cases, to begin with, the occurrence of the perturbation on a given trial was always certain. However, the odds of occurrence of a perturbation may itself influence postural planning; yet this factor has not been studied. To investigate how the odds of occurrence of a predictable perturbation affects APAs and CPAs, 7 healthy adults (24.86 ± 2.54 years; 4 males) were exposed to external predictable perturbations/hits via a pendulum at shoulder level while standing barefoot. The probability of occurrence of hits was varied across 7 conditions such that 100, 80, 60, 50, 40, 20, and 0 percent of the total trials were hits while remaining were non-hits (pendulum stopped just before subject contact); the order of conditions and of hits within conditions across subjects was pseudo-randomized. Prior to each condition, subjects were told about the total number of trials (10) and the percentage of hits. Integrated electromyographic (IEMG) activity over 4 epochs (APA1, APA2, CPA1, and CPA2) for several muscles and peak center of pressure (COP) displacements were computed. Repeated measures ANOVAs with post-hoc comparisons were conducted. In hit trials, for IEMG, while no main effect of interaction

(epoch x condition) was observed for tibialis anterior, rectus femoris, and biceps femoris muscles, a significant main effect was seen for gastrocnemius *(p = 0.014)*; post-hoc comparisons showed a larger inhibition during the APA2 phase in condition with 100% hits (-0.47 ± 0.33) than that in 50% hits (-0.19 ± 0.27) *(p = 0.005)*. Peak COP displacements did not differ across conditions. The preliminary findings suggest that even when the odds of occurrence of hits were less than 100%, the CNS seemed to largely use similar patterns of APA and CPA activity as seen in 100% hit condition. Maintaining the highest level of motor planning even when the odds of being hit are very low seems to reflect a prudent decision-making that enables maintaining postural stability at all times.

Disclosures: R. Sharma: None. **D. Gokani:** None. **N. Kanekar:** None.

Poster

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Topic: E.06. Posture and Gait

Support: NIH Grant R01NS100928
NIH Grant R01NS110550
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Title: Hindlimb musculoskeletal structure and mechanical demands of locomotion determine basic organization of spinal locomotor networks in the cat

Authors: S. RAHMATI[1], A. N. KLISHKO[1], S. N. MARKIN[2], N. SHEVTSOVA[2], A. FRIGON[3], I. A. RYBAK[2], *B. PRILUTSKY[1];
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Abstract: Assuming that the hindlimb musculoskeletal system and its neural control have evolved in parallel to satisfy behavioral demands, locomotor demands can be used to determine the required muscle activation patterns and propose the corresponding organization of hindlimb spinal locomotor networks. We defined hindlimb locomotor demands as (i) the required muscle moments of force produced at the ankle, knee and hip leading to the observed kinematics of walking and (ii) the necessity to maximize walking time at a given speed (or to minimize muscle fatigue). We considered that the spinal locomotor network controlling each hindlimb includes (i) a half-center rhythm generator (RG), (ii) a pattern formation network (PF), receiving RG input and sensory feedback to control the activity of synergistic muscle groups, and (iii) reflex pathways projecting to motoneurons of agonist and antagonist muscles. To quantify mechanical demands, we recorded hindlimb kinematics and ground reaction forces of 9 cats during overground walking (duty cycle > 0.6, speeds of 0.5 - 0.7 m/s) and computed the resultant muscle moments at hindlimb joints. Using these data, we then computed activations of 12 major

hindlimb muscles during each recorded walking cycle based on minimizing the cost function characterizing muscle fatigue (Crowninshield, Brand, 1981) and constrained by hindlimb musculoskeletal properties and the requirement to produce the obtained moments at hindlimb joints. Potential organization of the PF network was determined by extracting muscle synergies (groups of muscles receiving the same time-dependent input; Klishko et al., 2021) from the muscle activations. We then compared the predicted synergies with the synergies obtained from actual activation patterns recorded in the same muscles and in the same walking cycles. In both cases, the number of identified synergies was the same (n=5), they contained essentially the same muscles and had highly correlated activation patterns. The computed and recorded muscle activations had the following features: (i) reciprocal activity of one-joint antagonists, (ii) concurrent activity of joint agonists, and (iii) dependence of two-joint muscle activity on moments at both joints. We incorporated the revealed muscle synergies and features of muscle activity in a neuromechanical model of spinal locomotor control of a single hindlimb. The model generated realistic walking and muscle activity patterns. The results support the idea that the structure of the hindlimb musculoskeletal system and locomotor demands predefine and shape the organization of spinal locomotor networks.

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Poster

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Topic: E.06. Posture and Gait

Support: NSF Career Award 1847891
NSF-GRFP 2139321

Title: Modeling perception of leg speed differences during walking through drift-diffusion models

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[1]Bioengineering, [2]Univ. of Pittsburgh, Pittsburgh, PA; [3]Univ. de la Católica, Montevideo, Uruguay

Abstract: Sensation plays a crucial role in the planning, execution, and adaptation of movements. For example, people adapt their movements based on the difference between predicted and actual sensory consequences from our actions. However, quantifying human sensation is challenging because it does not have observable consequences like motor outputs. To measure perception of somatosensation during walking (e.g., leg speed differences), psychophysics tasks known as the 2-alternative forced-choice (2AFC) task have been used in the past. Additionally, from the recording of the task it is possible to apply computational modeling

through a variation of the drift-diffusion model to characterize the relation between accuracy and reaction times across different sensory stimulus magnitudes in this active task (Iturralde et al., 2023). This model differs from the traditional formulation of DDM (Bogacz et al., 2006) given that both the rate (drift) as well as the noise (diffusion) in the evidence accumulation process are scaled by the stimulus magnitude. Here, we wanted to assess model performance in a 2AFC task assessing sensitivity to speed differences during walking on a split-belt treadmill. Specifically, we used data from 26 participants who had mean walking speeds of 0.7, 1.05, or 1.4 m/s. We hypothesized that the new DDM formulation (drift and diffusion are stimulus-dependent) will outperform the traditional formulation in this broad dataset. To test this hypothesis, we fitted the models and computed the residual sum of squares (RSS) and Mallow's Cp (James et al., 2021) to compare between the two models. We expected that the new formulation will have a superior performance at explaining the relationship between accuracy, reaction time, and stimulus magnitude simultaneously, which would be indicated by smaller values of RSS and Cp. Consistently, the RSS for the reaction times was 42.92 for the traditional formulation vs. the 14.9445 in our proposed stimulus-dependent noise model. Likewise, Mallow's Cp had values of 0.373 vs. 0.127, respectively. Qualitatively, differences between the two models are not as striking as what was reported before (Iturralde ref). A possible explanation are the differences in protocol: 1) we limited the response window in our 2AFC task 2) we included an auditory cue warning participants when time was running up, 3) we included more repetitions per stimulus magnitude, and 4) we have a reduce set of stimulus magnitude tested. Taken together, our results indicate the parameters relevant to the evidence accumulation process leading to the perception of a difference in leg speed can be explained by magnitude-dependent noise.

Disclosures: **M. Gonzalez-Rubio:** None. **P.A. Iturralde:** None. **G. Torres-Oviedo:** None.

Poster

PSTR416. Posture and Gait: Control, Learning, and Biomechanics

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Topic: E.06. Posture and Gait

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NIH/NIDCD 1R01DC018287S1

Title: A role for vestibular lateral translation cues in postural control

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[3]Otolaryngology, Harvard Med. Sch., Boston, MA

Abstract: Imbalance and falls pose significant health risks, especially as people age, leading to hospitalizations and serious injuries. While many factors contribute to postural control, a key factor is sensory feedback from the vestibular, visual, and proprioceptive systems. In most prior

work, the predominant vestibular cue for postural control is presumed to be feedback about tilt orientation relative to gravity. In fact, postural control models, which have been used to further understanding about sensory contributors, typically include vestibular feedback about tilt orientation relative to gravity. However, the vestibular system provides crucial information about head tilt, translation, and rotation. In a recent experimental study (Karmali et al. 2021), we measured postural sway and vestibular function using vestibular perceptual thresholds. These vestibular thresholds are robust measures of vestibular function, and measure the smallest motion that can be reliably perceived. We measured thresholds for lateral translation, vertical translation, tilt relative to gravity, and yaw rotation. We found that lateral translation thresholds were correlated with postural sway across subjects, and not other thresholds. This supports a role for vestibular lateral translation cues in postural control. This finding is further supported because the typical tilt of the body during quiet stance is much less than roll tilt thresholds. To further support these findings, we developed a closed-loop postural control model that includes independent feedback channels for vestibular tilt and translation, using a single-link inverted pendulum. Since thresholds are related to neural variability/noise via signal detection theory, we implemented thresholds for each pathway as low-pass filtered white noise. Notably, when both tilt and translation cues are available in the model, the predicted postural sway is significantly reduced compared to using only tilt cues. This finding provides support for the hypothesis that translation cues play a role in postural control. Future modeling will include proprioceptive and visual feedback.
Karmali, Faisal, et al. "The role of vestibular cues in postural sway." Journal of neurophysiology 125.2 (2021): 672-686.
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Poster

PSTR416. Posture and Gait: Control, Learning, and Biomechanics

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Program #/Poster #: PSTR416.06/EE21

Topic: E.06. Posture and Gait

Support: NSF 1847891

Title: Similarity across walking contexts improves the generalization of adapted locomotor patterns.

Authors: *A. AWUAH, K. FJELD, G. TORRES-OVIEDO;
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Abstract: Generalization in motor control is the ability to transfer motor patterns from a training context, where patterns are developed, to a testing context, where patterns are tested. This ability is critical for rehabilitation because it is important that motor corrections with training devices

generalize to movements without them. However, device-induced training, for example, split-belt treadmill walking, in which one leg walks faster than the other, has limited generalization to walking without the training device. This limitation may be attributed to incongruent contextual cues, specifically, natural visual flow and the ability to self-regulate step initiation typically found in overground walking is absent in split-belt walking. To address this question, we manipulated the contextual similarity between devices inducing locomotor adaptation and overground walking. We hypothesized that devices with more contextual similarity to overground walking would improve the generalization of adapted patterns to walking without the device. To test this hypothesis, we adapted twenty young adults on either the split-belt treadmill (i.e., low contextual similarity group; n=10) or a pair of motorized shoes (i.e., high contextual similarity group; n=10) which can induce the same adaptation as the split-belt treadmill while walking overground. Following adaptation, we further divided the groups to test the generalization of adapted locomotor patterns (i.e., aftereffects) under two conditions: tested **with the training device** (i.e., treadmill or motorized shoes) or during overground walking **without the training device.** We anticipated to observe an interaction such that contextual similarity would regulate the reduction in aftereffects tested without the device relative to those tested with the device. Consistently, we found a trending interaction effect between contextual similarity and testing conditions ($p=0.054$). More specifically, we observed that groups with low contextual similarity exhibited less aftereffects without the training device than with the training device, whereas groups with high contextual similarity showed the opposite pattern (larger aftereffects without the device than with the device). Overall, due to large aftereffects observed in the groups with high contextual similarity (i.e., motorized shoe groups), our results suggest that increasing contextual similarity between training and testing contexts enhances the generalization of locomotor adapted patterns. This finding could be exploited to design rehabilitation devices that improve motor patterns in clinical populations during unassisted walking.

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Poster

PSTR416. Posture and Gait: Control, Learning, and Biomechanics

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Program #/Poster #: PSTR416.07/EE22

Topic: E.06. Posture and Gait

Support: OIST (institutional support)

Title: Exploring body configurations of mice during treadmill locomotion

Authors: *L. I. SWAMINATHAN[1], B. M. IGNATOWSKA-JANKOWSKA[1], A. G. OZER[1], M. Y. UUSISAARI[1], G. J. STEPHENS[2];
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Abstract: Animal locomotion comprises rhythmic spatio-temporal modules that are inherently variable. Quantifying animal movement and its variability can provide insight into underlying processes of the central nervous system. Previous studies on mouse locomotion focused on quantifying individual limb movement and inter-limb coordination using central metrics such as step-length, speed, angular excursions, and phase differences, despite locomotion being a whole-body movement. The aim of this study is to move beyond the classic metrics by describing whole-body mouse movements as body configurations changing through time. We utilized a novel marker-based 3D motion capture system comprised of 7 high-speed, high-resolution cameras and 10 custom-made retroreflective markers permanently attached to the skin of the mice (adult male, C57bl/6, n=9) at the shoulders, hips, knees and ankles. The 10 markers were tracked in 3D to obtain highly resolved (300 Hz, 200 micrometers) kinematic data that is a 30-dimensional readout of the body configurations (BCs) of the mouse as it locomoted, without restraints, on a treadmill set at different speeds. Principal component analysis (PCA) was used to decompose the trajectories into modes of deformation (MDs) around the mean BC. The analysis revealed 6 significant MDs explaining over 85% of the data variance; the remaining modes had contributions comparable to noise and were discarded. The 6 MDs form a basis set of deformations to the mean BC during treadmill locomotion, expressing the BCs during the task as a combination of these MDs. The MDs were classified into two main types: hopping and leg-alternation, each further divided into three subtypes based on differences in joint coordination. The number of significant MDs decreased from 6 to 3 at treadmill speeds above 24 m/min (associated with bounding and galloping), indicating that mice utilize only a subset of BCs seen with treadmill locomotion at lower speeds. At the highest speed, this subset could be expressed as a combination of only leg-alternation MDs with no hopping MDs. In this study, we obtained a description of whole-body mouse movements built of BCs and MDs. The space of BCs resulting from distinct leg-alternation and hopping modes can be further explored using delay-embedding techniques to unveil distinct classes of recurrent BC patterns (analogous to step-cycles) during mouse locomotion. Furthermore, this tractable representation allows the quantification of the variability of locomotory movements by assessing the spread of trajectories in recurrent BC patterns.

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Poster

PSTR416. Posture and Gait: Control, Learning, and Biomechanics

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Topic: E.06. Posture and Gait

Support: DFG DI 2907/1-1 (Project number 500615768)
NSF/CIHR/DFG/FRQ/UKRI-MRC Next Generation Networks for Neuroscience Pro-gram
NSF IIS 2113028

Title: The Effects of Variability in the Relative Arrangement of Mechanotransducive Sensors on Strain Processing

Authors: *G. F. DINGES, N. S. SZCZECINSKI;
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Abstract: A common aspect of locomotive coordination is the integration of sensory information to modify or reinforce motor patterns while the organism is adapting to its variable environment. Sensory information is measured and monitored by sensory organs that can be found on and within various limb segments. In the legs of insects, there are multiple sensory organs monitoring key phases of cyclic movements, such as stepping. One class of sensory organ in insects whose phasic inputs has been shown to influence locomotion is the Campaniform sensilla (CS). These measure forces that arise within the exoskeleton. Investigations have shown that the general locations of CS along the leg are consistent between individuals in *Drosophila melanogaster*. However, the precise arrangement of sensilla within fields and groups show interindividual differences. Recently, a mechanical approach was used to 3-D model *Drosophila* field CS using information from nano-computed tomography segmentation. This work showed that polymer resin exhibits viscoelastic properties, much like those shown to be present within the insect cuticle. This viscoelasticity affects CS firing within the animal. This experimental design enables circumventing the experimental restrictions currently limiting the investigation of CS within the animal itself. The limited availability of sparse leg-CS labelling Gal4 lines as well as the occurrence of movement artifacts that may arise during leg calcium imaging, makes this mechanical modeling approach useful for investigating the mechanical aspects of strain sensing. We used this setup to investigate the effects of field rotation and individual cap rotation on strain induced cap compression in simplified models of the femoral *Drosophila* CS field. Using these 3-D mechanical models allows the modulation of attributes, such as rotation, while capturing viscoelastic properties. In different test samples we rotated the whole field or two individual CS. While applying different tensile forces to the field, the strain each cap experienced was monitored using strain gauges. Our findings show that the rotation of the whole field affects which loading scenario can compress the most CS. Further, the rotation of individual CS by 30° can decrease the monitored strain but does not necessarily lead to tension instead of compression, suggesting that difference in arrangement may not lead to altered firing. Further, there are distal to proximal, and coherently large to small cap, gradients in monitored strain. Our findings underline that interindividual variability in sensilla orientation can affect which strains are monitored on a purely mechanical level.

Disclosures: G.F. Dinges: None. **N.S. Szczecinski:** None.

Poster

PSTR416. Posture and Gait: Control, Learning, and Biomechanics

Location: WCC Halls A-C

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Program #/Poster #: PSTR416.09/EE24

Topic: E.06. Posture and Gait

Title: Cortical brain dynamics associated with unanticipated changes in exoskeleton walking assistance

Authors: *J. BRADFORD[1], S. SONG[2], C. A. HAYNES[1];
[1]Humans in Complex Systems, US Army Res. Lab., APG, MD; [2]Texas A&M Univ., College Station, TX

Abstract: Little research has been performed to understand how the human brain and body respond and adapt to active exoskeleton assistance during walking. The goal of this study was to uncover cortical brain signals related to the user's adaptation to an autonomous, bilateral ankle exoskeleton. 22 subjects walked on a treadmill while wearing a powered bilateral ankle exoskeleton that assisted with plantarflexion at toe off. We measured EEG, EMG, kinematics, and ground reaction forces. During walking with exoskeleton assistance, we intermittently turned the exoskeleton power on and off without notifying the participant to determine the timescale of how the brain and body adapt to the new gait dynamics. We found increased theta band power in prefrontal brain areas only during the first stride when augmentation was turned on. We found decreased beta band power in the right primary sensorimotor cortex after the augmentation was turned on that persisted for many strides after the transition

Disclosures: J. Bradford: None. **S. Song:** None. **C.A. Haynes:** None.

Poster

PSTR416. Posture and Gait: Control, Learning, and Biomechanics

Location: WCC Halls A-C

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Program #/Poster #: PSTR416.10/EE25

Topic: E.06. Posture and Gait

Title: Gait characteristics in young adults during dual-task conditions.

Authors: *C. CHAU, T. COUGHLIN, K. DOWDALL, M. GELDER;
Nazareth Col. of Rochester, Rochester, NY

Abstract: This study investigates the gait characteristics of young adults while performing a concurrent cognitive task. Nine healthy young adults ages 18-25 years old were recruited from Rochester, NY. Participants performed two cognitive tests, serial subtraction (S) and an auditory Stroop test (A). Walking was recorded by the Vicon 3D motion capture system (Nexus) using eight cameras. Twenty-two 14 mm retro-reflective markers were placed on the skin of predetermined anatomical landmarks of the lower extremities, and seven body segments were reconstructed (CGM 1.1). Anatomical joint angles were calculated. Each participant completed 4 trials of walking (20 ft) without a concurrent task (Single-Task (ST)) or with a concurrent cognitive task (Dual-Task (DT)), either DTS or DTA, in a randomized order. The only constraint for all walking tasks is that the participant may not stop during walking trials. Spatiotemporal and kinematic gait parameters were processed by Visual3D software (C-Motion). Joint angular excursion for the pelvis, hip, knee, and ankle joints was calculated. Angle-angle diagrams were

plotted to examine intralimb coordination. A one-way repeated measures ANOVA was used to compare the gait difference between the three walking conditions. A paired t-test was used to compare the dual-task locomotor cost between DTS and DTA. The effect size between dual and single tasks was calculated using Cohen's D statistics. As compared to ST-walking, results showed a significant decrease in velocity (-0.15 m/s, -0.15 m/s), cadence (-6.9 steps/min, -5 steps/min), and stride length (-0.08 m, -0.1m) during DT-walking for both DTS and DTA, respectively. A significant increase in stance duration (+5.0 ms, +4.3 ms), double limb support (+3.9 ms, +3.4 ms), and step cycle duration (+6.9 ms, +5.5 ms) was found during DT-walking for both DTS and DTA, respectively, as compared to ST-walking. A significant decrease in joint angular excursion of the hip (-2º, -1.8º) and ankle (-2.3º, -1.9º) was found during DT-walking for both DTS and DTA, respectively. Hip-Knee and Knee-Ankle cyclogram were similar in shape between ST- and DT-walking suggesting the intralimb coordination remains robust. Cognitive errors during dual-task locomotion were comparable between DTS and DTA but with marked variability between participants. Our results suggest a decrease in gait stability in adults during both DTS and DTA walking compared to ST-walking support literature that suggests a decrease in attentional resources during dual-task conditions. A decrease in joint angular excursion may contribute to the decrease in spatiotemporal parameters.

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Poster

PSTR416. Posture and Gait: Control, Learning, and Biomechanics

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Program #/Poster #: PSTR416.11/EE26

Topic: E.06. Posture and Gait

Support: NIH NIDCD R01-DC002390

Title: Neck muscle-specific modulation for head stabilization by vestibular reflex during locomotion

Authors: *R. WEI, O. STANLEY, K. E. CULLEN;
Dept. of Biomed. Engin., Johns Hopkins Univ., Baltimore, MD

Abstract: Locomotion is a continuous, rhythmic oscillatory motor behavior. Vestibulospinal reflexes are believed to make an essential contribution to locomotion by controlling balance, especially in activating the neck musculature for head stabilization. However, the contribution of neck muscles to head stabilization and how this contribution is influenced by locomotion velocity remains unclear. To date, it had not been possible to directly assess the contribution of vestibular pathways from that of extra-vestibular pathways to head stability. Accordingly, we studied the head stabilization and motor unit activity in neck muscles using 3D motion capture and EMG recording during treadmill and ground walking in both intact and bilateral vestibular loss (BVL) monkeys. A head-mounted 6D sensor and a marker-based tracking system was used

to extract the animals' head rotation and position. The marker-based tracking system was also used to track trunk position, while 4 high-speed cameras captured synchronized limb motion. Motor unit activity was measured through acute intramuscular EMG recordings in the splenius capitis (SPL) and sternocleidomastoid (SCM) muscles. Overall, we observed that the head was well-stabilized in normal monkeys, as the head-on-body movement compensated for the body's movements. Both the head and body exhibited increased movement as speed increased. Single motor unit activity of SPL and SCM muscles showed antagonistic phase-dependent activity. Additionally, as speed increased, there was an accompanying increase in EMG activity in the neck muscles. In contrast, poor stabilization was observed after BVL. Pronounced head oscillations were observed, which failed to compensate for the body's movements, especially in the roll rotation. Furthermore, neck muscle activity revealed minimal modulation across all speeds, leading to limited phase-locked activity or antagonism. Taken together, our findings establish that vestibular sensory inputs play a crucial role in organizing neck motor activity to ensure compensatory head movement during locomotion. Furthermore, our study highlights the distinct phasic patterns and speed modulation of muscle activity generated by vestibular pathways, which contribute to enhanced head stability during locomotion.

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Poster

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Topic: E.06. Posture and Gait

Support: NSF DBI 2015317
NSF CRCNS 2113028

Title: Robotic Model of Carausius morosus Middle Leg Used to Predict Sensory Discharge During Walking

Authors: *W. P. ZYHOWSKI[1], S. N. ZILL[2], N. S. SZCZECINSKI[1];
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Abstract: Animals adapt their motion by means of a variety of sensory organs and neural systems. The campaniform sensilla (CS), sensory organs located in groups in high-stress areas of the exoskeleton of the leg, is one such organ. Extracellular recordings of these sensors' discharge in response to exoskeleton strain have improved our understanding of how they encode forces acting on the body. However, due to interference from muscle potentials and other limitations, these recordings are challenging to collect in walking animals. In order to further our comprehension of the insect nervous system during walking we built a dynamically scaled robotic middle leg of the stick insect *Carausius morosus* (*C. morosus*). We attached strain

gauges to the leg in morphologically correct locations and orientations, namely CS groups 3, 4, 6A, and 6B. The robotic leg stepped on a treadmill to mimic walking. To ensure the leg supports the weight of the body, it was attached to a vertically-oriented linear guide. A computational model of CS discharge that was previously published was used to process data from the strain gauges. Our research indicates that the CS sensory discharges 1) strongly and selectively signal the beginning and end of the stance phase and 2) are sustained at lower levels during stance. Such signals would be essential for a robot or insect to maintain intra- and inter-leg coordination while traversing terrain.

Disclosures: **W.P. Zyhowski:** None. **S.N. Zill:** None. **N.S. Szczecinski:** None.

Poster

PSTR416. Posture and Gait: Control, Learning, and Biomechanics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR416.13/EE28

Topic: E.06. Posture and Gait

Title: Identifying the naked mole-rat behavioral fingerprint using machine vision approaches

Authors: ***R. SCHWARK**[1], S. OGUNDARE[2], P. CHANG[3], P. SHENG[4], I. ABDUS-SABOOR[5];
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Abstract: The naked mole-rat (*Heterocephalus glaber*) is a eusocial species of rodent which lives in large subterranean colonies in arid regions of east Africa. Colonies consist of a single breeding female, 1-3 breeding males, and dozens of non-reproductive drones organized in a dominance hierarchy. These animals have evolved an endless variety of adaptations and behaviors, including an exquisitely sensitive somatosensory system, abilities tunes to social dominance interactions, and unique navigation behaviors suited to an underground lifestyle in pitch-darkness. Despite decades of work, these behaviors have not been examined in a rigorously quantitative fashion. Here we use machine learning—specifically markerless tracking via SLEAP and unsupervised behavioral classification via MoSeq—to assay behavior in these animals for the first time. We show that naked mole-rats exhibit a wide variety of behavioral syllables that reflect their subterranean lifestyle, including fast locomotory movements (in both the forward and reverse directions) and robust digging behaviors. Isolated animals engage in certain syllables to a lesser extent than when placed in a social context with other mole-rats. We also demonstrate their highly social nature by showing that they exhibit a robust place preference for interacting with another conspecific compared to inanimate objects in the environment. MoSeq reveals that the nature of this social interaction is based heavily on snout-to-snout interactions, and that mole-rats show highly motivated digging towards conspecifics if separated. Furthermore, the queen mole-rat appears to exhibit an upregulation in locomotory behaviors and

appears to have a more hyperactive phenotype than the sterile workers in the colony. The behavioral fingerprint of a given worker furthermore appears to be modulated in the presence of a queen vs. in the presence of another worker. This data illustrates that the naked mole-rat behavioral suite is more nuanced than previously described, is divergent from that of mice, and is highly dependent on social context and caste.

Disclosures: R. Schwark: None. **S. Ogundare:** None. **P. Chang:** None. **P. Sheng:** None. **I. Abdus-Saboor:** None.

Poster

PSTR416. Posture and Gait: Control, Learning, and Biomechanics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR416.14/FF1

Topic: E.06. Posture and Gait

Support: Exercise and Rehabilitation Science Research Assistantship

Title: Corrective stepping responses and motor adaptations in young healthy adults in response to mediolateral and anteroposterior treadmill perturbations

Authors: *M. ADAM, A. HYNGSTROM, B. SCHMIT;
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Abstract: Motivation: Individuals with neurologic disorders often fall because of external balance perturbations and an impaired corrective stepping (CS) response. Training CS strategies through repeated exposure to external perturbations has shown promise in neurologic and aging populations, yet the impact of block versus randomized protocols on CS modifications is not known and is needed to guide accurate exercise prescription. For the purposes of future comparisons to clinical populations, we aimed to quantify how neurologically intact participants adapt their CS response to random external balance perturbations during treadmill walking within a session. We hypothesized that participants would increase their CS length and velocity following exposure to a randomized perturbation protocol. **Methods:** Twenty participants (10F/10M), aged 25.7 (4.31), ambulated on a treadmill at a self-selected speed. During mid right or left single limb stance, the base of the treadmill moved anteriorly, posteriorly, or in a destabilizing mediolateral direction. 4 perturbation intensities were provided for each condition in which the amplitude and velocity of the treadmill movement increased linearly. Perturbation conditions were repeated 5 times throughout the trial, and intensity, limb, and direction were randomized. Perturbations occurred every 7-13 seconds for 20 minutes, totaling 120 perturbations. 40 reflective markers were recorded via a 16-camera optical motion capture system to quantify the biomechanical CS response. **Results:** By comparing the first to last CS response to the most intense perturbation condition during the randomized perturbation protocol using paired t-tests, it was noted that participants did not increase their CS length or velocity following mediolateral (Width: -0.033m (0.046), -0.039m (0.044), $p = 0.58$; Velocity: -0.067m/s

(0.098), -0.078m/s (0.092), p = 0.62) or posterior (Length: 0.57m (0.055), 0.59m (0.065), p = 0.17; Velocity: 1.34m/s (0.24), 1.38m/s (0.21), p = 0.38) perturbations. Participants did lengthen and quicken their CS response following anterior perturbations (Length: 0.29m (0.19), 0.44m (0.10), p = 0.005; Velocity: 0.61m/s (0.34), 0.88m/s (0.20), p = 0.002). **Conclusion:** Young neurologically intact adults adapt to a single session of randomized external perturbation gait training by lengthening their CS and increasing their CS velocity after anteriorly, but not mediolaterally or posteriorly, directed perturbations. External perturbation paradigms may benefit from a combination of block and randomized protocols to limit contextual interference and ensure sufficient motor learning.

Disclosures: **M. Adam:** A. Employment/Salary (full or part-time):; Marquette University. **A. Hyngstrom:** A. Employment/Salary (full or part-time):; Marquette University. **B. Schmit:** A. Employment/Salary (full or part-time):; Marquette University.

Poster

PSTR416. Posture and Gait: Control, Learning, and Biomechanics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR416.15/FF2

Topic: E.06. Posture and Gait

Support: Univeristy of Colorado Anschutz - Boulder Nexus Grant
NIH National Center of Neuromodulation for Rehabilitation
P2CHD086844

Title: Acute intermittent hypoxia mediates the reduction of net metabolic power during motor learning and motor savings

Authors: *A. BOGARD[1], M. HEMMERLE[1], G. MARZLOFF[2], S. RYDER[2], A. SMITH[3], A. Q. TAN[1];
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Abstract: Breathing mild bouts of low oxygen air (i.e., acute intermittent hypoxia, AIH) has been shown to improve walking performance after a spinal cord injury. Evidence in spinally injured rodents suggest walking improvements after AIH result from increased synthesis of the plasticity-promoting neural substrate, brain-derived neurotrophic factor (BDNF). Importantly, elevated BDNF is paralleled by improvements in motor learning in humans. Thus, gains in walking performance after AIH may be partially driven by enhancements in motor learning. To test this hypothesis, we investigated the effects of repetitive AIH on sensorimotor adaptation during a split-belt walking task. It is well documented that healthy individuals adapt spatial and temporal parameters of step symmetry in response to novel belt speed perturbations. Given that spatiotemporal adaptation is associated with reductions in metabolic cost, we investigated whether AIH would reduce net metabolic power during split-belt walking. Study participants

were randomly assigned to an AIH or control group. Participants in the AIH group received treatments for five consecutive days. A single AIH treatment consisted of breathing 90-second bouts of hypoxic air (9% O_2) alternated with 60 seconds of normoxic air (21% O_2) for 15 episodes. On the fifth day, participants adapted their walking to an unexpected, belt speed perturbation for four conditions in the following order: (1) baseline with a 1:1 speed ratio, (2) adapt 1 with a 1:1.5 ratio, (3) washout with a 1:1 ratio, and (4) adapt 2 with a 1:1.5 ratio. Step length asymmetry (SLA), step time asymmetry (STA), and double support time asymmetry (DSA) were calculated for each condition. Expired gas was collected through open circuit spirometry and used to quantify net metabolic power. In this study, the AIH group achieved greater reductions in net metabolic power that was retained upon subsequent exposure to split-belt walking. Consistent with previous work, we found that both groups adapted their walking to minimize SLA and DSA while STA remained elevated. Interestingly, the AIH group had significantly greater STA and lower DSA during each adaptation condition. These preliminary results indicate that AIH-induced enhancements in motor learning leads to the selection of more energetically favorable temporal coordination strategies. Discerning changes in motor learning may clarify how AIH modulates walking recovery after neurological injury and is important for the translation of AIH to walking rehabilitation paradigms.

Disclosures: A. Bogard: None. **M. Hemmerle:** None. **G. Marzloff:** None. **S. Ryder:** None. **A. Smith:** None. **A.Q. Tan:** None.

Poster

PSTR416. Posture and Gait: Control, Learning, and Biomechanics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR416.16/FF3

Topic: E.06. Posture and Gait

Title: A preliminary kinematic comparison of Vive and Vicon systems for the accurate tracking of lumbar motion

Authors: N. YAGHOUBI[1], Z. MOORE[1], S. M. VAN DER VEEN[1], P. E. PIDCOE[1], J. S. THOMAS[1], *B. DEXHEIMER[2];
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Abstract: Title: A preliminary kinematic comparison of Vive and Vicon systems for the accurate tracking of lumbar motion**Authors:**Yaghoubi, N.[1], Moore, Z.[1], van der Veen, S.M.[1], Pidcoe, P.E.[1], Thomas, J.S.[1], & Dexheimer, B.[2]
[1]Department of Physical Therapy, College of Health Professions, Virginia Commonwealth University, Richmond, VA 23220[2]Department of Occupational Therapy, College of Health Professions, Virginia Commonwealth University, Richmond, VA 23220
Abstract: Optoelectronic 3D motion capture systems, such as the Vicon kinematic system, are widely utilized in biomedical research to track joint motion. These systems are considered

powerful and accurate measurement tools with <2 mm average error. However, these systems are costly and may be difficult to implement and utilize in a clinical setting. 3D virtual reality (VR) is gaining popularity as an affordable and accessible tool to investigate motor control and perception in a controlled, immersive environment. The HTC Vive VR system includes puck-style trackers that seamlessly integrate into its VR environments. These affordable, wireless, lightweight trackers may be more feasible for clinical kinematic data collection. However, the accuracy of HTC Vive Trackers (3.0) when compared to optoelectronic 3D motion capture systems, remains unclear. In this preliminary study, we compared the HTC Vive Tracker system to a Vicon kinematic system in a simulated lumbar flexion task. A 6-DOF robot arm (SCORBOT ER VII, Eshed Robotec/RoboGroup, Rosh Ha'Ayin, Israel) completed various reaching movements to mimic increasing levels of hip flexion (15°, 30°, 45°). Light reflective markers, along with one HTC Vive Tracker (3.0) were placed on the rigid segment separating the elbow and shoulder of the robot. We compared position measures simultaneously collected from both systems. Our preliminary analysis shows no significant differences between the Vicon motion capture system and the HTC Vive tracker in the Z axis, regardless of hip flexion. In the X axis, we found no significant differences between the two systems at 15 degrees of hip flexion but minimal differences at 30 and 45 degrees, ranging from .047 cm ± .02 SE ($p = .03$) at 30 degrees hip flexion to .194 cm ± .024 SE ($p < .0001$) at 45 degrees of hip flexion. In the Y axis, we found a minimal difference for 15 degrees of hip flexion only (.743 cm ± .275 SE; $p = .007$). This preliminary analysis shows that the HTC Vive Tracker may be an appropriate, affordable option for gross motor motion capture when the Vicon system is not available, such as in clinical settings. Further research is needed to compare these two motion capture systems in different body poses and for different body segments.

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Poster

PSTR416. Posture and Gait: Control, Learning, and Biomechanics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR416.17/FF4

Topic: E.06. Posture and Gait

Support: FRQS Grant 324010
Praxis Grant G2021-36

Title: Modulation of leg trajectory by transcranial magnetic stimulation during walking in human.

Authors: *H. BOURGEOIS[1], R. GUAY HOTTIN[2], H. TONKOV[3], E.-M. MEFTAH[3], M. MARTINEZ[4], M. BONIZZATO[4,2], D. BARTHELEMY[5,3];
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Abstract: ln a recent study, using a rat model of incomplete spinal cord injury, we demonstrated that by delivering intracortical microstimulation during locomotion, at specific timings during the swing phase of gait, we were able to achieve precise control of leg trajectories and effectively alleviate gait deficits associated with incomplete paraplegia. Expanding on these findings, we here investigate whether cortical stimulation could similarly modulate human locomotion. ln this study, we used Transcranial magnetic stimulation (TMS), a non-invasive brain stimulation technique, to achieve this goal and determined the effect of TMS applied over the leg representation of the left motor cortex at different timings during the swing phase of the right leg in healthy subjects. Our goal was to characterize the varying effects that the timing of stimulation delivery during the swing phase may have. For a comprehensive characterization, we applied stimulation at random delays that encompassed the entirety of the right swing phase. These were done at intervals of 0, 100, 200, 300, and 400 ms after toe off. The stimulation intensity used was 120% of the movement threshold, which was defined as the minimum intensity required to elicit a dorsiflexion at the ankle during rest for at least 5 out of 10 stimuli. We found that stimulation delivered during the swing phase led to increased leg swing movement, particularly when administered early in the swing phase. lndeed, both the toe and knee excursion during walking significantly increased for four out of the five participants tested. On average, the maximum vertical toe position increased by 36.5 mm (54%,+/-30%) compared to the position during a spontaneous step, while the knee position increased by 45.4 mm (64.5%,+/-36%). However, it is noteworthy that in some subjects, stimulation delivered in the late swing phase altered foot placement moments before landing, leading to minor loss of balance. Our findings indicate that transcranial magnetic stimulation (TMS) can modulate leg trajectory during walking and highlight the potential of TMS as a valuable tool to investigate cortical control of locomotion. This can inform development of more invasive and specific neuromodulation intervention that may improve exercise-mediated recovery in gait rehabilitation therapy.

Disclosures: H. bourgeois: None. **R. Guay Hottin:** None. **H. Tonkov:** None. **E. Meftah:** None. **M. Martinez:** Other; Submitted an international patent application (PCT/CA2020/051047) covering a device allowing performing cortical stimulation during movement. They are also co-founders of 12576830 Canada Inc., a start. **M. Bonizzato:** Other; Submitted an international patent application (PCT/CA2020/051047) covering a device allowing performing cortical stimulation during movement. They are also co-founders of 12576830 Canada Inc., a start. **D. Barthelemy:** None.

Poster

PSTR416. Posture and Gait: Control, Learning, and Biomechanics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR416.18/FF5

Topic: E.06. Posture and Gait

Support: NSF CAREER Grant 1944207

Title: Momenta control during each phase of gait reveals a common strategy between straight-line gait and 90-degree turns.

Authors: *M. TILLMAN, A. ZAFERIOU;
Biomed. Engin., Stevens Inst. of Technol., Hoboken, NJ

Abstract: Transverse-plane linear and angular momentum must be managed simultaneously to accomplish a turn. We recently uncovered that young adults leverage existing control strategies used to change momentum (and the average values of the momentum derivatives, force and moment) during each phase of gait during straight-line gait to perform a left turn. In straight-line gait and left turns, right single support generated the largest change in leftward linear momentum, while left double support generated the largest change in leftward angular momentum (Tillman et al., 2023). The purpose of this study is to determine if older adults use the same phases of gait as young adults to generate transverse-plane linear and angular momentum – and force and moment – during straight-line gait and turns.Nine healthy and active older adults (2 m 7 f; 71±6 yrs) performed two tasks. First, they walked 10 m straight 10 times along the lab's +Y axis. Next, they performed 10 90° left turns (+Y axis for 5 m, then -X axis for 4 m). Whole-body kinematics were recorded via reflective markers attached to 13 segments (250fps, Optitrack). We identified four phases of gait (left and right single and double support) using the method from (Zeni et al., 2008) modified for turning gait (Ulrich et al., 2019). We computed the change in linear momentum in the global X direction (dP_x) and the change in angular momentum about the Z axis (dH_z) during each phase of gait. The average force (Fx_{avg}) and moment (Mz_{avg}) were computed by dividing dP_x and dH_z, respectively, by the gait phase durations. Sign tests were used to compare each metric at a group level between gait phases for straight-line gait and turns (α=0.05). During straight-line gait and turns, the largest dP_x and dH_z were generated during right single support and left double support phases, respectively (all comparisons $p<0.04$). The largest Mz_{avg} occurred during left double support (all $p=0.004$). During straight-line gait, Fx_{avg} of right single support was greatest (all $p<0.04$). During turns, right single support Fx_{avg} was greater than it was during left single support ($p=0.004$), but not other gait phases (all $p>0.18$). Sex has not yet been assessed as a biological variable.Older adults generated momenta during straight-line gait and turns during the same phases of gait, suggesting a common control strategy. Further, these results mimic those found in young adults (Tillman et al., 2023). Understanding momenta control within each gait phase could lead to targeted interventions applicable to various types of turns and potentially other nonlinear gait such as side-stepping. Future work will include studying a wider range of older adults, including those with a history of falls.

Disclosures: M. Tillman: None. **A. Zaferiou:** None.

Poster

PSTR416. Posture and Gait: Control, Learning, and Biomechanics

Location: WCC Halls A-C

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Program #/Poster #: PSTR416.19/FF6

Topic: E.06. Posture and Gait

Support: NSF-GRFP1747452
NIH 5T32GM081760-12
NSF 1847891

Title: Characterization of locomotor adaptation and generalization dynamics from high-dimensional neuromuscular data

Authors: *D. MARISCAL, K. FJELD, G. TORRES-OVIEDO;
Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Humans can adapt their gait to compensate for changes in environmental demands and generalize learned movements from one situation to another. One way to study the processes that underlie locomotor adaptation is by exposing participants to split-belt treadmill walking; this paradigm induces robust changes in gait kinematics and muscle activation patterns. Additionally, we can study generalization by contrasting the adaptation effects (i.e., aftereffects) that participants exhibit in the same (treadmill) or different (overground) contexts from the adaptation. Here, we propose a novel approach for characterizing locomotor adaptation by analyzing individual muscle activation patterns. We ask what processes underly the adaptation of muscle activation patterns and what aspects are observed during generalization (overground). We hypothesize that at least two processes with distinct dynamics underlie the changes in muscle activity during locomotor adaptation and generalization. Specifically, we posit that a fast reactive process will recruit a neuromuscular pattern to maintain balance at each transition between walking environments. Whereas a slow adaptive process will forge a contextual pattern meeting the demands of the novel split environment, this pattern will be slowly disengaged during post-adaptation (tied walking) on the treadmill and will not be used during overground post-adaptation due to environmental differences. We recorded the activity of 28 leg muscles of twenty-four young adults (<40 yrs. old) who experienced split belt walking during their adaptation, and their de-adaptation was measured on either the treadmill (n=12) or overground (n=12) walking. We used a data-driven approach to measure individual muscles' reactive and contextual patterns to reproduce the evolution of muscle activity during the split-belt walking paradigm. Our analysis showed that the reactive and contextual processes contribute to the adaptation and post-adaptation of muscle activity on the treadmill. However, during overground post-adaptation, 2 out of the 28 muscles generalized the contextual pattern and all other muscles exhibit the reactive pattern, suggesting that the kinematic effects previously reported overground are mostly induced by reactive processes in response to a small number of muscles generalizing the split pattern. These findings provide insights into locomotor adaptation features beyond those drawn from traditional kinetic or kinematic analyses, which can be leveraged to study the effect of aging and brain lesions on the carryover of muscle activity.

Disclosures: D. Mariscal: None. **K. Fjeld:** None. **G. Torres-Oviedo:** None.

Poster

PSTR416. Posture and Gait: Control, Learning, and Biomechanics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR416.20/FF7

Topic: E.06. Posture and Gait

Support: NSF Grant 2015317

Title: Embodying synthetic nervous system control in biomimetic robots with passive viscoelastic joints

Authors: *F. O. HOLMQUIST[1], C. GOLDSMITH[1], G. SUTTON[2], N. S. SZCZECINSKI[1]; [1]Mechanical and Aerospace Engin., West Virginia Univ., Morgantown, WV; [2]Univ. of Lincoln, Univ. of Lincoln, LINCOLN, United Kingdom

Abstract: Each nervous system is tuned to control and is constrained by the unique mechanics of its body. Understanding this harmony is necessary to understand and model how animals move their bodies. For example, while animals walk, mechanical energy delivered to the skeleton by the muscles is converted into kinetic energy of moving body parts, converted into gravitational potential energy of raised body parts, converted into elastic potential energy within muscles and tendons, and/or dissipated as heat due to viscosity within the body. To stabilize motion or reject perturbations, the nervous system monitors whether the body's mechanics can passively absorb energy and will activate muscles if they cannot. Prior modelling work suggests that the dominant form of energy is dictated by the size of an animal and speed of its stepping cycle. Because of this, we hypothesize that the nervous system's control strategies similarly depend on its size and speed. We use biomimetic legged robots modelled after insects to test this hypothesis in a real-world system. Stabilizing motion and rejecting perturbations is further complicated by sensorimotor delays due to synaptic transmission along sensory feedback pathways, muscle recruitment, and axon length and myelination. Legged robots face similar challenges regarding delays (although from different sources), and may struggle to maintain stability when subjected to perturbations and harsh impacts. Recent simulations suggest that mimicking the passive viscoelasticity within robots' joints increase stability in the face of such delays. Such delays may also increase animals' reliance on feedforward control strategies in which the passive viscoelasticity within their joints is largely responsible for stabilizing motions. To test the effect of passive viscoelastic joints on sensorimotor control and scale-driven nervous system strategies in a real-world system, we developed a device that introduces stiffness and damping to the motorized joints of robots. The device is a torsional spring infused with silicone whose viscoelastic properties mimic energy storage and dissipation an insect's joint. The device decreases the relative importance of the limb's kinetic energy by increasing the elastic potential energy stored and viscous energy dissipated during walking. The resulting dynamically-scaled insect-like robot may increase understanding of how scale and passive dynamics affect the nervous system.

Disclosures: F.O. Holmquist: None. **C. Goldsmith:** None. **G. Sutton:** None. **N.S. Szczecinski:** None.

Poster

PSTR416. Posture and Gait: Control, Learning, and Biomechanics

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Topic: E.06. Posture and Gait

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DFG NeuroNex Bu857/15
"iBehave" network funded by the Ministry of Culture and Science of the State of North Rhine-Westphalia

Title: Using a hexapod robotic model of Drosophila to investigate walking forces and strains

Authors: *C. GOLDSMITH[1], W. P. ZYHOWSKI[1], M. HAUSTEIN[2], G. F. DINGES[1], A. BÜSCHGES[2], N. S. SZCZECINSKI[1];
[1]Mechanical and Aerospace Engin., West Virginia Univ., Morgantown, WV; [2]Inst. of Zoology, Univ. of Cologne, Cologne, Germany

Abstract: Sensory feedback is an important part of how the nervous system controls adaptive walking. Feedback from sense organs continually tunes activity throughout the nervous system, which in turn shapes limb movements. Campaniform sensilla (CS), receptors located in high stress areas of the exoskeleton that measure load, are one such type of sensory organ in insects. Leg CS have been found to have effects on walking behaviors in several species of insect. However, electrophysiological recording from CS in free-walking animals poses technical challenges, particularly in smaller insects like the fruit fly, *Drosophila melanogaster.* Biomimetic robotic models of insect legs can circumvent these constraints by facilitating strain recordings during biomimetic stepping. Affixing strain gauges in leg CS locations produces data that increases our understanding of the load feedback signaled by CS in freely moving animals. We have previously developed physical robotic models of stick insect and fruit fly middle legs with biomimetic strain sensing. These legs were used to compare CS discharge between insects and the role of tarsal morphology in strain the during stepping on a treadmill.We have also developed Drosophibot II, a 140:1 robotic model of an adult fruit fly. The proportions and degrees of freedom in each leg of Drosophibot II have been modeled after *Drosophila* 3D motion capture data, producing a robot that is kinematically similar to the animal. We developed a program to solve the inverse kinematics necessary for walking and solve the inverse dynamics necessary for mechatronic design. By applying this solver to a fly-scale body structure, we have demonstrated that the robot mimics the animal's dynamics and can hypothesize insect joint torques, internal limb forces, and ground reaction forces. The robot has been validated for straight-line forward and backward walking with biologically inspired foot trajectories. Including strain sensing on Drosophibot II allows us to make hypotheses about CS discharge in all six legs for a variety of walking directions. This data can then be used to investigate how the sensory

discharge in each leg pair may differ depending on their role in stepping, and how this discharge may change with walking direction.

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Poster

PSTR416. Posture and Gait: Control, Learning, and Biomechanics

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Program #/Poster #: PSTR416.22/FF9

Topic: E.06. Posture and Gait

Support: NIH Grant NIA 5R01AG054621-05

Title: Faster walking speeds are retained after experiencing discrete mediolateral perturbations

Authors: *L. LEE[1], C. R. CASTANO[1], H. J. HUANG[2];
[2]Mechanical and Aerospace Engin. Dept., [1]Univ. of Central Florida, Orlando, FL

Abstract: Locomotor adaptation is a motor learning process that can be used to alter ones gait. Uncertain environments, such as perturbations to disrupt ones walking pattern, are a common approach to induce locomotor adaptation processes. Previous locomotor adaptation studies used fixed speed treadmills that do not allow subjects to change their walking speed, which is not reflective of a real-world response. Perturbations can be applied on a self-paced treadmill, which allows participants to experience uncertain environments without constraining walking speed. In a previous study, we implemented discrete mediolateral perturbations on a self-paced treadmill with varying levels of predictability and found walking speed increased compared to a self-paced walking condition. However, we did not analyze if the faster walking speeds were retained after the perturbations were removed. The purpose of this study is to conduct follow up analyses from a locomotor adaptation perspective. We can explore adaptation because our perturbation conditions contained periods of self-paced walking before and after the perturbations. We hypothesized that young and older adults would retain faster walking speeds after the perturbations were removed and that these speeds would carry over to later conditions.Young (n=10) and older adults (n=9) walked on a self-paced treadmill as we recorded lower limb movements using motion capture. There were 4 perturbation conditions randomized across subjects that were combinations of magnitudes (1, 3 or 5cm) of the mediolateral treadmill shifts and timings during the gait cycle (loading response, terminal stance, or mid-swing). Each condition was in self-paced mode for 80 strides before (PRE) and after (POST) the 200 perturbations were applied every other stride (PERT). Conditions were analysed in chronological order to determine if faster speeds were retained from one condition to the next. Walking speed was also compared within a condition between PRE, PERT and POST phases.
Walking speed generally increased with conditions in chronological order such that the walking speeds PRE and POST of the final condition were the fastest. Comparing walking speed between

PRE and PERT, young adults walked faster during PERT compared to PRE whereas older adults walked slower during PERT. In young adults, POST speeds were slower than PERT but faster than PRE. In older adults, POST speeds were faster than PERT and either as fast or faster than PRE. Our results show that when given the freedom to change walking speeds, subjects opted to walk faster after perturbations were removed and these faster walking speeds were retained and carried over to later conditions.

Disclosures: L. Lee: None. **C.R. Castano:** None. **H.J. Huang:** None.

Poster

PSTR416. Posture and Gait: Control, Learning, and Biomechanics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR416.23/FF10

Topic: E.06. Posture and Gait

Support: U.S. Army Medical Research Award No. W81XWH2210684

Title: Is Micro-Doppler radar effective as a standalone measure of gait kinematics?

Authors: *E. I. JONES[1], V. VENEZIA[1], B. SIMONE[2], M. YANKELLO[2], C. ONKS[3], J. NYLAND[4], R. NARAYANAN[2], R. CREATH[1];
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Abstract: Introduction: Micro-Doppler radar (MDR) has emerged as a cost-effective method for human gait analysis. Combined with machine learning, MDR can discern subtle movement differences making it a potentially valuable tool for assessing neuromechanical gait conditions associated with disease or injury. MDR works the same as “police” radar. Movements create modulations in the reflected MDR signal known as the micro-Doppler effect. By analyzing frequency modulations, one can infer movement characteristics. While several studies have demonstrated MDR capabilities, radar signal consistency has not been assessed. Since inconsistencies can alter signal magnitude or cause frequency shifts, variation would affect movement characterization. The purpose of this study is to compare MDR signal consistency between a mechanical pendulum (Pd) and subjects performing a step-in-place task (STP) to determine differences in signal strength and Doppler frequency shift.
Methods: 6 adults (2f; 21.8 yrs) performed a STP task (60 sec @ 60 spm) at 6 locations on a radial grid. The Pd (height 44 cm) rotated from horizontal to vertical in 1.4 sec. Antennae were 3m above the floor facing 45 deg downward. The MDR consisted of a horn antenna emitting a 10 GHz signal at 1 watt. The reflected signal was received by an identical antenna with a sample rate of 20 KHz. Data were collected at 44.1 KHz. Radial grid locations (6) relative to the antennae were at 2 distances (4.2 and 5.0m) and 3 radial angles (0 deg, 10 deg right and left of center). Fast Fourier transforms (FFTs) of the time series were calculated which were normalized

to the maximum FFT amplitude of location 4.2m and 0 deg. The integrals of the amplitude spectra were compared to determine amplitude and frequency changes for the Pd and STP conditions for all 6 locations.
Results: For the Pd trials, MDR amplitude decreased 27% for 10 deg left, 78% for 10 deg right, and 4% from 4.2m to 5.0m. For the STP trials, the MDR signal decreased 51% for 10 deg left and 71% for 10 deg right, but increased 11% from 4.2m to 5.0m. The Pd FFT integrals showed a shift towards lower frequencies while the STP FFT integrals were similar between higher and lower frequencies.
Conclusions: The MDR signal is most consistent along the 0-deg radial path. Angular deviations of ±10 deg produce changes in signal amplitude. Frequency shifts are likely specific to the type of task. Given the narrow path of the strongest signal (0 deg), gait studies could be affected by deviations from the 0-deg path. Slight shifts ±10 deg could place one side of a subject's body in a zone of weaker signal, creating an asymmetrical Doppler shift that appears as an artifact of signal strength rather than a movement component.

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Poster

PSTR416. Posture and Gait: Control, Learning, and Biomechanics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR416.24/FF11

Topic: E.06. Posture and Gait

Support: R01NS125863

Title: Heterogeneous and flexible control of triceps surae during standing balance in humans

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Abstract: During bipedal standing, the triceps surae modulate their activity considerably with anteroposterior oscillations of the center of mass. This has led to the assumption that the triceps surae act primarily to generate of antigravity plantarflexion torques during standing balance. The heterogeneous architecture of the triceps surae suggests each constituent muscle can contribute to ankle torques outside of the sagittal plane, but is not necessary during bipedal standing since mediolateral control of sway is accomplished largely by loading and unloading the hips. However, during tasks that challenged mediolateral stability, the triceps surae may be controlled independently to generate corrective torques in the mediolateral plane. This study examined how the muscles of the triceps surae tuned their activity to centre of pressure (COP) movement about 360 degrees during one- and two-legged standing. Ten healthy young adults completed a total of 16 one- and two-legged standing trials lasting 30 and 120-s, respectively. Muscle activity was recorded from high-density electrode arrays (64 channels) adhered to the skin overlying the right

soleus (SOL) and medial (MG) and lateral (LG) gastrocnemii, while COP was recorded from independent force plates. A rotation matrix was iteratively applied to the 2-dimensional COP data, generating 360, 1-D timeseries corresponding to COP movement about 360 degrees. Prominent peaks were identified in each COP timeseries. EMG were then triggered-averaged to each peak and the amplitude of event-related EMG was calculated to construct EMG tuning curves. During two-legged standing, all muscles of the triceps surae showed uniform EMG tuning curves, with maximal activity oriented primarily in the anteroposterior plane. During one-legged standing, significant deviation of the tuning curves were observed, with LG showing a near-opposite pattern of activation compared to SOL and MG. In particular, LG was maximally activated during COP deflections that were consistent with eversion, but inhibited during inversion, while SOL and MG were maximally activated during COP deflections consistent with inversion, but inhibited during eversion. These results demonstrate that, depending on the nature of the balance task, muscles of the triceps surae can contribute to corrective ankle torques outside of the sagittal plane. The triceps surae act as a functional unit during bipedal standing, but remarkably independent during unipedal standing, such that muscles that normally behave as synergists behave as antagonists. Future work will examine if this flexible neural control of postural musculature is modulated through training and disease.

Disclosures: **M. Zaback:** None. **M. Topley:** None. **C.K. Thompson:** None.

Poster

PSTR416. Posture and Gait: Control, Learning, and Biomechanics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR416.25/FF12

Topic: E.06. Posture and Gait

Support: Casio Science Promotion Foundation

Title: Non-contractive tissue is an important component in regulating the degree of freedom of fingers during the tapping task

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Abstract: 1. Motivation
The central nervous system constrains the degree of freedom of joint to simplify the regulating for accurate performance. Previous studies have revealed the role of muscle contraction, which is the coordination of multiple muscles or motor units, in evaluating movement stability (Ting+, 2005; Madarshahian+, 2022). However, focusing on only muscle contraction does not fully explain the control of joint movements. Joints are also composed with non-contractive tissues;

ligament, meniscus, and joint capsule. These contribute to regulating a joint degree of freedom. Here we aimed to clarify the relationship between the stability of finger movements and non-contractive tissues, collateral ligament, to constrain the motion of the metacarpophalangeal (MP) joint when the MP joint is flexed during tapping.
2. Methods
Eight healthy adults provided informed consent after a detailed explanation of the purpose and risks of the study based on the Declaration of Helsinki. 3D motion capture system was used to obtain the position of the markers attached to the right hand. We measured two tasks; (Exp. A) the subjects placed their right hand on a desk and moved the index finger as widely as possible; (Exp. B) the subjects repeatedly tapped 7 and 9 on a keypad with the index finger in time to a metronome at 180 bpm. The marker trajectories, joint angles, and tapping times were analyzed using Python 3.7. This study was approved by the Ethical Review Committee.
3. Results
In Exp. A, the fingertip trajectories of all subjects showed an elliptical shape with a long vertical direction. As the flexion angle of the MP joint increased, the abduction angle of the MP joint showed a decreased ($r = -0.65$ to -0.98). In Exp. 2, which focused on the lateral movements, subjects were classified into two groups: a group that moved the MP joint dominantly (MPD, $n = 4$) and a group that moved the MP joint and the wrist joint (MPW, $n = 4$). The variation in tapping times of the MPW was less than that of the MPD.
4. Conclusion
The collateral ligaments of the MP joint have the role of constraining lateral motion when the MP joint is flexed. This property of ligament was selected to constrain the degree of freedom of the MP and wrist joint. This function contributed to accomplishing the temporal accuracy of task performance with the low cost of central nervous systems. Analyzing each joint's movement pattern depending on the role of contractive and non-contractive tissues, which exploits the effect of the ligament, may facilitate the learning of efficient movements.

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Poster

PSTR416. Posture and Gait: Control, Learning, and Biomechanics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR416.26/FF13

Topic: E.06. Posture and Gait

Support: NIH 1P41EB018783
VA Merit Award 01 CX001812

New York State Spinal Cord Injury Research Board SCIRB C32236GG
New York State Spinal Cord Injury Research Board SCIRB C33279GG
Stratton VA Medical Center, Albany, NY

Title: Studying the Negotiated Equilibrium Between the Substrate of a New Motor Skill and the Substrate of an Old One: Protocol Design and Testing

Authors: *J. R. WOLPAW[1], R. L. HARDESTY, Jr.[2], H. MOJTABAVI[3];
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Abstract: The substrate of a motor skill is a distributed network of neurons and synapses that produces the skill and changes continually to maintain the key features of the skill, the attributes that make it satisfactory (Neuroscientist 16:532-549, 2010; JPhysiol 596:3469-3491, 2018; JPhysiol 2022, DOI:10.1113/JP283291). This network has recently been given the name *heksor* from the Greek *hexis*. Because heksors share the CNS, their concurrent changes constitute a negotiation; they negotiate the properties of neurons and synapses they all use. They keep the CNS in a state of negotiated equilibrium that allows each heksor to maintain its skill. We hypothesize that the negotiated equilibrium between a new skill and an old skill that share spinal motoneurons; (1) comprises plasticity at many sites from cortex to spinal cord; (2) entails EMG/kinematic changes in the old skill; (3) maintains key features of the old skill. To test this hypothesis, we are studying the impact on normal locomotion of learning to walk on a splitbelt treadmill with belts going in opposite directions. Participants are healthy adults. Each of the 12 study sessions (2-3/wk) has three parts. First, the person walks on the treadmill for 2 min with both belts going in the same direction at 80% of a self-selected speed. Second, right-belt speed is gradually decreased and then reversed until is equal but opposite to left-belt speed. The person walks on these opposing belts for four 5-min periods separated by 1-min breaks. Third, the participant again walks on the treadmill for 2 min with both belts going in the same direction. Before, after, and/or during the sessions, we collect: locomotor EMG and kinematics; soleus and tibialis anterior H-reflexes and motor evoked potentials to transcranial magnetic stimulation; and somatosensory evoked potentials to tibial and common peroneal nerve stimulation. The protocol has functioned well in 6 initial participants. Each arrived over the first split-belt period at a satisfactory locomotor pattern. Two companion abstracts present initial data analyses.

Disclosures: J.R. Wolpaw: None. **R.L. Hardesty:** None. **H. Mojtabavi:** None.

Poster

PSTR416. Posture and Gait: Control, Learning, and Biomechanics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

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Topic: E.06. Posture and Gait

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New York State Spinal Cord Injury Research Board SCIRB C32236GG
New York State Spinal Cord Injury Research Board SCIRB C33279GG
Stratton VA Medical Center, Albany, NY

Title: Aftereffects of Locomotor Adaptation to Split-belt Training in Opposing Directions: Initial Results

Authors: *R. L. HARDESTY, Jr.[1], H. MOJTABAVI[1], J. R. WOLPAW[2];
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Abstract: Locomotion is a highly adaptive behavior, allowing us to traverse new environments and accommodate changing demands. Split-belt protocols are widely used to study adaptation to asymmetric walking conditions; they have frequently found aftereffects with return to symmetrical walking (e.g., Choi & Bastian Nat NS 2007). The existing literature focuses on single-session studies. We plan multi-session studies to define the long-term impact of asymmetric split-belt training (see Wolpaw et al. abstract). This initial report examines, for our first session, the aftereffects of training with the belts moving in opposite directions. We recruit healthy adults. First, they walk for 2 min on a Bertec split-belt treadmill with both belts at 80% of their self-selected speed to define baseline gait. Then, they walk for four 5-min Training blocks separated by 1-min breaks while left-belt movement is the same as baseline but right-belt movement is in the opposite direction at equal speed. Finally, they walk again for 2 min with both belts in the same direction. We track movement with 41 reflective markers and motion capture (Qualysis). We record ground reaction forces, center-of-pressure, and soleus and tibialis anterior EMG activity in both legs. We compare symmetrical walking during baseline and post-training to assess whether adaptations adopted in the Training block persist. The 6 initial participants performed the training blocks well. Preliminary analyses show kinematic and EMG changes that enabled the training walk (see Mojtabavi et al abstract). With return to symmetric locomotion, most of these adaptations disappeared; stride length and inter-step variability quickly returned to their pre-training values. In contrast, double-stance time (% of the step cycle in which both feet touch the ground), which showed a participant-specific change in the Training blocks, remained altered after return to symmetric locomotion. This is our first clear indication of an aftereffect. Further analyses and data collection are underway.

Disclosures: R.L. Hardesty: None. **H. Mojtabavi:** None. **J.R. Wolpaw:** None.

Poster

PSTR416. Posture and Gait: Control, Learning, and Biomechanics

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VA Merit Award 01 CX001812
New York State Spinal Cord Injury Research Board (SCIRB) C32236GG & C33279GG
Stratton VA Medical Center, Albany, NY

Title: Locomotor Adaptation to Split-belt Training in opposing Directions: Initial Kinematic Analyses

Authors: *H. MOJTABAVI[1], J. R. WOLPAW[2], R. HARDESTY[3];
[1]Natl. Ctr. for Adaptive Neurotechnologies, Albany, NY; [2]Natl. Ctr. for Adaptive Neurotechnologies, Natl. Ctr. for Adaptive Neurotechnologies, Delmar, NY; [3]Natl. Ctr. for Adaptive Neurotechnologies, Sauquoit, NY

Abstract: Humans can readily adapt their gait to different environments. Split-belt treadmill protocols have helped to elucidate these adaptations and their underlying neural correlates. Studies to date have focused on adaptations that occur in a single session. We are developing a protocol to examine long-term plasticity induced by multi-session walking on a treadmill with belts moving in opposing directions (Wolpaw et al. abstract). This initial report assesses, for our first session, the kinematic adaptations to the belts moving in opposing directions. We are studying healthy adults. First, they walk for 2 min on a Bertec split-belt treadmill with both belts at 80% of their self-selected speed. This defines baseline gait. They then walk for four 5-min Training blocks separated by 1-min breaks with left-belt movement the same as baseline but right-belt movement in the opposing direction at equal speed. Lastly, they again walk for 2 min with both belts in the same direction. We record: movement using 41 reflective markers and motion capture (Qualysis); ground reaction forces; center-of-pressure; and soleus and tibialis anterior EMG activity in both legs.During the Training blocks, our initial 6 participants maintained coordination and balance with minimal difficulty. Each adopted a cyclical gait pattern within the first Training block. At the same time, inter-step variability in both kinematics and EMG was greater than in the baseline and post-training blocks, presumably due to the novelty and challenge of walking with the belts opposed. Stride length was shorter than during baseline. Double-stance (% of step cycle with both feet on the ground), was significantly altered compared to the pre-training block. Interestingly, this change in double-stance time varied across participants; it increased in some and decreased in others. This suggests that different people reach different solutions for walking with the belts moving oppositely. Current analyses seek to delineate the strategy adopted by each participant.

Disclosures: H. Mojtabavi: None. **J.R. Wolpaw:** None. **R. Hardesty:** None.

Poster

PSTR416. Posture and Gait: Control, Learning, and Biomechanics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR416.29/FF16

Topic: D.05. Auditory & Vestibular Systems

Support: NIH/NICHD T32HD007414-29 (RA)
NIH R01-DC018061 (KEC)
NIH R01-DC002390 (KEC)
NIH 1UF1NS111695-01 (KEC)

Title: Head motion kinematics in pre-surgical superior canal dehiscence syndrome patients

Authors: *R. ARYAN[1], J. L. MILLAR[2], J. P. CAREY[3], M. C. SCHUBERT[3], K. E. CULLEN[1,3,4];
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Abstract: Introduction: Superior Canal Dehiscence Syndrome (SCDS) is characterized by the abnormal thinning or formation of an opening on the bony structure of the superior semicircular canal. It is well-documented that SCDS causes sound/pressure-induced dizziness and nystagmus, resulting in chronic balance difficulties. Here we asked whether impaired function of the superior canal alters head motion generation in these patients. Specifically, we hypothesized that head kinematics in the pitch axis would be the most altered, since this axis corresponds to that which is maximally activated by stimulation of the superior semicircular canal. Accordingly, we measured head kinematics between healthy controls and pre-surgery SCDS patients while performing gaze stabilization exercises. **Methods:** Using an inertial measurement unit, 6D head movements of SCDS patients (n=17) were measured and compared with healthy controls (n=14). Participants performed standard of care gaze stabilization exercises in yaw and pitch planes with far and near earth-fixed targets. We then computed range, standard deviation, and asymmetry of linear and rotational kinematics for each exercise. **Results:** No significant between-group differences were found during pitch gaze stabilization exercises. However interestingly, our results revealed significant between-group differences in pitch velocity during yaw gaze stabilization exercises. Notably, while performing yaw gaze stabilization using a near target, our SCDS group showed higher variability and asymmetry in head pitch velocity. Furthermore, in the same task, these SCDS patients showed reduced range of yaw velocity. Additionally, correlational analysis showed significant correlations between the head kinematics and clinical measures, including logMAR (logarithm of the minimum angle of resolution, which measures visual acuity) and Beck anxiety scores. **Conclusion:** Our results confirmed that head kinematics alter in pitch axis in pre-surgery SCDS, but surprisingly during yaw head movements. Furthermore, our study suggests that impaired visual acuity and high level of anxiety are associated with more variable head kinematics in SCDS. Overall, we conclude that an objective and multiplanar examination of head kinematics in SCDS is necessary, which can ultimately lead to more effective treatment interventions.

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Poster

PSTR417. Neuromodulation in Rhythm Generating Networks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR417.01/FF17

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant R21NS111355

Title: Mechanisms underlying generation of episodic bursting activity in a half-center oscillator

Authors: *M. FOMENKO[1], E. SMITH[2], Y. SHAMS[3], G. CYMBALYUK[2];
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Abstract: Repertoire of animal locomotor behaviors includes episodic locomotion, where episodes of movement are separated by periods with no movement. Recent experimental and modeling studies in vertebrates like mouse and fish spinal cords suggest that spinal circuits, called central pattern generators (CPGs), can produce episodic locomotor rhythm (Sharples et al., 2021). Invertebrate preparations like the leech heart CPG offer a cellular-level understanding of circuit dynamics and a biophysical model that has a high predictive capacity. This model is capable of representing experimental recordings and suggesting new hypotheses that are testable in experiments (Ellingson et al., 2021). The rhythm of this CPG is based on two pairs of mutually inhibitory bursting neurons, half-center oscillators (HCOs). Here, we found the episodic bursting regime in the model of the leech heart HCO. Relatively to the canonical parameter set (Ellingson et al., 2021), the new regime exists in the domain with upregulated h-current (Ih) and downregulated persistent sodium current (INaP) and Na+/K+ pump current (IPump). We found that the episodic bursting generation is based on slow oscillations of reversal potential of Na+ (ENa). During a bursting phase, ENa decreases due to increase in intracellular Na+ concentration, [Na+]i, reducing all the Na+ inward currents, and eventually terminates an episode. During the interepisode interval, when inward Na+ currents are small, but IPump is active, ENa increases due to decrease in [Na+]i which eventually leads to the start of new episode. The episodic bursting is affected by parameters determining [Na+]i. The episode duration and the number of bursts per episode positively correlate with the parameter of intracellular Na+ diffusion (Naidiff) and maximal conductance of h-current (Gh), and negatively correlates with conductances of leak current (gleak), maximal conductance of INaP (GNaP) and maximal pump current (IPumpMax). The interepisode interval positively correlates with gleak and Gh and negatively correlates with Naidiff and IPumpMax. Both burst duration and interburst interval notably positively correlate with GP and negatively with gleak. Importantly, manipulations of the Naidiff parameter allowed us to have an arbitrary number of bursts per episode and, therefore, episode duration. Manipulations of the IPumpMax parameter allowed us to have interepisode interval of an arbitrary long time.

Disclosures: **M. Fomenko:** None. **E. Smith:** None. **Y. Shams:** None. **G. Cymbalyuk:** None.

Poster

PSTR417. Neuromodulation in Rhythm Generating Networks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR417.02/FF18

Topic: E.07. Rhythmic Motor Pattern Generation
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NSERC grant RGPIN/04394-2019 to PJW

Title: The interaction of calcium-activated potassium current and NMDA receptor current contributes to the episodic locomotor rhythms produced by mice and zebrafish

Authors: M. FOMENKO[1], J. MILLA CRUZ[3], B. MERCIER[4], S. M. KOROGOD[2], M. A. MASINO[5], P. WHELAN[6], *G. CYMBALYUK[1];
[1]Neurosci. Inst., [2]Ctr. for Behavioral Neurosci., Georgia State Univ., Atlanta, GA; [3]Univ. of Calgary, Calgary, AB, Canada; [4]Neurosci., Univ. of Minnesota, Twin Cities, Minneapolis, MN; [5]Dept. of Neurosci. and Rehabil. Sci. Program, Univ. of Minnesota, Minneapolis, MN; [6]Univ. Calgary, Calgary, AB, Canada

Abstract: Animals employ a range of motor behaviors to survive amidst changing environmental conditions. Among these behaviors, episodic locomotion, which is characterized by alternating periods of movement and pauses with no motion, is the least understood. Central pattern generators (CPGs) are capable of generating diverse rhythmic patterns, one of which is the episodic locomotor rhythm. Recent experimental and modeling studies indicate that different ion channels produce degenerate mechanisms which can support episodic bursting patterns (Sharples et al., 2021). We developed biophysical models that reproduce temporal properties of locomotor episodic bursting rhythms in postnatal mice and in zebrafish larvae. The models predict that the interaction of the small conductance calcium-activated potassium current (I_{SK}) and the NMDAR current (I_{NMDA}) could support fictive locomotor activity at the burst and episode time scales. We tested these predictions in two animal models: dopamine (DA)-evoked episodic activity in the *in vitro* spinal cord preparation of postnatal mice and optogenetically-evoked fictive locomotor activity in spinalized transgenic (*Tg(vglut2a:Gal4;UAS:ChR2)*) larval zebrafish. Two pharmacological agents were tested; apamin (5 μM) was used to block I_{SK} alone or in combination with APV to block I_{NMDA}. In mice, application of apamin alone, after DA-evoked episodic activity was stable, led to a decrease of the interepisode interval and an increase of the episode cycle period coefficient of variation. In zebrafish, the application of apamin alone resulted in a decrease in episode duration, a decrease in the number of bursts per stimulus, and an increase in the burst duration. Subsequent application of APV in combination with apamin resulted in a decrease in the burst duration. Our biophysical models recapitulate these results. Overall, our results suggest that I_{SK} plays a key role in the generation of locomotion at the bursting and episodic scales in both mouse and zebrafish models.
Reference
Sharples SA, Parker J, Vargas A, Milla-Cruz JJ, Lognon AP, Cheng N, Young L, Shonak A, Cymbalyuk GS, Whelan PJ (2021) Contributions of h- and Na(+)/K(+) Pump Currents to the Generation of Episodic and Continuous Rhythmic Activities. Front Cell Neurosci 15:715427.

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Poster

PSTR417. Neuromodulation in Rhythm Generating Networks

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Program #/Poster #: PSTR417.03/FF19

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant R21NS111355 to GC and RC

Title: Co-existence of a high spike-frequency and low spike-frequency bursting regimes in a Central Pattern Generator: the special role of interaction of the Na^+/K^+pump current and persistent Na^+current

Authors: *Y. SHAMS[1], M. FOMENKO[2], P. ELLINGSON[4], J. PARKER[5], R. L. CALABRESE[6], G. S. CYMBALYUK[3];
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Abstract: Essential rhythmic motor functions, such as heartbeat in invertebrates require a persistent generation of a functional pattern by Central Pattern Generators (CPGs). Neuromodulation regulates the dynamics of CPGs in order to align with environmental constraints. The CPG controlling leech heartbeat is based on two pairs of mutually inhibitory interneurons, half-center oscillators (HCO). Using our biophysical model, we showed that, through coordinated adjustments, neuromodulation guides HN-HCOs to avoid dysfunctional states by decreasing the Na^+/K^+ pump current (I_{pump}) and enhancing the h-current (I_h) (Ellingson et al., 2021). Under a condition of a high state of neuromodulation, we found a domain of coexistence of two functional bursting patterns. They have almost the same cycle periods but are distinct in terms of the intra-burst spike frequency: one has a high frequency (HF) above 17 Hz, and the other has significantly lower frequency (LF). Here, we investigated the properties of short perturbations of the HCO by pulses of conductance of inhibitory current and identified domains of phase and amplitude of the pulses leading to the switch from LF to HF bursting and vice versa. We showed that HF bursting requires dynamic interaction of I_{pump} and persistent Na^+ current, I_{NaP}. I_{NaP} is a low-voltage-activated Na^+ current, that does not inactivate and is the major source of Na^+ influx. I_{pump} is an outward current activated by $[Na^+]_i$ and is the main source of Na^+ efflux. Both currents are active and counteract each other between and during bursts. Here, we explored the role of I_{NaP} by investigating comodulation maps of HCO stationary and oscillatory regimes under variation of maximal pump activity and maximal conductance of I_h (G_h) similar to (Ellingson et al., 2021) but with smaller values of G_{NaP} . We found a large domain exhibiting the silent regime where previously we recorded functional and dysfunctional regimes. This emphasizes the necessity of an increase of G_h for the generation of bursting. The domain of

the functional activity shifted down at smaller values of maximal pump activity compared to (Ellingson et al., 2021). We also found a domain of coexistence of functional HF bursting with LF bursting. We further apply this approach to investigate the emergence of the HF bursting along comodulation of I_{pump} and I_{NaP}. We show that the dynamic interaction of I_{pump} and I_{NaP} offers a mechanism for the robust generation and flexible control of the two functional bursting patterns. We highlight the significance of multistability and this control over regime switching is vital for the effectiveness of neuromodulation and coordination of rhythmic motor programs.

Disclosures: Y. Shams: None. **M. Fomenko:** None. **P. Ellingson:** None. **J. Parker:** None. **R.L. Calabrese:** None. **G.S. Cymbalyuk:** None.

Poster

PSTR417. Neuromodulation in Rhythm Generating Networks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR417.04/FF20

Topic: E.09. Motor Neurons and Muscle

Title: Neuromodulation of sodium potassium ATPase pumps dynamically regulate mammalian spinal networks

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Abstract: Mammalian spinal motor networks are sensitive to descending neuromodulatory input, which can fine tune and adapt motor output. Neuromodulators often act by targeting, either directly or indirectly, ion channels and pumps to change membrane potentials and thus affect the likelihood or rate of action potential generation. One potential target, the sodium/potassium pump, has recently been suggested to be linked to an afterhyperpolarisation termed the ultra-slow after-hyperpolarisation (usAHP). Recent work has also highlighted a potential role for the neuromodulator dopamine in modulating this usAHP. The presented work utilises whole cord electrophysiology to interrogate the effect of Na/K pump activation and dopaminergic signalling on network activity. Lumbar ventral root recordings were taken of evoked rhythmic activity in neonatal mice both with and without the presence of dopamine. The sodium ionophore monensin was used to cause electroneutral influx of Na ions and maximally activate the pumps. This caused a strong inhibition of network activity which appeared to be enhanced by the presence of dopamine. Additionally a mutant mouse model carrying a mutation on the ATP1A3 gene was used to investigate if this relationship between dopamine and pump activity was associated with the more dynamically activated alpha3 Na/K pump which may be tied to the usAHP. The presence of the mutation, which caused a 50% reduction in functional ATP1A3 expression, appeared to disrupt the dopamine induced enhancement of pump related network inhibition. The mutation also appeared to disrupt some of the rhythmic stabilisation effect observed with application of dopamine. Work is ongoing using whole cell patch clamp

techniques to interrogate underlying components of the network. The single cell work is aimed at creating a heatmap of usAHP behaviour in lumbar spinal interneurons. Currently of 16 cells studied 7 (43.8%) displayed the usAHP behaviour in response to 10 second high frequency stimulus. The application of dopamine is also studied for effect on the usAHP and of 6 cells studied before and after addition of dopamine 4 either lost previously displayed usAHPs or had reduced afterhyperpolarisation behaviour, 1 appeared to have a usAHP unchanged, and 1 appeared to gain a usAHP after addition of dopamine. Other conductances such as Ih and PICs are also being recorded to investigate for potential correlations with the usAHP. Altogether the current data highlights that the presence of dopamine can have heterogenous effect on usAHPs but appears to have an enhancing effect of inhibitory pump currents involved in excitatory network output as a whole in a manner tied to the alpha 3 Na/K pump.

Disclosures: **S.J. Nisbet:** None. **S.A. Sharples:** None. **G.B. Miles:** None.

Poster

PSTR417. Neuromodulation in Rhythm Generating Networks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR417.05/FF21

Topic: E.07.a. Cellular properties – Interneurons and motor neurons

Support: R01-NS029436

Title: Hormonal tuning of a specific circuit state

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Abstract: We aim to establish how feeding state-specific hormonal milieux influence the activity patterns of feeding-related circuit states. Here, we focus on the gastric mill (chewing) circuit state configured by Gly[1]-SIFamide [G-SIF: 1 µM] in the isolated stomatogastric ganglion (STG) of the crab *Cancer borealis*. G-SIF is a peptide cotransmitter in two STG innervating projection neurons. The STG is dissected from a crab not fed for ≥24 hr [‘unfed’], with recordings done during 15 min incubations with G-SIF in saline vs. hemolymph [hemo] obtained from a different, unfed or 1 hr post-fed [‘fed’] crab. Neither saline, unfed hemo or fed hemo alone activated the gastric mill rhythm (n=8-24) but when co-applied with G-SIF a consistently coordinated gastric mill rhythm was elicited for the entire 15 min incubation in fed hemo (n=9/9), and for all (n=5/15) or some (n=6/15) of that time with unfed hemo. In the remaining G-SIF plus unfed hemo incubations (n=4/15) coordination was incomplete due to one or more neurons generating tonic instead of rhythmic activity. These rhythms included repeated bursting in the IC, LG, DG, AM and LPG neurons, plus IC-timed interruptions in the pyloric rhythm. The rhythm cycle period was similar in both hemo conditions (Unfed, 18.7±8.2s, n=24; Fed, 18.5±12.3s, n=9; p=0.45, Mann-Whitney U Rank Test). This rhythm was elicited less often in

saline plus G-SIF (7/15), with only two lasting 15 min. The greater effectiveness of fed than unfed hemo suggests it contains more of the relevant hormone(s). Notably, G-SIF is not detected in unfed or fed hemo (DeLaney et al, 2022 Analyt Bioanalyt Chem). As a first step to identify the effective hormone(s), we used HPLC to separate unfed hemo into three Fractions (F1, F2, F3). F2 alone did not activate the gastric mill rhythm but when paired with G-SIF it elicited this rhythm for the entire 15 min incubation (n=4/4), with a comparable cycle period to unfed hemo plus G-SIF (18.0±8.6s, n=4). Neither F1 nor F3 consistently mimicked the effect of unfed hemo plus G-SIF. G-SIF plus unfed or fed hemo also often prevented gastric mill rhythm activation by subsequent G-SIF applications, even after saline washes of 1-3 hr (n=24/30). No such effect occurred after most single Fractions plus G-SIF (n=1/12), suggesting the source of this effect spans HPLC fractions and is different from the hormone(s) that enhance the gastric mill rhythm response. Because there were no qualitative changes to the gastric mill rhythm in hemo plus G-SIF, and G-SIF is not detected in unfed or fed hemo, we hypothesize that the hemo effect results from a hormone-mediated increase in the local G-SIF concentration (e.g. by preventing its enzymatic degradation).

Disclosures: L.J. Fickling: None. **A.P. Cook:** None. **W. Wu:** None. **L. Li:** None. **M.P. Nusbaum:** None.

Poster

PSTR417. Neuromodulation in Rhythm Generating Networks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR417.06/FF22

Topic: E.07.a. Cellular properties – Interneurons and motor neurons

Support: NIH Grant MH-060605

Title: Flexibility vs. consistency of circuit output: a push-pull mechanism through diverging and converging comodulation

Authors: N. DAUR, E. CRONIN, F. NADIM, ***D. BUCHER**;
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Abstract: Neuromodulation lends flexibility to neural circuits, as different neuromodulators can sculpt circuit activity into different patterns, allowing adaptation to different behavioral contexts. Comodulation potentially increases the number of possible output patterns. However, in a single neuron, overlapping effects of two modulators on ion channels or synaptic release may either be divergent or convergent. If comodulators converge on the same signaling pathway, their effects may be occluding and therefore potentially redundant. In the crustacean stomatogastric nervous system, amine neuromodulators have divergent actions on neuronal excitability and synaptic function, whereas neuropeptide and muscarinic cellular actions are predominantly convergent. However, different neuropeptides act on different subsets of circuit neurons and therefore have divergent actions at the circuit level. If convergence at the cellular level is predominantly

additive, increasing numbers of comodulators could eventually activate all circuit neurons in the same way, rendering the identity of the modulator unimportant.
We used recordings of the pyloric rhythm of the crab, *Cancer borealis*, to test whether different combinations of convergent neuromodulators at tonic non-saturating levels produce progressively similar circuit outputs. We applied three converging modulators as a singlet, doublet, and triplet (A, A+B, A+B+C), then washed and applied a second set of modulators (D, D+E, D+E+F). We quantified a range of circuit output attributes (cycle frequency, burst phases, spike numbers and spike frequencies) under each condition. We then determined the Euclidean distance in the multidimensional space of normalized output attributes to obtain a single measure of difference between the singlet applications (A vs. D), the doublet applications (A+B vs. D+E), and the triplet applications (A+B+C vs. D+E+F). Circuit outputs became increasingly similar from singlet to triplet applications, independent of the order of modulators. We then tested non-converging neuromodulators. We started with neuropeptide applications and added one and then two different amine modulators. Comodulation with non-converging modulators did not increase circuit output similarity, and in some cases decreased it.
Our results suggest that complex comodulation can either pull circuits towards some common baseline output activity that may correspond to a baseline state or push it towards different output patterns that reflect circuit flexibility. The actual circuit output then depends on the balance of converging and diverging modulatory actions.

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Poster

PSTR417. Neuromodulation in Rhythm Generating Networks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR417.07/FF23

Topic: E.07.a. Cellular properties – Interneurons and motor neurons

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Title: Modulation of the stretch feedback pathway by neuropeptides in the heart of the American lobster, Homarus americanus

Authors: *K. VAN HASSEL[1], M. P. THIES[2], M. A. PADILLA SOTO[2], G. G. GRIESMAN[2], D. J. POWELL[2], E. S. DICKINSON[3], X. QU[2], P. S. DICKINSON[2];
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Abstract: The cardiac ganglion (CG), a central pattern generator, is a neural network that produces the patterned motor output of the cardiac muscle in the American lobster, *Homarus americanus*. The CG in the lobster is made up of nine electrochemically coupled neurons: four premotor neurons that send signals to five motor neurons, causing bursts of action potentials from the motor neurons. These bursts cause cardiac muscle contractions that vary in strength based on the burst duration and burst frequency. Underlying these bursts of action potentials are driver potentials (DPs), which are slow and sustained Ca2+ based depolarizations that allow for the patterned motor output from the CG. We have established a generally excitatory response to cardiac muscle stretch that is mediated by stretch-sensitive dendrites that respond to hemolymph entering the heart. Experimentally, we induce this response by isolating the CG along with the surrounding muscle and applying ramp shaped stretches. These stretches have three phases: the rising phase when the muscle is stretched and the bursting phase is delayed, the hold phase when the stretch length is held constant and the bursts become shorter and more frequent, and the release phase when the stretch length returns to the baseline and the bursts increase in duration. Previous research has established that numerous neuropeptides modulate the cardiac neuromuscular system by acting directly on the CG and peripheral sites such as the neuromuscular junction and cardiac muscle. We have found that GYSNRNYLRFamide and SGRNFLRFamide, two neuropeptides with similar effects on the cardiac muscle activity, suppress the excitatory stretch response. Myosuppressin, an endogenous neuropeptide, decreases the cardiac muscle contraction and CG burst frequency and increases the contraction amplitude. Myosuppressin modulates the stretch feedback pathway by increasing the phase delay during the rising phase, suppressing the stretch response during the hold phase, and inducing no changes in burst parameters during the release phase. Additionally, myosuppressin seems to increase the time in between DPs during the rising phase, increase the DP duration during the hold phase, and induce no changes in DP parameters during the release phase. The rate of depolarization between DPs tends to decrease from saline to myosuppressin during the rising and hold phases. We are currently continuing to work on understanding how myosuppressin modulates the driver potentials underlying the stretch response and whether changes in membrane resistance may mediate the stretch response.

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Poster

PSTR417. Neuromodulation in Rhythm Generating Networks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR417.08/FF24

Topic: E.07.a. Cellular properties – Interneurons and motor neurons

Support: R35 NS097343

Title: Differential role of I_h in the Cancer borealis pyloric rhythm across temperature

Authors: *K. SCHAPIRO[1], J. RITTENBERG[3], M. KENNGOTT[2], E. E. MARDER[4];
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Abstract: Neural circuits must maintain their functions across a variety of environmental challenges, but the mechanisms underlying the execution of these functions may differ to compensate for specific challenges. We studied the role of the hyperpolarization-activated inward current, I_h, in maintaining the pyloric rhythm of the stomatogastric ganglion of male *Cancer borealis* crabs across temperature. The lab has previously shown that the firing phases of pyloric neurons are maintained across temperature, though the overall frequency of the triphasic rhythm increases (Tang et al, 2010). We asked whether, across temperature, the slowly activating I_h plays a different role in maintaining frequency or phase and/or whether the properties of this current change to allow the circuit to operate at different frequencies. Using a combination of extracellular and intracellular recordings, we assessed pyloric frequency as well as phase and membrane waveform of constituent pyloric neurons at temperatures ranging from 11°C to 21°C in both control and I_h-blocked (Cs^+ or ZD 7288) conditions. We repeated these investigations in picrotoxin to isolate the pyloric pacemaker to determine the dependence of temperature-sensitive, I_h-dependent activity on inhibitory synaptic feedback from follower neurons. We found that while the phase of each pyloric neuron's firing differed between control and I_h-blocked conditions, these differences did not change with temperature, suggesting a constant role of I_h in controlling the relative timing of each neuron regardless of temperature. However, we found that I_h blocking agents slowed the pyloric rhythm more high temperatures. This differential slowing suggests that I_h plays a larger role in maintaining pyloric frequency at high temperatures than low temperatures. This finding is particularly intriguing because I_h is canonically slowly-activating, yet it seems to be playing a more critical role in maintaining fast activity. Preliminary results suggest that this effect persists in picrotoxin.

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Poster

PSTR417. Neuromodulation in Rhythm Generating Networks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR417.09/FF25

Topic: E.07. Rhythmic Motor Pattern Generation

Support: BBSRC Grant BB/T015705/1

Title: The modulatory role of the NO-cGMP signalling pathway on spinal neuronal properties and locomotor output in Xenopus laevis tadpoles

Authors: *L. HACHOUMI, N. MCLAUGHLIN, M. KUEHNER, K. T. SILLAR;
Sch. of Psychology and Neurosci., Univ. of St Andrews, St. Andrews, United Kingdom

Abstract: Rhythmic locomotor behaviours, such as swimming in *Xenopus laevis* tadpoles, are flexible to allow organisms to adapt their movements. This flexibility is partly mediated by neuromodulators that alter intrinsic properties of locomotor central pattern generator (CPG) neurons and network output. In tadpoles, nitric oxide (NO) is an important neuromodulator that exerts an overall inhibitory effect on swimming (McLean and Sillar, 2002) and impairs short-term motor memory (STMM; Hachoumi et al., 2022). STMM is a mechanism that influences future swimming behaviour based on past network activity and is mediated by dynamic Na^+/K^+ pumps that produce an ultra-slow afterhyperpolarisation (usAHP) in spinal CPG neurons (Zhang and Sillar, 2012). In this investigation, the NO-cGMP pathway was pharmacologically manipulated to elucidate its role in locomotor control. We performed ventral root recordings of fictive swimming and patch clamp recordings of spinal CPG neurons in immobilised stage 42 *Xenopus* tadpoles. The cGMP analogue, 8-Bromo-cGMP (100 μM), was utilised to examine the involvement of PKG as a downstream target of NO. 8-Bromo-cGMP did not affect swim episode duration or intra-swim parameters such as burst duration or cycle period (n=8). However, it did impair STMM (n=11) and recordings from spinal neurons indicate smaller usAHPs (n=3). Applications of the NOS substrate, L-arginine (L-arg; 2 mM) were conducted to increase NOS activity. If L-arg levels were below saturation, it should increase NO generation and consequently decrease swimming. Contrary to expectations, L-arg increased swim episode duration (n=10) and induced a slow modulation of swimming in which ventral root frequency and burst intensity waxed and waned. These rhythmic oscillations in swimming occurred with a cycle period of ~1-6 sec, reminiscent of NMDA receptor-mediated oscillations reported previously in tadpoles (Reith and Sillar, 1998). L-arg also depolarised the resting membrane potential of spinal neurons and decreased input resistance (n=7). Interestingly, L-arg did not alter STMM or the usAHP (n=7) suggesting its actions do not involve the NO pathway. In summary, we propose that the actions of NO on tadpole swimming are partly mediated via PKG and hypothesise that L-arg, a substrate also for arginase enzymes that generate polyamines, can facilitate NMDA receptor-mediated currents (Williams et al., 1991).

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Poster

PSTR417. Neuromodulation in Rhythm Generating Networks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR417.10/GG1

Topic: E.07.a. Cellular properties – Interneurons and motor neurons

Support: NIH Grant NS094176
University of Minnesota Foundation Grant

Title: Cellular and molecular mechanisms underlying dopaminergic mediated maturation of locomotor activity in larval zebrafish

Authors: B. MERCIER[1], M. L. BECKMAN[2], *M. A. MASINO[3];
[1]Rehabil. Sci., Univ. of Minnesota, Twin Cities, Minneapolis, MN; [2]Biol., Augsburg Univ., Minneapolis, MN; [3]Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: The refinement of gross motor skills, such as locomotion, during development is conserved across vertebrate species. Previously we reported, in larval zebrafish, that dopaminergic (DAergic) signaling through dopamine 4 receptors (D4Rs) was necessary for the developmental transformation of behaviorally relevant locomotor activity from an immature state (erratic, long duration swim episodes) to a mature state (goal-directed, short duration swim episodes) between 3 and 4 days post-fertilization (dpf) (Lambert et al., 2012). The primary source of spinal dopamine is a subset of ventral diencephalic DAergic neurons (DDNs), which project to the spinal cord forming the DAergic diencephalospinal tract (DDT) (Takada et al., 1988; Kastenhuber et al., 2010). Although the DDNs and the DDT are present as early as 1 dpf and the DDT projects along the rostrocaual extent of the spinal cord by 3 dpf (Kastenhuber et al., 2010) and both dopamine transporter and tyrosine hydroxylase are present in the DDNs at 3 dpf (Xi et al., 2011), the cellular and molecular mechanisms underlying the developmental change in locomotor state are not well understood. Recently, we demonstrated that exogenous application of dopamine to 3 dpf larvae produced mature-like locomotor output (unpublished), which suggests both the presence of functional dopamine receptors and a lack of dopamine release in the spinal cord at this developmental stage. Thus, we hypothesized that the developmental transformation of locomotor activity may be due to increased DDN activity, greater release of dopamine in the spinal cord, increased spinal D4R receptor expression, or a combination of these. To measure and compare DDN activity and spinal dopamine release at different developmental stages, calcium or dopamine transients were recorded from larvae that expressed calcium indicator in DDNs (*Tg(th1:Gal4;UAS:GCaMP6s)*) or dopamine sensor pan-neuronally (*Tg(elavl3:Gal4;UAS:GRAB$_{DA}$)*), respectively. The calcium (n=8) and dopamine (n=3) signals were more frequent and larger in amplitude in 5 dpf larvae than in 3 dpf larvae. In addition, the calcium signals were more periodic and synchronous in 5 dpf larvae than in 3 dpf larvae (n=8). Finally, qRT-PCR demonstrated that D4Rs were expressed in the spinal cords of 3 dpf larvae (N=1); however, whether D4Rs were functional and whether their expression levels changed during development have not been determined. In summary, these data support the hypotheses that differences in DDN activity, dopamine release, and D4R expression between 3 dpf (immature) and 5 dpf (mature) larvae are correlated to the DAergic-mediated transformation of locomotor activity during development.

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Poster

PSTR417. Neuromodulation in Rhythm Generating Networks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR417.11/GG2

Topic: E.07.a. Cellular properties – Interneurons and motor neurons

Support: The Edward Jekkal Muscular Dystrophy Research Fellowship (DLGR)
NIH R01 NS104194 (KJD & SG)
NIH R21 NS118226 (KJD)

Title: Early, but not late, epidural stimulation alters spinal cord injury-induced plasticity of serotonergic modulation of lumbar Shox2 interneurons

Authors: *D. GARCIA-RAMIREZ, J. R. MCGRATH, N. J. STACHOWSKI, S. J. ATOCHE, L. YAO, S. F. GISZTER, K. DOUGHERTY;
Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: The spinal locomotor circuitry, located in the thoracolumbar spinal cord, is able to produce the rhythm and the patterning of locomotion even in the absence of brain descending inputs. However, supraspinal control is necessary to initiate and adapt the locomotion. Spinal cord injury (SCI) disrupts the supraspinal descending control, including the pro-locomotor serotonergic modulation of the spinal circuitry. Current promising clinical therapies to recover motor control, such as epidural stimulation (ES), aim to activate the spinal locomotor circuitry by targeting proprioceptive afferents. However, chronic SCI induces plasticity, and the state of the spinal locomotor circuits after SCI and further plasticity following rehabilitation is poorly understood. Interneurons (INs) expressing the transcription factor Shox2 are part of the locomotor circuitry. Previously, we showed that Shox2 INs are modulated by serotonin producing inhibitory and excitatory actions, depending on the concentration. However, in mice that received a complete T8/9 transection 7-weeks before the terminal experiment, serotonin only increased the excitability of Shox2 INs. Furthermore, Shox2 INs from SCI mice that received 6 weeks of sub-motor threshold ES, starting one week after the injury, did not display the SCI-induced changes. The aim of this study was to determine if ES delivered early or late would prevent or reverse the SCI-induced plasticity in the serotonergic modulation of Shox2 INs. Therefore, we performed experiments on adult Shox2::Cre;R26-lsl-tdTomato mice 4 weeks after a complete spinal T8/9 transection with or without 3 weeks of ES beginning one week post-surgery. We performed whole-cell patch clamp recordings from Shox2 INs and tested serotonin modulation. Effects at 4 weeks post-SCI were similar to those observed at 7 weeks post-SCI. Next, to test if ES can reverse the SCI-induced plasticity, mice developed chronic SCI for 4 weeks, followed by 3 weeks with ES treatment. The serotonergic modulation of Shox2 INs observed from these mice was no different from the SCI mice. This result suggests that ES does not reverse the SCI-induced plasticity in chronic conditions. Finally, to test if early ES effects are maintained when ES is discontinued, mice received ES for 3 weeks, followed by 4 weeks without ES. We found that the ES prevention of the SCI-induced changes was maintained over 4 weeks following termination of ES. Our results suggest possible therapeutic implications of an early treatment with ES to preserve spinal locomotor circuitry after SCI.

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Poster

PSTR417. Neuromodulation in Rhythm Generating Networks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR417.12/GG3

Topic: E.07.a. Cellular properties – Interneurons and motor neurons

Support: WMLundgren Grant 2022-4070
CCI University of Gothenburg, NMI, VR-RFI 2019-00217

Title: Contribution of non-inactivating calcium channels to the long-lasting effects of epidural polarization of afferent fibres

Authors: I. H. J. HAMMAR, ***L. I. M. M. KARLSSON SUNDBERG**, E. JANKOWSKA;
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Abstract: Epidural polarization may increase the excitability and shorten the refractory period of nerve fibres within the dorsal column. These effects are most potent at the levels of branching of afferent fibres as they enter the spinal cord. They depend on GABA but also on other factors, in particular persistent Na^+ or Ca^{2+} currents as they are long-lasting ($\geq$ one hour).
We therefore investigated the contribution of non-inactivating Ca^{2+} channels to direct current (DC) evoked changes in excitability and refractory period of fibres within the dorsal column. In one series of experiments in deeply anesthetized adult rats, changes in compound action potentials evoked in hindlimb peripheral nerves by epidural stimulation applied at lumbar segments via a thin tungsten electrode were compared in untreated animals and during local application of nifedipine, a persistent calcium current blocker.
In the presence of nifedipine, nerve fibre excitability was slightly reduced (to 85 ±10%, $p<0.01$, $n=11$). Only minor changes in the relative refractory period were observed but during epidural DC the shortening of the refractory period was somewhat counteracted.
The presence of persistent calcium current channels within the relevant spinal segments was analysed using confocal imaging and immunohistochemistry in preparations where afferents were labelled by Neurobiotin, with antibodies directed towards L-type calcium channels ($Ca_V1.3$).
Preliminary data indicate that $Ca_V1.3$-receptors are present within and ventral to the dorsal column, in the vicinity of labelled afferents but not overlapping with them.
The results suggest that L-type channels are present in the lumbar dorsal columns but are expressed in structures adjacent to the afferent fibres rather than in the fibres themselves. The weak effects of nifedipine in the dorsal column may thus be primarily indirect.

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Poster

PSTR417. Neuromodulation in Rhythm Generating Networks

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Topic: E.07.a. Cellular properties – Interneurons and motor neurons

Support: NIH NINDS (5R01NS064004)
Craig H Neilsen Foundation (547040)
NYSDOH SCIRB Pre-Doctoral Fellowship

Title: Harnessing neuromodulation to prevent transneuronal degeneration of premotor interneurons after spinal cord injury

Authors: *J. PATHAN[1,2], H. SHARIF[1], T. BETHEA[1,2], J. MARTIN[1,2];
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Abstract: Producing skilled movements in humans and many animals requires the corticospinal tract (CST), the direct motor pathway connecting the cerebral cortex with the spinal cord. Spinal cord injury (SCI) results in CST damage and leads to transneuronal degeneration, whereby undamaged premotor interneurons (IN) denervated of CST synaptic input are vulnerable to degeneration (J Neurosci 38:8329, 2018). Complement protein C1q triggers this process, leading microglia to phagocytose non-apoptotic cholinergic (ChAT) and glutamatergic (Chx10) INs. These INs receive direct CST inputs and synapse onto motoneurons to produce muscle contraction. In a bilateral CST lesion mouse model, we found that chemogenetic neuromodulation of motor cortex or spinal cord immediately after injury abrogates innate immune responses and mitigates IN transneuronal degeneration. However, it is unknown if abrogation of transneuronal degeneration and inflammation persists after neuromodulation ends. We hypothesize that neuromodulation will provide sustained neuroprotection, modulating microglia phagocytosis and ameliorating transneuronal degeneration after SCI. Using motor cortex intermittent theta burst stimulation (iTBS) and/or cathodal trans-spinal direct current stimulation (ts-DCS) to modulate neural activity in a C4 midline contusion SCI model in Sprague Dawley rats, we determined if ChAT and Chx10 INs are protected and if inflammation subsided during the post-stimulation period. Neuromodulation began 2 weeks post injury (wpi), daily for 10 days (30 min/day) in the following groups: 1) iTBS only, 2) ts-DCS only, and 3) combined iTBS and ts-DCS (N=18 total for all groups). Animals were perfused 8wpi to examine if neuroprotection persists after stimulation ends. After SCI, the number of ChAT and Chx10 INs decreased (ChAT 24% reduction; Chx10 40% reduction). While iTBS only or ts-DCS only provided minimal protection of ChAT INs, the dual neuromodulation protocol provided enhanced neuroprotection (3.7% ChAT reduction). For Chx10 INs, all stimulation protocols significantly reduced Chx10 IN degeneration (iTBS 1.5% reduction; ts-DCS 1.9% reduction; iTBS and ts-DCs 1.9% reduction). Intriguingly, while dual neuromodulation protected INs, there was a persistent 1.3-fold increase in activated microglia numbers. Our findings support our hypothesis and imply that electrical neuromodulation ameliorates transneuronal degeneration and modulates the inflammatory response during the post-stimulation period after SCI. Determining how augmenting activity prevents transneuronal degeneration and modulates inflammation can lead to better SCI therapeutic targets.

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Poster

PSTR418. Motor Neurons: Activity, Sensory, and Central Control: Exercise, Injury, and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR418.01/GG5

Topic: E.09. Motor Neurons and Muscle

Support: NIH Grant NS45248
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Title: Proprioceptive and cutaneous modulation of locomotor circuitry in the isolated spinal cord of the neonatal mouse

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Abstract: During locomotion proprioceptive and cutaneous information controls amplitude and timing of the step cycle to adjust walking to the terrain and unexpected obstacles. It is known that enhancement of flexion or extension phases, resetting of the locomotor cycle or the stumbling correction responses occur through selective pathways and could provide insights on how sensory feedback modulates the different interneurons comprising the Central Pattern Generator (CPG) circuitry. This study was aimed to investigate how stimulation of proprioceptive and cutaneous afferents modulates the CPG circuitry in the neonatal mouse, considering that this approach is still suitable to disclose CPG organization. Experiments were carried out on the isolated bulbospinal neuraxis, and in some cases including one hindlimb with peripheral nerves, from C57BL/6 mice (P1-3). Fictive locomotor activity was induced by bath application of NMDA (5-7 μM), 5-HT (4 μM), DA (50 μM) and veratridine (10 nM). Stimulation strength of dorsal roots (DRs) and peripheral nerves was based on afferent volley or L5 ventral root (VR) responses, expressed in times threshold of the most excitable fibers (xT). Electroneurographic locomotor activity was recorded bilaterally on L2 and L5 ventral roots by means of glass suction electrodes. DRs, peripheral cutaneous and muscle nerves were stimulated with trains of 30 pulses at 100 Hz with strengths 2-4 xT, triggered off VR-L2 (flexor) or VR-L5 (extensor) locomotor phases every 6 cycles, in order to activate low threshold (group I muscle or group Aβ cutaneous) afferents. Preliminary results show that in general, stimulation of low threshold afferents in the ipsilateral DR-L2, triggered off the ipsilateral VR-L5 activity, evoked resetting to flexion, i.e. a shortening of the ongoing bursting and an early onset of the subsequent L2 activity. On the other hand, stimulation of ipsilateral DR-L5, triggered off the ipsilateral VR-L2 activity, evoked resetting to extension, i.e. a shortening of the ongoing bursting and an early onset of the subsequent L5 activity. As to cutaneous afferents effects, stimulation of ipsilateral Tibial and Sural nerves evoked mixed or negligible effects either in flexor or extensor phases. These data suggest that similarly to the neonatal rat, sensory information is able to modulate the CPG circuitry throughout selective pathways at early stages of development in a similar way as in adult mammals. Investigating afferent inputs on interneurons identified by molecular techniques in the mouse will disclose more thoroughly the control of the CPG circuitry by sensory feedback in mammals.

Disclosures: **A. Salcedo-Carrillo:** None. **J.R. Calvo:** None. **J.N. Quevedo:** None.

Poster

PSTR418. Motor Neurons: Activity, Sensory, and Central Control: Exercise, Injury, and Disease

Location: WCC Halls A-C

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Program #/Poster #: PSTR418.02/GG6

Topic: E.09. Motor Neurons and Muscle

Support: DFG - 233886668/ GRK1960

Title: Task-dependent control of coxal motor neurons in an insect leg

Authors: ***A. RUTHE**, C. MANTZIARIS, P. ROSENBAUM, A. ÖZYER, A. BÜSCHGES; Inst. of Zoology, Univ. of Cologne, Cologne, Germany

Abstract: Animal behavior needs to be highly adaptive and finely tuned to meet the ever-changing environmental demands. In terrestrial locomotion, such adaptive behaviors include straight and curve walking, climbing obstacles or changing the walking direction. The latter seems simple, but requires a number of modifications in the locomotor system. For example, in the middle legs of stick insects, forward and backward stepping differ primarily in the activity of coxal motor neurons and muscles, i.e. the retractor (Ret) and protractor (Pro) coxae: While in forward stepping Ret motor neuron activity generates leg stance and Pro motor neuron activity leg swing, the reverse is true for backward stepping. Interestingly, the activity of those muscles innervating distal leg joints, i.e. the coxa-trochanter and the femur-tibia joints are not different in principle (Rosenbaum et al. 2010). However, the underlying neuronal mechanisms are not yet understood (e.g., Tóth et al. 2011; Akay et al. 2007). In our study, we used pharmacology, extra- and intracellular recording techniques and sensory stimulation paradigms in the stick insect species *Carausius morosus* and *Cuniculina impigra* to investigate the neural mechanisms underlying the task-depend control of coxal motor neuron activity. First, we analyzed the synaptic drive underlying the rhythmic activation of the coxal motor neurons in the deafferented mesothoracic ganglion. We found that rhythmic alternating activity in those motor neurons is based on a tonic depolarization that is patterned by phasic inhibitory synaptic input from the premotor rhythm-generating networks. In the next step, we examined the role of local premotor nonspiking interneurons in reversing the activity of coxal motor neurons during backward stepping. So far, our recorded nonspiking interneurons can be classified into two groups, namely those whose activity pattern remains the same during forward and backward stepping and those that change their activity pattern depending on the stepping direction. Currently, we are analyzing whether they provide synaptic drive to coxal motor neurons during both stepping directions. Additionally, we investigate how load signals from leg load sensors contribute to the generation of forward and backward stepping.

Disclosures: **A. Ruthe:** None. **C. Mantziaris:** None. **P. Rosenbaum:** None. **A. Özyer:** None. **A. Büschges:** None.

Poster

PSTR418. Motor Neurons: Activity, Sensory, and Central Control: Exercise, Injury, and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR418.03

Topic: E.09. Motor Neurons and Muscle

Support: NIH Grant R01

Title: Role of haltere basalare muscles in free flight

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Abstract: Fruit flies have evolved the ability to execute a series of sophisticated aerial maneuvers in response to oncoming obstacles, such as gusts of wind, within milliseconds. To achieve fast and precise control of the wing muscles, the flight motor control system relies on rapid mechanosensory feedback from the halteres. Halteres are a pair of dumbbell-shaped organs that oscillate at the same frequency as the wings and are thought to encode the angular velocity resulting from Coriolis forces acting on the fly's body during rotation. Additionally, previous studies have demonstrated that haltere muscles, which are analogous to the wing steering muscles, alter the phase spike timings of the wing muscles in tethered flies. However, the resulting wing kinematics from the manipulation of the haltere motor neurons in free flight remains unclear. To answer this question, we employed split Gal4 lines to target neurons innervating the haltere basalare muscles and optogenetically activated or silenced the muscles during free flight. Our initial findings reveal that bilateral activation of the haltere basalare muscles induces a pitch up response and an increase in wing stroke amplitude. Conversely, silencing these muscles results in a pitch down response and an overall decrease in wing stroke amplitude. This suggests that the haltere basalare muscles are essential in body pitch control and maintaining stable flight. Furthermore, unilateral silencing of the muscles produces a body roll maneuver, suggesting that the haltere basalare muscles may encode multiple degrees of freedom in flies. Additionally, prior studies have shown that haltere muscle activity is also modulated by visual perturbations. To further investigate the function of the haltere basalare muscles, we propose optogenetic activation or silencing during free flight in the presence of mechanical perturbations or visual perturbations as a different testing approach. This investigation will shed light on the relationship between sensory inputs and motor control mechanisms mediated by the haltere basalare muscles.

Disclosures: **A. Leung:** None.

Poster

PSTR418. Motor Neurons: Activity, Sensory, and Central Control: Exercise, Injury, and Disease

Location: WCC Halls A-C

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Program #/Poster #: PSTR418.04/GG7

Topic: E.09. Motor Neurons and Muscle

Support: NIH/NIBIB Grant T32EB025816
NIH NICHD R01HD090642

Title: Differential force encoding in primary and secondary muscle spindle afferents

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Abstract: Proprioceptive signals are vital for the precise coordination of movement. However, muscle spindle feedback is considerably complex and is signalled through primary (group IA) and secondary (group II) afferents that have different stretch response properties. Thus, to better understand the role of muscle spindle feedback in movement control, it is necessary to develop models that account for the complexities of muscle spindle firing across both group IA and II pathways. The differences in firing responses have been attributed to these two afferent types terminating on different regions of the intrafusal fiber (muscle fibers within the spindle) and terminating on different intrafusal fiber types, namely the bag_1 type fiber that gives rise to the initial burst in IAs (group II afferents only terminate on bag_2 and chain type fibers). We hypothesize that IA and II afferents respond to the resistive force (F) and yank (dF/dt) on the muscle spindle during stretch, and predict that group II afferents will have reduced responses to the force on the spindle and substantially reduced responses to the yank on the spindle compared to group IA afferents.We tested this in ramp stretches (3 mm, 20 mm/s) in deeply anesthetized rats using the MTU force and yank models outlined in Blum et al 2017 and 2019. In short, we estimated the force and yank on the muscle spindle from measurements of the force on the muscle-tendon unit (MTU). We fit the force and yank to the recorded firing rate using constrained optimization, producing sensitivities to force and yank to characterize the spindle response. This analysis over 11 afferents from 3 animals showed a 25.5% lower force sensitivity and a 99.7% lower yank sensitivity of group II afferents with respect to group IA. However, this model relies on fitting forces measured from the MTU, thus it is only applicable in passive stretches when similar forces are being generated by the extrafusal skeletal muscle and intrafusal spindle fibers and offers little predictive capacity. Thus, we aim to repeat a similar analysis with the mechanistic muscle spindle model described in Blum et al 2020 and Housley et al 2023. This model uses recorded muscle stretch to simulate the force and yank in bag and chain type intrafusal fibers and generate a prediction of muscle spindle firing. By accounting for muscle fiber mechanics, this model will offer a greater mechanistic insight into the physiological differences and further our understanding of the functional differences of primary and secondary afferent pathways.

Disclosures: **J.D. Stephens:** None. **S. Simha:** None. **P. Nardelli:** None. **T.C. Cope:** None. **L.H. Ting:** None.

Poster

PSTR418. Motor Neurons: Activity, Sensory, and Central Control: Exercise, Injury, and Disease

Location: WCC Halls A-C

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Program #/Poster #: PSTR418.05/GG8

Topic: E.09. Motor Neurons and Muscle

Support: NIH R01 HD90642-06

Title: Biophysical muscle spindle model predicts gamma sculpting of muscle spindle sensory signals during rhythmic behaviors

Authors: ***S. N. SIMHA**, L. H. TING;
Emory Univ., Atlanta, GA

Abstract: Current knowledge about the gamma motorneuron drive and its role in tuning the muscle spindle sensory output during behavior is limited. To understand muscle spindle function during behaviour, we developed a biophysics-based computational muscle spindle model that simulates the effects of two classes of gamma drive on muscle spindle firing. While it is hypothesized that gamma drive shapes muscle spindle Ia afferent firing differently across tasks, we know little about how it shapes the resulting information. Our model simulates the effects of gamma dynamic drive to bag1 intrafusal fibers and gamma static drive to bag2/chain fibers using cross-bridge dynamics. The muscle spindle's receptor potential that drives the Ia afferent's instantaneous firing rate is modeled as a linear sum of the force and rate change of force (yank) from bag1 fiber, and the force from chain fiber. We modelled three hypothetical tasks with the same rhythmic stretch-shorten cycles but different patterns of gamma drives. First, we simulated a postural sway task with gamma drives set to a constant low value. Similar to human spindles in Day 2017, the "posture" receptor potential showed an initial burst on the first stretch, rose sharply with each stretch, dropped slowly through the rest of the stretch, and disappeared soon after the onset of shortening. Next, we simulated an active movement task by simulating rhythmic alpha-gamma coactivation synchronized to the stretch-shorten length changes. The "voluntary" receptor potential was also rhythmic but with a slower rise and fall and no initial burst. Finally, we simulated an active locomotion task by matching the gamma drives to that measured in decerebrate cats by Taylor 2000; gamma static was synchronized to the length changes while gamma dynamic was on tonically only during lengthening. The "locomotor" receptor potential rose sharply with each stretch, continued to rise slowly for the rest of the stretch, and dropped immediately on shortening, with only the first stretch showing an initial burst. These differences in spindle firing solely from differing gamma drives suggest that we cannot estimate the muscle spindle signal during a task without understanding how neural control of gamma drive sculpts the resulting information.

Disclosures: **S.N. Simha:** None. **L.H. Ting:** None.

Poster

PSTR418. Motor Neurons: Activity, Sensory, and Central Control: Exercise, Injury, and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR418.06/GG9

Topic: E.09. Motor Neurons and Muscle

Title: Physical exercise improves functional recovery through attenuation of apoptosis after MCAo in rats

Authors: J. YOO[1], E. JANG[2,3], J. HWANG[2], D. KIM[2], J. CHOI[2], *H.-S. JEONG[2,4], S. JANG[2,4], D. KIM[1];
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Abstract: Ischemic stroke, one of the world's leading fatal diseases, has a high recurrence and incidence that can lead to severe mortality and disability. In this study, we investigated whether treadmill exercise has an important treatment for ischemic stroke to improve functional impairment. Experimental cerebral ischemia was induced by middle cerebral artery occlusion in rats and the effect of 10- or 30-minute training for two weeks was evaluated. To investigate the improvement of motor function, behavioral tests were conducted with cylinder, rota-rod, and elevated body swing tests. The region of the ischemic brain was measured by TTC staining. The expressions of the apoptosis-related genes and proteins were investigated by qPCR, western blotting analysis, TUNEL staining, and immunohistochemistry. Following cylinder, rota-rod, and elevated body swing tests, the motor function in both exercises 10- and 30-minute was improved compared to the non-exercise group. In addition, the brain infarct volume was decreased after exercise following TTC staining. Further examination of the cell signaling mechanisms involved in the improvement showed a significantly decreased expression of the pro-apoptotic proteins, Bax and caspase-9, and increased that of the anti-apoptotic proteins, Bcl-2 and Bcl-xl. Our results suggest that exercise has a beneficial effect on ischemic stroke for short- or long-term training by regulation of the signaling mechanisms such as apoptosis.

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Poster

PSTR418. Motor Neurons: Activity, Sensory, and Central Control: Exercise, Injury, and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR418.07/GG10

Topic: E.09. Motor Neurons and Muscle

Support: Grant-in-Aid for Young Scientists (B) 17K13069
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Grant-in-Aid for Scientific Research (B) 16H05443

Title: Three-dimensional kinematical analysis revealed spatiotemporal changes in ankle motor function in both the unaffected and affected sides of brain damage mice after undergoing therapeutic mild-intensity running

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Abstract: Motor dysfunction such as gait impairment is a major disability induced by traumatic brain injury or stroke. Rehabilitation is one of the therapies for recovery of lost function after neural damage. Treadmill running is often used as a physical exercise task in clinically and experimentally for their recovery. In rodents, although several behavior experiments such as ladder waking and footprint test are performed to assess the motor function, there are no objective measurement for the motor evaluation. In rodents, although dynamic behavioral deficits can be evaluated using scoring system such as ladder waking and footprint test are performed to assess the motor function, local and minor behaviors are difficult to determine using objective measurement for the motor function. The purpose of this study is to evaluate the motor dysfunction and recovery after brain damage (BD) with/without exercise (Ex) in 8 weeks male mice using 3D kinematic analysis. To determine mild-intensity running, mice were examined an incremental running test while the pulmonary gas exchange of O_2 and CO_2 were measured. The BD was produced by aspiration of left sensorimotor cortical region, and BD with Ex mice performed mild-intensity running (10 m/min for 30 min 5 times/wk) for 4 weeks. The BD with Ex, and BD or sham-operated mice (sham) without Ex were recorded their gait by four-synchronized cameras, and gait was evaluated by 3D-kinematic analysis. The mice of BD without Ex significantly differed stride, step, and stride width in the both limbs comparing to those of sham without Ex. The BD with Ex mice were improved them. The BD without Ex mice had restricted ankle movements, and were observed impairment in dorsal/planter flexing using trajectory analysis. Consistent with the impairments, the nonaffected side also exhibited a different trajectory, suggesting compensatory movements. These results suggest that the appropriate Ex after BD recovered motor function. The present study also suggests that 3D-

kinematic analysis become a powerful tool for detecting minor behavioral alterations due to the impairment of the affected side and the compensation of the unaffected sides as well.

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Poster

PSTR418. Motor Neurons: Activity, Sensory, and Central Control: Exercise, Injury, and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR418.08/GG11

Topic: E.09. Motor Neurons and Muscle

Support: R01 HL161696
NIH OT2OD030534

Title: Plasticity of cardiac vagal motoneurons to remote ischemic preconditioning

Authors: *E. HORNUNG, S. ROBBINS, A. SRIVASTAVA, J. SCHWABER, R. VADIGEPALLI;
Thomas Jefferson Univ., Philadelphia, PA

Abstract: The significance of the Dorsal Motor Nucleus of the Vagus (DMV) to the maintenance of cardiac health was previously ambiguous and underappreciated and has only recently been found to be profound (i.e. DMV activity is required for and sufficient to emulate the cardioprotection induced by physiological interventions, specifically remote ischemic preconditioning (RIPC)). Due to the recency of this evidence, little is known of the molecular, anatomical, and functional basis for the key cardioprotective role of DMV. Findings emerging from our single cell and spatial transcriptomic methods combined with heart to brain neural tracing in 12 week old male and female Sprague Dawley rats highlight pituitary adenylate cyclase-activating polypeptide (PACAP), as well as several other functionally relevant transcripts, as markers specifically of cardiac-projecting DMV neurons. We have also found several transcripts, including PACAP, to be neuroanatomically DMV-specific relative to the surrounding brainstem tissue, supporting the potential for DMV fiber-specific molecular identifiers innervating neurons of the intrinsic cardiac nervous system (ICN). Given the vast literature suggesting the cardioprotective effect of PACAP on ICN neurons, we hypothesize that RIPC increases PACAP levels in DMV. Preliminary RT-qPCR data from DMV tissue micro punches suggests differential expression of various neuromodulatory markers, including upregulation of the neurotrophic and cardioprotective neuropeptide PACAP, in response to RIPC. To build upon these preliminary RT-qPCR results, we have been collecting brainstem samples post-RIPC and post-sham surgery in an effort to validate these transcriptomic findings at the protein level with immunofluorescence staining and imaging measuring expression levels of

PACAP, and other neuromodulatory markers. This work fills a major gap in the understanding of the cellular mechanisms by which parasympathetic drive provides cardioprotection and provides a future translational path to emulating PACAP's vagal neuromodulatory effects on heart health.

Disclosures: E. Hornung: None. **S. Robbins:** None. **A. Srivastava:** None. **J. Schwaber:** None. **R. Vadigepalli:** None.

Poster

PSTR418. Motor Neurons: Activity, Sensory, and Central Control: Exercise, Injury, and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR418.09/GG12

Topic: E.09. Motor Neurons and Muscle

Support: NIH NINDS. 2R01NS064004
NIH Diversity Supplement. 2R01NS064004

Title: Investigation of mechanistic determinants of spinal segmental excitability following CST injury.

Authors: *T. BETHEA[1], T. ADEGBENRO[2], J. MARTIN[1,2];
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Abstract: Investigation of mechanistic determinants of spinal segmental excitability following CST injury.
Authors: Thelma Bethea, Temitope Adegbenro, John Martin
SCI damages the corticospinal tract (CST), a motor pathway key to skilled movement. Selective CST injury produces aberrant proprioceptive afferent (PA) sprouting and increased spinal excitability, leading to hyperreflexia and spasticity. We have observed following a pyramidal tract lesion (PTX) an increase in PA terminals that synapse onto motoneurons. Loss of inhibitory GABAergic presynaptic (GABApre) regulation of PA afferent terminals is a second mechanism of hyperreflexia; GABApre is reduced after bilateral PTX in rats. Chloride homeostasis dysregulation is a third mechanism underlying hyperreflexia. A reduction of membrane-bound, and an increase in cytosolic, KCC2 has been observed after CST injury. In this study, we use selective CST injury to determine the individual contributions of these mechanisms to hyperreflexia.We compared naïve and PTX animals. Injury was followed by 2 weekly H-reflex assessments using rate-dependent depression (RDD). We labeled motoneurons using retrograde CTB tracing. Spinal cord sections were labeled with: VGlut1, a PA terminal marker; GAD65, a GABApre marker; and KCC2. Motoneuron 3-D reconstructions were made using Imaris, as were counts of Vglut1 and GABApre terminals. The ratio of GAD65 to Vglut1 was used to indicate the proportion of PA terminals receiving presynaptic inhibition. Pixel intensities for motoneuron membrane-bound and cytosolic KCC2 were made (ImageJ).We observed significant hyperreflexia in the cervical cord represented as increased RDD percentages (pre: 10%; post

40%), and the absence of hyperreflexia in the lumbar cord(pre: 4%; post 4%). We also see significant increases in PA synapses on motoneurons in both the cervical and lumbar cord, indicating sprouting. GAD65/Vglut1 ratios revealed a significant reduction in GABApre in the cervical, but not lumbar cord. This suggests impaired GABApre regulation in the cervical, but maintained regulation in the lumbar cord. Surprisingly, there was no significant difference between membrane-bound and cytosolic KCC2 in both spinal segments. Using the PTX model, our findings suggests that changes in KCC2 activity are disassociated from hyperreflexia. Instead, hyperreflexia can be explained by PA sprouting and associated loss of GABApre regulation in the cervical cord.

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Poster

PSTR418. Motor Neurons: Activity, Sensory, and Central Control: Exercise, Injury, and Disease

Location: WCC Halls A-C

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Program #/Poster #: PSTR418.10/GG13

Topic: E.09. Motor Neurons and Muscle

Support: ONR/ ILIR

Title: Repeated Occupational Exposure to the Organophosphate Pesticide Malathion Underlies Locomotor Performance Changes and Peripheral Neuropathy in Sprague Dawley Rats.

Authors: *D. MCMEANS[1,3,4], K. MILLER[2,3,4], M. J. SONNER[2,5], H. M. PROCOPIO[2,5], J. L. STRICKER[2,5], H. I. WARWICK[2,5], E. A. PHILLIPS[2,5], V. ETHRIDGE[2,5], K. L. MUMY[2], J. G. ROHAN[2], S. H. ROMER[2,5];
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Abstract: Although organophosphates are known neurotoxicants, they remain in use as flame retardants, additives to lubricants and plasticizers, and are heavily used as pesticides. Acute exposure to organophosphates inhibits cholinesterase activity (>80%) and can lead to muscle paralysis and in some cases death. There is also evidence that low-level repeated exposures, such as those that may occur working with or around pesticides, are linked to adverse neurobehavioral effects in humans and animals even though they only impact cholinesterase levels by <10 %. Malathion is an organophosphate insecticide commonly used in the United States. Despite known neurobehavioral impacts, there are little data available as to how repeated malathion exposures impact locomotion and the structure of motoneurons and neuromuscular junctions. We hypothesized that low-level repeated exposure to malathion would result in locomotor deficits with anatomical alterations in the motor unit consistent with neurodegeneration. To test our hypothesis, we exposed male and female adult Sprague Dawley rats to 50 mg/kg of malathion

via subcutaneous injections once daily for 5 days a week for 4 weeks total. Locomotor behavior was assessed at approximately 1- and 4-weeks following exposure and included open field motor activity, rotarod/accelerod and quantitative gait analysis. Malathion exposed animals were significantly less active in the open field, with activity times for control animals of 1488 ± 150 seconds (mean ± standard deviation) while animals exposed to malathion displayed mean activity times of 1253 ± 151 seconds. Rats exposed to malathion also fell off the accelerod approximately 54 seconds earlier than control animals suggesting impairment of locomotor coordination. Consistent with this, malathion exposed rats also required a 25% increase in base of support during voluntary locomotor gait suggesting additional stability requirements. To examine neuromuscular changes that may underpin the locomotor changes, we analyzed neuromuscular junctions and found evidence of peripheral neuropathy in 50% of the endplates in the extensor digitorum longus muscle in malathion exposed animals. Altogether, our results suggest that the effects of repeated low-level exposure to malathion can cause peripheral neuropathy effects with locomotor changes.

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Poster

PSTR418. Motor Neurons: Activity, Sensory, and Central Control: Exercise, Injury, and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR418.11/GG14

Topic: E.09. Motor Neurons and Muscle

Support: Central States ACSM Doctoral Research Grant

Title: Four weeks of resistance training may improve peripheral nerve function in both young and older adults

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Abstract: It is well known that the natural progression of age can result in motor neuron degeneration. Consequently, this leads to slowing of nerve conduction, denervation, and reduced motor function. Slowing nerve speed can alter an individual's response time and could subsequently lead to increased fall risk and injury. Given the potentially detrimental effects of age-related motor axon degeneration and its role in sarcopenia, it is important to find interventions that improve peripheral nerve speed and size. Therefore, the purpose of this study is to determine if four weeks of resistance training could elicit positive adaptations in peripheral

nerves in healthy adults, with a secondary purpose of identifying the extent to which age could influence the adaptations. We hypothesized that training would result in faster nerve speed in both younger and older adults, albeit the magnitude of change would be greater in younger participants. Since larger axons are faster, we also hypothesized that any increases in nerve speed may be accompanied by axonal growth. Thirty subjects (18-71 yrs.) have completed this ongoing study so far (young training: n= 13, young control: n = 10, older training: n =7). Median nerve motor conduction velocity (NCV) was recorded before (PRE) and after (POST) four weeks of hand resistance training in both arms. Training was conducted 3×/week with the use of hand grippers, bands and rings. Cross-sectional area (CSA) measures of the median nerve from both arms were taken pre- and post-training using ultrasound imaging. Separate 3-way mixed factorial ANOVA's (limb × time × age) and (limb × time × group) were used to analyze the findings. For both NCV and nerve CSA, there were significant time (pre vs. post) × group (training vs. control) interactions ($p = 0.001$) and main effects for time ($p < 0.001$), with other comparisons being non-significant ($p > 0.05$). Our preliminary findings suggest that 4 weeks of resistance training improves nerve conduction velocity (~ 6.5%). To our surprise, age does not seem to be a factor, as the older adults improved at the same rate as the younger group. This type of training modality may be useful in counteracting diminished nerve speed with age. This study also shows that the nerve grows (~ 5.4%) with training. However, more importantly, it shows that nerve CSA, as measured by ultrasound, may be sensitive enough to identify large-scale changes in the size of motor neuron axons. Further sampling, along with the inclusion of older controls, will provide necessary insight into the efficacy of resistance training for improving nerve function, but the preliminary results are promising.

Disclosures: J.E. Shields: None. **M.L. Dos Santos:** None. **C.M. Smith:** None. **S.M. Reese:** None. **J.M. DeFreitas:** None.

Poster

PSTR418. Motor Neurons: Activity, Sensory, and Central Control: Exercise, Injury, and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR418.12/GG15

Topic: E.09. Motor Neurons and Muscle

Support: NICT 21701
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JSPS KAKENHI JP19K04289
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JST SPRING JPMJSP2106

Title: Investigating the efficacy of low-gravity VR training on juggling skill improvement

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Abstract: Juggling, an intricate motor skill, has garnered considerable attention in the study of motor skill acquisition mechanisms within the brain. However, the majority of investigations have relied on MRI or fMRI techniques, which are limited in their ability to directly observe neural activity during juggling. Therefore, this study aims to capture changes in brain activity associated with juggling improvement using electroencephalography (EEG) as well as fMRI, ball trajectory and arm movement, and to verify the effectiveness of slow-tempo training, which has been difficult to achieve in juggling, by realizing through a visuo-haptic virtual reality system. Participants who have never practiced juggling before were divided into two groups: the training group who practiced three-ball cascade juggling through the VR training program starting with slow tempo, and the control group who practiced it at a continuous highest speed. The participants practiced the program for 10 consecutive days. In each day, the participants also performed 3-ball juggling in the real world before and after the VR training, and 2-ball juggling with shutter-glasses in order to assess their juggling skills. As the result, behavioral data analysis showed a significant improvement in the juggling performance of the slow-tempo training group compared to the control group. Also, from EEG data acquired on days 1, 5, and 10 of the training period, it has been shown that slow-tempo VR juggling training led to changes in the event-related potential (ERP) images and event-related spectral perturbations (ERSPs) of the independent components (ICs) extracted from the EEG data. As an additional experimental approach, we conducted a comprehensive assessment of juggling experts affiliated with the juggling club at the Tokyo Institute of Technology. This assessment encompassed measurements of EEG, EMG, as well as anatomical and functional MRI data. A comparative analysis of fMRI images between juggling experts and beginners revealed a noteworthy finding: negative functional connectivity within the occipital area was observed among the juggling experts.

Disclosures: W. Cho: None. **M. Kobayashi:** None. **H. Kambara:** None. **H. Tanaka:** None. **T. Kagawa:** None. **M. Sato:** None. **H. Kim:** None. **M. Miyakoshi:** None. **S. Makeig:** None. **J. Iversen:** None. **N. Yoshimura:** None.

Poster

PSTR418. Motor Neurons: Activity, Sensory, and Central Control: Exercise, Injury, and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR418.13/GG16

Topic: E.09. Motor Neurons and Muscle

Support: National Science and Technology Innovation 2030 Major Program (2021ZD0204500)

the National Key Research and Development Program of China (2018YFA0108000

Title: A central mechanism for generation and maintenance of vigilance state in zebrafish and mice

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Abstract: After successfully escaping from predators into a safer space, or standing with head up in the grassland to surveil the surrounding bush for approaching predators, animals stay quietly and are alertly watchful as well as persistently attentive to avoid potential threats. Besides the behavioral phenomenon, neural signature and underpinning of vigilance state controlling generation and maintenance of vigilance state are still elusive. Here we demonstrated that in adult zebrafish conspecific alarm substance (CAS) could reliably change an actively behaving state into a quiescent vigilance state, in which neurons in the central zone of dorsal pallium (Dc) (homolog to mammalian cortex) displayed low-frequency high-amplitude synchronization, during which stage animal showed decreased threshold to aversive stimuli. The vigilance state was generated and maintained by tonic activation of raphe 5-HT neurons, which released and maintained the high concentration of 5-HT acting on the glutamatergic neurons located in the Dc zone via 5-HT7a receptors. Knockout 5-HT7a receptors diminished the synchronized state as well as vigilance behavior.
This ancestral mechanism also applies to the mammals. Persistent optogenetic activation of raphe 5-HT neurons in mice could reliably trigger the vigilance behavior state with low-frequency high-amplitude neuronal synchronization in the prefrontal cortex and decreased response threshold to aversive stimuli. This study sheds lights on understanding neural underpinning of vigilant state and may help to develop new strategies to treat with hypervigilance diseases, such as PTSD.

Disclosures: Y. Zhao: None.

Poster

PSTR418. Motor Neurons: Activity, Sensory, and Central Control: Exercise, Injury, and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR418.14/GG17

Topic: E.09. Motor Neurons and Muscle

Support: NSTC Grant 111-2314-B-182-035-MY3
NSTC Grant 112-2425-H-011-001

Title: Frequency dependent modulation of cortical excitability by peripheral nerve stimulation in patients with ataxia

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Abstract: Previous studies demonstrated that peripheral electrical stimulation has the ability to modulate cortical excitability in individuals with spinocerebellar ataxia (SCA). The effects of stimulation frequency on facilitation/inhibition were observed in individuals without disabilities. This study aimed to investigate whether individuals with SCA could modulate their cortical excitability based on the frequency of stimulation and whether this modulation correlated with the symptoms of ataxia. Twenty individuals with SCA received median nerve stimulation at high (150Hz) and low (25Hz) frequencies in two consecutive weeks. Measurements of motor evoked potentials (MEP), intracortical inhibition (ICI), and intracortical facilitation (ICF) were conducted before and after the electrical stimulation intervention. Additionally, the clinical finger-to-nose test was used to assess coordination performance. The results revealed that after high frequency stimulation, MEP decreased, ICI decreased, and ICF increased. Conversely, after low frequency stimulation, MEP increased without any changes in ICI or ICF. Furthermore, a weak but significant correlation was observed between ICF and the timed repetition of the finger-to-nose test. In conclusion, this study demonstrates that peripheral electrical stimulation can modulate cortical excitability in individuals with ataxia, and these effects are dependent on the frequency of stimulation. High frequency peripheral electrical stimulation increases ICF, which is correlated with the symptom of ataxia. Long-term training effects should be further investigated in future studies.

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Poster

PSTR418. Motor Neurons: Activity, Sensory, and Central Control: Exercise, Injury, and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR418.15/GG18

Topic: E.09. Motor Neurons and Muscle

Title: Role of contralesional non-primary motor areas in the recovery of proximal and distal upper-extremity muscles after stroke: A scoping review

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Abstract: Targeting non-primary motor areas (NPMAs) is an alternative approach to improve upper extremity (UE) motor function after moderate-to-severe damage to the primary motor cortex (M1). Hence, determining the contribution of NPMAs to UE muscle activations is critical

to help the largest cohort of stroke survivors with moderate-to-severe stroke (65%) to improve UE function. Thus, the objective of this review was to examine the literature regarding the roles of NPMAs in proximal and distal UE muscles after stroke. We developed an a priori scoping review protocol before undertaking this review. We reviewed human and non-human primate literature using PubMed, Cochrane Library, MEDLINE, and hand-searching strategies. We initially screened 458 references, which met the eligibility criteria. One hundred forty-five studies were included for data abstraction, and 43 of the 145 studies (human studies n=16; non-human studies n=27) were included in the final review. Human studies showed that facilitating ipsilesional M1 or inhibiting contralesional M1 contributes to proximal and distal UE muscle activations after mild-to-moderate stroke. On the other hand, facilitating the contralesional dorsal premotor area (cPMd) contributes to proximal and distal UE muscles after moderate-to-severe stroke. The contralesional superior parietal lobule (cSPL) contributes to distal UE flexor muscles, while cPMd, contralesional anterior intraparietal sulcus (aIPS), contralesional posterior parietal cortex (cPPC) contribute to distal UE abductor muscles in severely impaired patients. Furthermore, human studies showed that cPMd contributes to bimanual arms coordination after moderate-to-severe stroke, while M1 contributes to bimanual arms coordination after a mild stroke. Non-human studies showed that supplementary motor area (SMA), cingulate motor areas (CMAs), and SPL have direct or indirect connections with M1 and other cortical areas and have direct or indirect projections to the spinal cord contributing to the movement of the proximal flexor and extensor UE muscles. The ventral premotor area (PMv), inferior parietal lobule (IPL), PMd, and aIPS have connections that contribute to the movement of the distal flexor and extensor UE muscles. In conclusion, the connections of the NPMAs mentioned above with M1 and other cortical areas, as well as the direct or indirect projection of these areas to the spinal cord, contribute to the movement of the proximal and distal UE muscles. These results may inform future rehabilitation protocols by laying the foundations for developing a new mechanism-based approach for proximal and distal UE recovery after moderate-to-severe stroke.

Disclosures: G. Almalki: None. **A. Sethi:** None.

Poster

PSTR418. Motor Neurons: Activity, Sensory, and Central Control: Exercise, Injury, and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR418.16/GG19

Topic: E.09. Motor Neurons and Muscle

Title: Amplitude variation of motor evoked potentials with spinal cord temperature change ; rat local hypothermia model

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Dept. of Anesthesiol. and Intensive care medicine, Univ. of the Ryukyus, Nakagami-Gun, Japan

Abstract: [Background] Intraoperative motor function monitoring is important for safe spine and spinal cord surgery. The motor evoked potentials (MEPs) can assess the function of corticospinal tracts, which are altered by various factors such as body temperature, anesthesia, etc. In this study, we investigated how the amplitude of motor evoked potentials changes when the spinal cord temperature is changed. [Methods] Male SD rats (300-350 g) were used. All procedures were performed under general anesthesia with intramuscular and intraperitoneal administration of ketamine. A copper perfusion plate was inserted under the dorsal thoracolumbar skin and local cooling of the spinal cord was performed until the spinal cord temperature reached 20 degrees Celsius(20℃=68℉). MEP and F waves were measured during cooling and heating.All measurements were performed in the left hindlimb. MEPs were derived in the gastrocnemius muscle by transcranial stimulation, and F waves were derived in the plantaris muscle by gastrocnemius stimulation. [Results] Spinal cord temperature was varied at 20, 23, 27, 30, 34, and 37 ℃. For MEPs, an increase in MEP amplitude was observed as spinal cord temperature decreased (Amp37℃ : Amp20℃ = 1 : 62.21); for F waves, the F/M ratio decreased as body temperature decreased (F/M37 ℃ : F/M20 ℃=21.5 : 14.7), and the frequency of appearance also decreased (P37 : P20=100 : 5). [Discussion] The present study showed that the amplitude of MEPs in the rat spinal cord increases when spinal cord temperature is decreased. Furthermore, the increase in amplitude with spinal cord temperature change was reversible, and the amplitude decreased with return of spinal cord temperature. The F-wave results also indicated that the anterior horn cell function was reduced by the decrease in spinal cord temperature.These results suggest that the increase in MEP amplitude is not due to an increase in motor nerve excitability, but that other mechanisms are involved.The amplitude of MEPs may increase even when motor nerve function is impaired, and caution should be exercised in their clinical determination, i.e., intraoperative motor function determination.

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Poster

PSTR418. Motor Neurons: Activity, Sensory, and Central Control: Exercise, Injury, and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR418.17/GG20

Topic: E.09. Motor Neurons and Muscle

Support: CIHR PJT-148626
Abdul Majid Bader Graduate Scholarship-Dalhousie University

Title: A novel strategy to restore meaningful function to permanently denervated skeletal muscles

Authors: *A. YANS, V. F. RAFUSE;
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Abstract: Spinal cord injuries, peripheral nerve injuries and motoneuron diseases, cause permanentparalysis when the affected skeletal muscles are completely and permanently denervated due to motor neuron death. Even with advances in neuroprosthetics, functional movement of completely denervated muscles is extremely difficult to achieve because non-innervated muscle fibers cannot be efficiently activated via electrical stimulation. To circumvent this problem, we previously showed that permanently denervated muscles can be functionally activated with blue if the muscles are genetically engineered to express channelrhodopsin 2 (ChR2). Here, we are developing a novel strategy to express genes in denervated skeletal muscles without the use of transgenic technology. Briefly, shank muscles in adult C57/B6 mice were subjected to a high dose of gamma irradiation (18Gy). Days later, snake venom was then injected into the irradiated soleus muscles. Analysis of these muscles, up to one month later, showed that this procedure kills all the soleus muscle fibers and resident satellite cells. In another cohort of animals, the irradiated and venom injected soleus muscles were injected with cultured mouse embryonic stem cell-derived myoblasts (ESCMs) expressing a gene of our choice. To date, our results show that injected ESCMs expressing Td-Tomato survive the transplant procedure, fused to form multinucleated muscle fibers, and restored muscle mass as well as whole muscle cross-sectional area. Interestingly, the injected soleus muscles contained significantly more muscle fibers compared to non-operated muscles because the Td-Tomato expressing muscle fibers were significantly smaller than normal. Finally, we showed that transplanted ESCMs can become functionally innervated when endogenous motor nerve regeneration is permitted. Together, these findings indicate that ESCMs can repopulate irradiated/venom-injected mouse soleus muscles with new, exogenously derived fibers that are capable of functional reinnervation. We are currently conducting a similar series of transplant studies with ESCMs expressing ChR2. We anticipate that the exogenously formed ChR2 muscle fibers will functionally contract when illuminated with blue light. Ultimately, we hope that these preliminary studies will form the basis of using this technology to restore meaningful function to permanently and completely denervated muscles.

Disclosures: A. Yans: None. **V.F. Rafuse:** None.

Poster

PSTR418. Motor Neurons: Activity, Sensory, and Central Control: Exercise, Injury, and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR418.18/HH1

Topic: E.09. Motor Neurons and Muscle

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Title: Motion and signal analysis of the Paw Withdrawal Learning paradigm

Authors: F. MORENO[1], E. ALDANA[2], J. ARAIZA[3], M. JOSEPH[2], *C. WANG[1];
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Abstract: Following spinal cord injury (SCI), sensory and motor functions are severely disrupted yet the spinal circuitry below the injury site continues to maintain active and functional neuronal properties (Edgerton, 2004). The spinal cord is endogenously capable of several forms of adaptive plasticity, including functional re-training with exercise, instrumental, and Pavlovian learning. The paw withdrawal learning (PaWL) paradigm represents a simple spinal instrumental learning model (Jindrich 2019). In this study, we utilize high-speed, high-resolution cameras (Basler AG, Ahrensburg, Germany) to capture the motion and electromyography (EMG) to capture tibialis anterior (TA) muscle activity during the paw withdrawal learning paradigm in mice and rats. Our hypothesis is that the motion patterns from the contingent stimulation (CS) group differs greatly from non-contingent simulation (non-CS) group, especially once the learning has occurred. Joint angles of the hind leg form the raw motion data. Power spectra, acceleration/deceleration speed, and other features of the motion data of pre-stimulation, post-stimulation, and post-learning sequences were compared between the CS and Non-CS group. We successfully recreated the PaWL paradigm using a high-framerate camera and achieved synchronized data collection between the CS and Non-CS groups with EMG at the tibialis anterior muscle. Preliminary data shows successful EMG activity corresponding to the hindlimb activity throughout the length PaWL training. The successful formulation of the paradigm using improved technology will validate the instrumental learning model. The CS group successfully learned to paw dorsiflexes while the non-CS group failed to dorsiflex above the imposed threshold. Development of this spinal learning paradigm will help answer physiological and molecular mechanism of plasticity in simple neuromuscular preparation in an isolated spinal cord from any input from the brain.

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Poster

PSTR418. Motor Neurons: Activity, Sensory, and Central Control: Exercise, Injury, and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR418.19/HH2

Topic: E.09. Motor Neurons and Muscle

Title: Variability in motor unit activity for a functional stationary cycling task

Authors: *A. M. WIGGINS, G. R. EVANS, V. VENEZIA, S. DARDZINSKI, E. FILBERT, R. CREATH;
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Abstract: Introduction: Under isometric conditions, the orderly recruitment of motor units (MUs) occurs by motoneuron size. By comparison, functional recruitment sequences display variation suggesting an alternative mechanism that depends on muscle and task mechanics. Previously, observed EMG frequency spectra displayed seemingly random correlations between consecutive pedal power strokes (PPS) during stationary cycling at constant effort and cadence (rpm). We expected a dominant subset of motor units to emerge by time-averaging many PPS under constant rpm conditions which would serve to identify the average MU requirements for the task. Instead, averages of increasing numbers of PPS failed to converge on a dominant subset of MUs suggesting a random component to selection. The purpose of this preliminary study was to characterize the random characteristics of MU recruitment for stationary cycling at constant effort and rpm.
Methods: 6 subjects (4m; 21.3 yrs) performed 70-sec trials on a Concept2 bike for 2 conditions: 1) 60 rpm at low effort (bike setting 2); and 2) 80 rpm at low effort. Rpm and watts were recorded to ensure cycling consistency. EMG was recorded for Vastus lateralis at 2K Hz using a Delsys EMG system. Data was resampled at 20K Hz and band-pass filtered between 20 and 1K Hz. Fourier transforms (FFTs) were calculated for each PPS. PPS Spectra were normalized to the maximum amplitude. Spectra were averaged for N=5, 10, 20, and 50 PPS. Pairwise correlations of frequency spectra were calculated for all unique pair combinations for 50 PPS from each trial.
Results: Coefficients for the pairwise correlations varied between -0.99 and 0.98 with the majority between 0.0-0.5 regardless of rpm. The correlation distribution results suggested that each PPS produced a unique frequency spectrum. For averages of 5 spectra, each trial contained frequency elements between 20 - 200 Hz, but without dominant frequencies. Averaging greater numbers of PPS spectra (10, 20, & 50) produced seemingly random MU frequencies in the 20-200 Hz range. Greater PPS time-averaging produced progressively smoother distributions as variable frequency elements between 20-200 Hz filled gaps to create a continuous spectrum with fixed limits. For larger averages, a peak activity level emerged between 100 - 120 Hz for the otherwise seemingly random MU spectra.
Conclusions: The absence of consistently high pairwise correlations between PPS suggests that MU recruitment varies randomly within a fixed distribution during cycling at constant effort and rpm. It appears that MUs for individual PPS are selected from a larger distribution that is task specific but can only be identified through repeated PPS.

Disclosures: A.M. Wiggins: None. **G.R. Evans:** None. **V. Venezia:** None. **S. Dardzinski:** None. **E. Filbert:** None. **R. Creath:** None.

Poster

PSTR418. Motor Neurons: Activity, Sensory, and Central Control: Exercise, Injury, and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR418.20/HH3

Topic: E.09. Motor Neurons and Muscle

Support: NIH UG3NS12313501A1

Title: Spinal cord stimulation in stroke patients shows suppression of cooperative movement circuitry recruited for compensatory action during movement

Authors: *R. FAKHREDDINE[1], D. M. GRIFFIN[2], N. VERMA[3], E. SORENSEN[4], E. CARRANZA[5], A. BOOS[6], G. F. WITTENBERG[6], P. GERSZTEN[4], E. PIRONDINI[7], M. CAPOGROSSO[7], D. J. WEBER[2];
[2]Carnegie Mellon Univ., [3]Mechanical Engin., [1]Carnegie Mellon Univ., Pittsburgh, PA; [4]Neurolog. Surgery, [5]Bioengineering, [6]Neurol., [7]Univ. of Pittsburgh, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Despite stroke being a leading cause of serious long-term disability, post-stroke rehabilitation is often unable to promote recovery of arm and hand function. Patients with chronic hemiplegia often use compensatory strategies in the unaffected limb to augment function in the affected limb. These strategies can be in the form of mirror movements (unaffected hand displays a scaled version of affected hand activity) or cooperative movements (antagonist-agonist pattern of activity between both hands) to better perform a task. Recently, we demonstrated that cervical epidural spinal cord stimulation (SCS) targeting the dorsal root entry zone improves upper extremity function in people with stroke related hemiplegia. The effects of SCS are attributed to activation of primary afferent neurons that provide excitatory input to motor neurons. This may compensate for the loss of descending input after stroke and reduce the need for compensatory strategies. To test this hypothesis, we examined EMG and reach-related kinematics in a patient with chronic stroke related hemiplegia during cervical epidural SCS. We instructed the subject to perform a point-to-point reaching task in a 2-D plane with their affected limb. A session consisted of trials with (stim on) and without (stim off) SCS. Although the task required only movement of the affected limb, we recorded EMG and kinematics from both limbs. Our analysis focused on the biceps and triceps of both arms, revealing consistent patterns of muscle activation in the unaffected arm while reaching with the affected arm. However, we found no signs of mirroring or coherence in the EMG, but rather an antagonist-agonist pattern of muscle activation between limbs during stim off trials. Averages of EMG activity revealed that activation of the tricep in the affected limb was paired with activation of the bicep in the unaffected limb with ~100ms latency during the reach phase of the task. During stim off trials, the participant often failed to reach the left and right targets. Motor performance of the affected limb improved with stim on, as seen by smoother kinematic traces and increase in the number of targets reached. As motor behavior improved, the need for compensatory action decreased, revealed by consistent reduction of average EMG in biceps and triceps on the unaffected side during stim on trials. EMG traces in individual trials also revealed lower levels of muscle activity in the unaffected limb with SCS, while in the affected limb EMG activity became more closely locked to reach onset with clearer activation peaks. Our analysis further reveals that SCS improves motor performance and reduces the need for compensatory behavior.

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Poster

PSTR418. Motor Neurons: Activity, Sensory, and Central Control: Exercise, Injury, and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR418.21/HH4

Topic: E.09. Motor Neurons and Muscle

Support: MnDrive Brain Conditions
CEHD seed grant

Title: Vibro-tactile Stimulation of the Neck as a Non-invasive Treatment for Cervical Dystonia

Authors: *J. KONCZAK[1], J. XU[2], J. OH[4], S. STANDAL[3], P. SALEHI[3], D. MARTINO[5], L. AVANZINO[6], A. CONTE[7];
[2]Kinesiology, [3]Physical Med. and Rehabil., [1]Univ. of Minnesota, Minneapolis, MN; [4]Children's Hosp., Los Angeles, CA; [5]Neurol., Univ. of Calgary, Calgary, AB, Canada; [6]Exptl. Med., Univ. of Genoa, Genoa, Italy; [7]Neurol., Univ. of Rome, Rome, Italy

Abstract: Cervical dystonia (CD) is a form of focal dystonia associated with involuntary cervical muscle contractions that lead to sustained or intermittent abnormal, painful head movements or postures, which severely affect a CD patient’s daily life. Current treatment opportunities for CD are limited and mainly consist of Botulinum toxin injections (Botox) in the dystonic muscles or deep brain stimulation. Both methods are invasive, and Botox is not tolerated by all CD patients. This multi-center clinical trial examined if superficial, vibro-tactile stimulation (VTS) of the cervical muscles can be an alternative, non-invasive method to provide temporary symptom relief for people with CD. **Method:** A total of 67 patients with CD (44 females) participated. The mean age was 61.1 ± SD 12.5 years. All participants were seen within two weeks before or one week after their new BoNT injection (i.e., their symptomatic period). The most often affected cervical muscles, sternocleidomastoid and trapezius, were stimulated. Participants completed up to 9 stimulations under different conditions (stimulating a single muscle or the combination of two muscles) in randomized order and based on their clinical manifestations (e.g., torticollis, laterocollis). Under each stimulation condition, VTS was applied continuously for 5 minutes. A head angle index (HAI), a composite measure reflecting the head deviation across the three head axes was and a self-reported pain score (100-point scales) were the primary outcome measures. **Results:** First, 82% (55/67) of participants showed an

improvement in head righting by at least 10% as measured by HAI in one or more stimulation condition. Second, of those experiencing pain, 66% (29/44) showed at least 10% relative improvement in pain score with 39% (17/44) of participants achieved a relative improvement in pain score of at least 50% during VTS-ON for the best stimulation condition. Retention of pain relief was observed after the cessation of VTS. Third, several stimulation conditions could either induce larger relative improvement in dystonic symptoms or higher responding rate than the other conditions for each CD manifestation. **Conclusion:** In summary, the findings of this study provide evidence that VTS can be used as a potential new treatment method for CD. Improvements in abnormal head posture and pain were observed among large portion of participants during the application of VTS.

Disclosures: J. Konczak: None. **J. Xu:** None. **J. Oh:** None. **S. Standal:** None. **P. Salehi:** None. **D. Martino:** None. **L. Avanzino:** None. **A. Conte:** None.

Poster

PSTR418. Motor Neurons: Activity, Sensory, and Central Control: Exercise, Injury, and Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR418.22/HH5

Topic: C.01. Brain Wellness and Aging

Title: Benefits of regular physical activity and polyphenol supplementation on locomotor function and lifespan in Drosophila melanogaster

Authors: *M. MORGAN[1], G. CELAYA[1], Z. LOPEZ[2], R. E. HARTMAN[2];
[2]Psychology, [1]Loma Linda Univ., Loma Linda, CA

Abstract: Physical exercise and dietary adjustments are non-pharmacological interventions that have been shown to protect against cognitive decline. Regular participation in physically demanding activities positively influences cognitive health compared to a sedentary lifestyle. Regular consumption of a healthy, polyphenol-rich diet can likewise positively influence cognitive health compared to unhealthy diets. Several human and animal studies have demonstrated that adopting these lifestyle factors can safeguard against cognitive and motor deficits associated with neurodegenerative disorders. This study aims to explore the exercise-mimetic effects of EA dietary supplementation, investigate EA's neuroprotective potential, and provide insights into the combined use of a polyphenol-rich diet and induced exercise to enhance cognitive outcomes against the deleterious effects of neurodegeneration. Adult fruit flies were exposed to ellagic acid via diet while engaging in regular exercise three times a week. Exercise was induced in the flies through repetitive movement of their vials. Control flies were not exposed to the repetitive movement. Flies were fed either a control diet consisting of instant Drosophila medium mixed with water or 10% ellagic acid solution throughout their lifetime. Climbing performance was assessed using the negative geotaxis assay, and longevity was evaluated as a measure of survival rate. Results demonstrated that, on average, flies with a

polyphenol-rich diet climbed higher and lived longer compared to flies on a control diet. The findings provide support for the use of ellagic acid in improving longevity outcomes and its potential neuroprotective qualities against motor function decline associated with aging and neurodegeneration. Further research will look at the effects of ellagic acid and exercise on other measures of cognitive function, such as learning and memory, and the use of different doses of these two interventions.

Disclosures: **M. Morgan:** None. **G. Celaya:** None. **Z. Lopez:** None. **R.E. Hartman:** None.

Poster

PSTR419. Vocal/Social Communication—Avian II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR419.01/HH6

Topic: F.01. Neuroethology

Support: R56NS110951
R01NS128286

Title: Does song preference formation in female zebra finches require mTOR signaling?

Authors: *K. V MAHESHWAR, O. CLARKE, S. E. LONDON;
Univ. of Chicago, Univ. of Chicago, Chicago, IL

Abstract: Song learning in zebra finches during development influences their social behavior in adulthood. The song structure of adult male zebra finches reflects their tutor experiences during a critical period; song preference formation and mate choice in females are shaped by song exposure during development. Overlaps in developmental learning timelines and brain regions involved in auditory processing suggest that males and females might share molecular mechanisms important for formation of song memories in juveniles as in adults. However, there are molecular data indicating the potential for sex differences in learning mechanisms. Constitutive activation and selective inhibition of the mechanistic Target of Rapamycin (mTOR) signaling cascade in the auditory forebrain of juvenile males during tutor experience disrupts tutor song copying. Interestingly, while the mTOR machinery is present in both sexes, song playback induces mTOR signaling in males, but not females, at ages when song experience influences adult behaviors in both sexes. To functionally test if formation of sensory song memories in juvenile females depends on mTOR signaling, we selectively inhibited or constitutively activated mTOR signaling in the auditory forebrain during tutor experiences, as we have done previously in males. We assayed the effect of these manipulations on adult song preference with an operant conditioning perch-trigger assay where perches were assigned to play either the tutor song or a novel song when the bird hopped on them. Preliminary results suggest that bidirectional manipulation of mTOR signaling in juvenile female zebra finches does not abolish their preference to the tutor song. These results may then add to the evidence that the

molecular mechanisms underlying sensory song learning in juvenile female zebra finches are distinct from those known to be essential for tutor song memorization in juvenile males.

Disclosures: K. V Maheshwar: None. **O. Clarke:** None. **S.E. London:** None.

Poster

PSTR419. Vocal/Social Communication—Avian II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR419.02/HH7

Topic: F.01. Neuroethology

Support: R56 NS110951

Title: Developmental shifts in epigenetically-defined regulatory regions reveal putative controllers of critical period learning

Authors: *G. KUNZELMAN[1,2], S. E. LONDON[2,1];
[2]Psychology, [1]Univ. of Chicago, Chicago, IL

Abstract: In zebra finches, juvenile song experience has meaningful and lasting consequences on adult behavior. Evidence suggests that molecular mechanisms underlying sensory song learning in males and females developmentally differ. During development, maturation- and experience-dependent mechanisms of plasticity intersect to regulate neural properties (e.g., cell number, subtype, functionality, connectivity) that temporally constrain sensory song learning. These properties are largely determined by the complement of protein-coding and noncoding RNAs transcribed from the genome. Transcription is regulated by transcription factors (TFs), proteins that coordinate the expression of gene sets that orchestrate shifts in functional and structural cell properties through binding transcription factor binding sites (TFBSs) in regulatory regions of the genome (i.e., enhancers, promoters, repressors). To determine the properties that influence juvenile song learning, we performed chromatin immunoprecipitation for H3K27ac, a histone modification that denotes accessible regulatory regions, and high-throughput DNA sequencing (ChIP-Seq) on auditory forebrains (AL) of males and females spanning developmental time points at which song experience has differing influence on adult behavior. To parse the roles of maturation, experience, and sex in establishing regulatory region accessibility, we compared birds differing in developmental state of receptivity (i.e. capable and incapable of learning) and age-matched birds of the opposite sex. We identify TFBSs enriched in differentially accessible regions, identified their putative genes of regulation, and performed GO ontology analysis to examine how enriched TFBSs and putatively regulated genes may influence AL properties. Our data suggest that male and female regulatory region accessibility profiles differently shift across development to support overlapping and distinct cellular and molecular processes of brain development and function. Comparisons of age-matched males and females revealed that sexually-dimorphic regions were largely located on sex chromosomes and more accessible in males. GO terms enriched in genes annotated to regions of greater accessibility in

males suggest increase regulatory potential for upstream initiators of the ERK and mTOR signaling cascades, each of which developmentally differ in song responsivity between males and females. Together, our evaluation of epigenetically-defined regulatory region profiles promotes further insight into the emergence of neural learning circuits in a brain area required for the developmental learning of complex behavior.

Disclosures: G. Kunzelman: None. **S.E. London:** None.

Poster

PSTR419. Vocal/Social Communication—Avian II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR419.03/HH8

Topic: F.01. Neuroethology

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European Research Council Starting Grant (ERC)-2017-StG-757459 MIDNIGHT
Deutsche Forschungsgemeinschaft VA742/2-1
Deutsche Forschungsgemeinschaft 327654276–SFB 1315

Title: Call-evoked activity in the zebra finch vocal premotor nucleus HVC

Authors: *C. GOMEZ-GUZMAN, D. VALLENTIN, J. BENICHOV;
Max Planck Inst. for Biol. Intelligence, Starnberg, Germany

Abstract: Zebra finches are highly vocal and engage in vocal turn-taking when interacting with social partners. The cortical premotor nucleus HVC has been associated with the context-dependent control of vocal timing during these interactions. Additionally, zebra finches are capable of differentiating calls from multiple individuals, and vocal exchanges most often occur between bonded partners, even within a group of calling birds. To regulate call production with respect to the heard calls of particular conspecifics, the vocal motor pathway must integrate relevant information about incoming auditory signals. We have previously reported that HVC interneurons in particular exhibit a variety of auditory-evoked activity patterns in response to call stimuli. However, it is unclear whether HVC activity is differentially affected by the calls of different birds. Here, we investigate the auditory influences on neural activity in HVC by performing high density Neuropixels probe recordings while awake birds listen to playbacks of calls from a cage mate and an unfamiliar conspecific. The study aims to characterize the patterns of HVC neural activity in response to acoustically similar stimuli that differ in behavioral relevance. To achieve a fine-grained temporal decomposition of auditory evoked neural patterns, clustering algorithms were used, and the activity during the presentation of different stimuli was compared. Results demonstrate various characteristic firing patterns of HVC neurons when calls are heard. These patterns are often shared for similar call types, produced by different individuals, however some neurons exhibit caller-specific activity, evidencing two different

coding strategies in HVC. These results suggest a mechanism by which caller-specific and caller-invariant representations can influence vocal responses during interactions. In sum, heterogeneous call-evoked activity patterns in HVC demonstrate the integration of behaviorally relevant auditory information in this premotor vocal area. The identification of these patterns will enable further investigation into how they influence premotor activity in the vocal-motor pathway and subsequent vocal responses.

Disclosures: C. Gomez-Guzman: None. **D. Vallentin:** None. **J. Benichov:** None.

Poster

PSTR419. Vocal/Social Communication—Avian II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR419.04/HH9

Topic: F.01. Neuroethology

Support: HHMI
Life Science Research Fellowship

Title: Inter-individual differences in linked vocal phenotypes

Authors: *E. SCHUPPE[1,2], D. METS[3], K. J. LI[2], M. BRAINARD[2];
[1]Ctr. for Integrative Neurosci., UCSF, San Francisco, CA; [2]UCSF/HHMI, San Francisco, CA; [3]UCSF/HHMI, Berkeley, CA

Abstract: Many organisms use suites of tightly linked behaviors to navigate diverse social interactions, with individuals often exhibiting different behavioral tendencies. Although it is increasingly appreciated that phenotypes are organized as suites of behaviors that change together, the genetic and neural mechanisms that shape linked behavioral phenotypes remains unknown. Here, we use the Bengalese finch (BF; *Lonchura striata domestica*) to investigate whether multiple vocal traits exhibit such linked co-variation across individuals. Songbirds have been widely studied because they learn to produce songs in a way that is similar to how humans acquire speech. However, they also exhibit other distinct vocal phenotypes, including both learned and innate ‘calls’, that can be produced in vocal exchanges resembling conversations. While song is flexibly learned during development, heritable inter-individual differences can shape and constrain the structure of learned song, including the tempo at which syllables are produced (Mets & Brainard, 2018). Since neural circuitry for song and other vocalizations overlaps, and because genetic variation contributing to differences in song tempo might also influence other phenotypes (pleiotropy), we investigated whether inter-individual differences in song tempo were linked to variation in other vocal and behavioral phenotypes.
We found that birds with faster versus slower song tempos also generate call trains with shorter gaps between calls, and respond with shorter reaction times during vocal turn-taking. Moreover, through computer tutoring and cross-fostering, we demonstrated that these linked phenotypes have a strong heritable component. To investigate molecular mechanisms that might contribute

to these differences, we used bulk RNA-seq and spatial transcriptomics to determine whether pre-motor vocal control regions, including HVC and RA, exhibit a transcriptional signature correlated with inter-individual differences in vocal performance. Our preliminary results identified differential expression of genes associated with GABAergic signaling between birds with faster and slower vocal phenotypes. Given that modulation of inhibitory dynamics within pre-motor regions can influence song timing (Isola et al., 2020) and call precision (Benichov et al., 2020), we suggest that genetically driven differences in GABAergic signaling may serve as a pleiotropic mechanism to promote inter-individual differences in multiple aspects of vocal behavior. Together, our findings provide insight into the mechanisms whereby genetic and experiential influences give rise to linked behavioral phenotypes.

Disclosures: E. Schuppe: None. **D. Mets:** None. **K.J. Li:** None. **M. Brainard:** None.

Poster

PSTR419. Vocal/Social Communication—Avian II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR419.05/HH10

Topic: F.01. Neuroethology

Support: HHMI
UCSF Discovery Fellowship
UCSF Sandler program for breakthrough biomedical research

Title: Coordination of breathing and calls in Bengalese finches

Authors: *E. KISH, W. H. MEHAFFEY, K. YACKLE, M. S. BRAINARD;
UCSF, San Francisco, CA

Abstract: Vocal communication is crucial for successful social interactions. Vocal turn-taking is an important form of vocal communication in which vocal partners engage in collaboratively coordinated vocal exchanges, such as human conversations. Although conversational response times are usually quite precise, deviations outside of the expected response window can be used to convey nuanced and salient information about the speaker's internal world, whereas unintended deviations lead to miscommunication and social awkwardness. Our ability to exert such temporal control and flexibility in conversations is impressive considering that vocal production relies on patterning respiratory machinery vital for sustaining life that is otherwise controlled by autonomous mechanisms in the medulla. Songbird calls, brief and discrete vocalizations, are also employed with great temporal precision and flexibility during vocal exchanges, providing us with a tractable model system to study how vocal precision is coordinated with breathing. To address this question, we simultaneously recorded audio and respiratory pressure in freely moving male Bengalese finches during spontaneous and antiphonal calling, as well as in head-fixed bird where calls were evoked by electrical stimulation of the midbrain calling nucleus DM. We found that while sound is only produced during expiration,

calls are always preceded by a brief and silent inspiration. Correspondingly, DM stimulation evoked calls were always preceded by a brief inspiration, demonstrating that the call motor pattern begins with an inspiration. Although this initial inspiration can interrupt breathing at any phase, spontaneous calls are more likely to be initiated during inspirations. Congruent with the temporal precision that has been observed during vocal turn-taking, this respiratory gating is overcome during antiphonal calling. Since the vocal forebrain has been shown to shape the timing of response calls, we wanted to test if manipulation of the vocal forebrain could shape calls initiated by DM, which can continue to drive calls even in the absence of input from the vocal forebrain. Pharmacological manipulation of the vocal forebrain could bidirectionally shape the efficacy of DM stimulation in evoking calls, demonstrating the ability of the forebrain to shape and gate reflexive brainstem circuits, which may be crucial for successfully navigating complex and nuanced social environments.

Disclosures: E. Kish: None. **W.H. Mehaffey:** None. **K. Yackle:** None. **M.S. Brainard:** None.

Poster

PSTR419. Vocal/Social Communication—Avian II

Location: WCC Halls A-C

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Program #/Poster #: PSTR419.06/HH11

Topic: F.01. Neuroethology

Support: NIH UF1NS115821

Title: Low-threshold and calcium-dependent potassium currents regulate the intrinsic firing properties of forebrain-projecting HVCRA neurons in zebra finches

Authors: *A. DAOU[1,2], D. MARGOLIASH[2];
[1]Biomed. Engin., American Univ. of Beirut, Beirut, Lebanon; [2]Dept. of Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL

Abstract: In zebra finch, the song system nucleus HVC produces stereotyped instructions leading to precise, learned vocalizations. The HVC contains multiple neural populations, including neurons that project to nucleus RA (robust nucleus of arcopallium; primary motor cortex analog), to Area X (of the avian basal ganglia), to nucleus Avalanche (higher order auditory structure), and interneurons. These four populations exhibit complex patterns of excitatory and inhibitory connectivity, yielding characteristic patterns of neuronal activity both *in vivo* and *in vitro*. Premotor HVC_{RA} neurons play a critical role in orchestrating the neural circuitry that guides the bird's song production. We performed whole cell current-clamp recordings on zebra finch HVC_{RA} neurons in brain slices to examine their intrinsic firing properties, determine which ionic currents are responsible for their characteristic firing patterns, and characterize firing properties of any observed classes of neurons. We show that HVC_{RA} neurons exhibit diversity in their spiking activity when stimulated with current pulses in slices, ranging from transient to stuttering patterns. Simple features of the raw data clearly subdivide the

neurons into two major classes, and a third. Morphological analysis of filled cells independently confirms these categorical distinctions. We developed conductance-based models for the different neurons in each subtype and calibrated the models using data from the slice recordings, yielding mechanistic descriptions of how the interplay of ion currents gives rise to the response properties of each neuronal class. These predictions were then tested and verified in the slice with pharmacological manipulations. The models and the pharmacology highlighted low-threshold potassium currents (D-type Kv1 channel and M-type Kv7 channel) as well as the Ca^{2+}-dependent K^+ current in driving the characteristic neural patterns observed in HVC_{RA}. The relative strengths of different currents give rise to the physiological features observed across the three classes of HVC_{RA} neurons. The data suggest that the intrinsic properties for one of the HVC_{RA} subtypes exhibit a within-bird homogeneity and across-birds heterogeneity, suggesting a role of learning in shaping the firing properties of this class of neurons. These results begin to establish a mechanistic basis for examining much debated circuit and network properties of HVC_{RA} neurons.

Disclosures: A. Daou: None. **D. Margoliash:** None.

Poster

PSTR419. Vocal/Social Communication—Avian II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR419.07/HH12

Topic: F.01. Neuroethology

Support: Simons Foundation
HHMI Gilliam Fellowship
NIH U24 NS126936
NIH R01NS109237
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Robert W. Woodruff Fellowship

Title: Emergence of crystallized neural patterns during vocal learning

Authors: L. M. PASCUAL, S. J. SOBER;
Biol., Emory Univ., Atlanta, GA

Abstract: Learned motor skills are essential to everyday function. Skill learning involves repeated practice of motor acts, a process which requires the brain to integrate sensorimotor feedback to refine behavior until consistent high levels of performance is achieved. Young songbirds undergo this process whereby its undifferentiated, babbling-like vocalizations develop into categorical vocalizations (syllables) which form a highly precise and stereotyped crystallized song that mimics the song of its adult “tutor”. During this long period of motor skill acquisition, neural activity patterns are reshaped; however, it remains poorly understood how

changes in neural activity patterns enable skill learning. To examine whether and how the neural control of song vocal behavior changes during the process of song acquisition, we recorded spiking activity from individual units in the premotor nucleus of the arcopallium (RA) in Bengalese finches across song development and quantified changes in the neural vocabulary (the unique spike patterns produced) as well as in the motor code (how spike patterns influence behavior). Our chronic neural recordings span multiple weeks of vocal learning in individual animals, which we then examine with novel mathematical tools. This unique approach advantages us with new insights into the individuality of motor codes used across animals as well as changes in motor codes within animals during skill learning. Our preliminary results show changes in the statistics of spike pattern vocabularies as song syllables mature and diverge into acoustically distinct syllables. We also ask whether and how the timescale of motor coding changes during learning -that is, whether developing song is impacted by variations in spike patterns on the scale of 1-millisecond, as it has been demonstrated in adult song, or by variations on slower timescales. Furthermore, we examine whether changes in the motor code is dissociable, even at the level of a single neuron's activity, into factors like age and amount of sensorimotor practice. Broadly, our study provides a framework for investigating how transformations in spike patterns drive learning across different species.

Disclosures: L.M. Pascual: None. **S.J. Sober:** None.

Poster

PSTR419. Vocal/Social Communication—Avian II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR419.08/HH13

Topic: F.01. Neuroethology

Support: Studienstiftung des deutschen Volkes
HHMI Grant
IMPRS-MMFD travel support

Title: Lesions of pallial nucleus mMAN increase variability in birdsong syntax

Authors: *A. KOPARKAR[1], T. L. WARREN[2], J. D. CHARLESWORTH[2], S. SHIN[2], M. S. BRAINARD[2], L. VEIT[1];
[1]Inst. for Neurobiology, Univ. of Tuebingen, Tuebingen, Germany; [2]Dept Physiol., UCSF Ctr. For Integrative Neurosci, San Francisco, CA

Abstract: Complex motor skills such as speech and dance are composed of ordered sequences of simpler elements, but the neuronal basis for syntactic ordering of individual actions into sequences is poorly understood. Birdsong is a learned vocal behavior composed of syntactically ordered sequences of individual elements called syllables. Song premotor nucleus HVC has been linked to the control of syllable sequencing, but its role has primarily been studied in the zebra finch (ZF), a species with highly stereotyped syllable sequencing (Vu et al. 1994, Long & Fee

2008), and little is known about how the complex and variable sequencing of syllables in other species is controlled. In Bengalese finches (BFs) and canaries, unique patterns of HVC neural activity can encode the variable sequential context of syllables (Fujimoto et al. 2011, Cohen et al. 2020), but the extent to which the sequencing of these patterns reflects the internal neural dynamics of HVC (Zhang et al. 2017) or is shaped by recurrent inputs to HVC (Hosino & Okanoya 2000) is poorly understood. One major recurrent input to HVC is nucleus mMAN, which is thus well-positioned to influence patterned activity in HVC and syllable sequencing. Previous studies found that lesions of mMAN had little effect on the stereotyped sequencing of adult ZF song (Foster & Bottjer 2001, Horita et al., 2008), but we reasoned that any contributions of mMAN to sequencing might be better revealed in birds with more complex syntax. We therefore assessed the effects of bilateral mMAN lesions on the variable songs of adult BFs (Lonchura striata domestica). The syntax of BF song includes several patterns: 1) chunks, where syllables follow stereotypical order 2) branch points, where a given syllable can be followed by two or more different syllables in a probabilistic manner and 3) repeat phrases, where an individual syllable is repeated a variable number of times. We found that after lesions of mMAN, the acoustic structure of syllables remained intact, but sequencing became more variable for each of these patterns; chunks developed 'breaks' in which previously stereotyped sequences could be interrupted by novel transitions, branch points exhibited increased variability, quantified as increased transition entropy, and repeat phrases became more variable in the number of syllable repetitions. These results demonstrate that mMAN contributes to variability of syllable sequencing in the BF and highlight the potential importance of regions projecting to HVC in the ordering of vocal elements. More broadly, they indicate the utility of species with more complex song syntax in investigating neural control of motor sequences.

Disclosures: **A. Koparkar:** None. **T.L. Warren:** None. **J.D. Charlesworth:** None. **S. Shin:** None. **M.S. Brainard:** None. **L. Veit:** None.

Poster

PSTR419. Vocal/Social Communication—Avian II

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Title: Transformation of premotor neural activity and respiratory pressure during the repetition of introductory notes in the male zebra finch

Authors: D. RAO[1], R. P R[2], S. KALRA[1], S. CHOROL[2], M. UPADHYAYA[2], A. DUTTA[2], S. CHITNIS[3], ***R. RAJAN**[2];
[1]Indian Inst. of Sci. Educ. and Res., Pune City, India; [2]Biol., Indian Inst. of Sci. Educ. and Res. Pune, Pune, India; [3]HHMI Janelia Res. Campus, HHMI Janelia Res. Campus, Ashburn, VA

Abstract: The song of the adult male zebra finch is a well-studied example of a learned movement sequence. Song consists of a stereotyped sequence of sounds interleaved by silent gaps and is controlled by the sequential and precise bursting of premotor neurons. This bursting activates vocal and respiratory muscles ensuring perfect coordination between respiration and vocalization during song production. Song bouts begin with the repetition of a variable number of short sounds, called introductory notes (INs), before actual song production. Compared to song syllables, INs have more variable gaps between them and more variable acoustic properties. This variability reduces as song approaches, with the last IN representing a more stereotyped "state" compared to the first IN in a song bout. This suggests the possibility that INs reflect a "preparatory" phase that drives respiratory and vocal coordination. To test this hypothesis, we first characterized neural activity in awake, singing zebra finches. In premotor nucleus HVC, putative interneurons (n=13 from 2 birds) and HVCX-projecting neurons (n=12 from 2 birds) showed complex IN-related activity patterns. Some neurons were active only before the last IN, while others were active for combinations of INs (all but the first or all but the last). As a population, this data suggests that different overlapping subsets of HVC neurons are active for each of the INs. Activity during the silent gaps between INs did not show correlations with the length of the gap. In a separate set of birds, multi-unit recordings (n=15 sites from 3 birds) from downstream motor nucleus RA showed equal activity for each IN. Finally, to determine whether the coordination between respiration and vocal production changes, as INs repeat, we are currently recording respiratory pressure changes in awake, singing, male zebra finches. Overall, our preliminary results suggest a reconfiguration of the HVC network during INs, possibly reflecting a switch towards the sequential and precise bursting that is necessary for song production.

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Poster

PSTR419. Vocal/Social Communication—Avian II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR419.10/HH16

Topic: F.01. Neuroethology

Title: Female vocal feedback promotes song learning in juvenile zebra finches

Authors: *L. BISTERE[1], C. M. GOMEZ-GUZMAN[1], Y. XIONG[2], S. WILCZEK[1], D. VALLENTIN[1];
[1]MPI For Biol. Intelligence, Seewiesen, Germany; [2]Univ. of Tübingen, Tübingen, Germany

Abstract: Social interactions promote vocal learning but little is known about how social feedback affects the vocal learning process and its underlying neural circuitry. To address this issue, we explored song imitation in juvenile zebra finches raised either in the presence or absence of females providing vocal feedback. We found that male zebra finches raised with a female copied the spectral and temporal features of the tutor song more accurately than compared to birds, that were raised socially isolated. To explore whether female zebra finches provide juvenile males with vocal feedback during song practice, we tracked vocalizations emitted by female birds with a small microphone attached to the back of the female. Females emitted more calls as young birds improved their song performance, indicating that females can provide practice-specific feedback. To decipher whether female vocal feedback has an impact on the neural activity within the song learning pathway, we performed intracellular recordings in HVC, a premotor area involved in song learning and production, in singing and listening zebra finches. In juvenile zebra finches, we found that female vocalizations can modulate neural activity in HVC during passively listening and singing. In contrast, in singing adult zebra finches female calls do not have an impact on the song-related neural activity pattern. Interestingly, we found female call-evoked responses outside of the context of singing to persist after development suggesting an age-independent mechanism for the representation of behaviorally relevant vocal feedback. These results highlight the contribution of female vocal feedback to developmental song learning and how vocal input other than the tutor song can influence the neural circuit involved in song learning and production.

Disclosures: L. Bistere: None. **C.M. Gomez-Guzman:** None. **Y. Xiong:** None. **S. Wilczek:** None. **D. Vallentin:** None.

Poster

PSTR419. Vocal/Social Communication—Avian II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR419.11/HH17

Topic: F.01. Neuroethology

Title: Characterizing auditory responsivity in female zebra finch HVC

Authors: *A. SAVOY[1], D. MARGOLIASH[2];
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Abstract: The zebra finch is a champion species of vocal learning and production, with males producing highly precise songs and females evaluating individual differences in male singing. The foundation of male song includes auditory learning early in development and auditory

feedback-mediated sensorimotor learning during development that extends into adulthood. Studies in females, who do not sing, have demonstrated their extraordinary auditory perception abilities as adults, as well as their sensitivity to developmental auditory experience and social context. Many studies in males have focused on sensorimotor area HVC, which expresses state-dependent higher-order song-related auditory responses. Despite the much smaller volume of female zebra finch HVC as compared to males, recent evidence shows robust anatomical connectivity between HVC and other "song system" areas in females. We hypothesize that this circuitry is involved in female auditory processing, song evaluation, and call interactions, which has been investigated only in very few studies. Our study aims to characterize the auditory responsivity of HVC in female zebra finches when exposed to male vocalizations. To date we have used Neuropixels probes for high-density extracellular recordings in HVC of awake female zebra finches. Birds were exposed to a variety of male vocalizations, as well as to artificial sounds such as noise bursts and other stimuli. Our data indicate that female HVC exhibits distinct neural responses to specific male songs or calls. Preliminary data indicate that the response patterns implicate involvement of HVC in the nuanced perceptual discrimination capability that female zebra finches are known to possess, with varying responsivity depending on her familiarity with the stimulus and relationship to the male. These findings potentially offer new insights into the role of HVC in female auditory perception and contribute to our understanding of the neural bases of social communication and mate choice.

Disclosures: A. Savoy: None. **D. Margoliash:** None.

Poster

PSTR419. Vocal/Social Communication—Avian II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR419.12/HH18

Topic: F.01. Neuroethology

Support: Simons Collaboration on the Global Brain

Title: Vocal representation of acoustic features in a budgerigar forebrain motor area

Authors: *Z. YANG[1], M. A. LONG[2];
[1]New York Univ. Langone Med. Ctr., New York, NY; [2]NYU Sch. of Med., New York, NY

Abstract: Parrots have extraordinary vocal mimicry abilities, yet the neural mechanisms responsible for effectively reproducing sounds with diverse acoustic qualities remain unclear. Here we investigate neural dynamics underlying vocal production in the budgerigar, a highly social parrot species. We use high-density silicon probes to record vocalization-related activity from neuronal populations in the central nucleus of the anterior arcopallium (AAC), a forebrain motor region that directly projects to brainstem phonatory motoneurons. We found that AAC neurons demonstrate robust and consistent network activity associated with both stereotyped contact calls and flexible warble songs produced during social interactions. Furthermore, AAC

activity exhibits a distinct neural signature that aligns with the complex acoustic structure presented in warble songs. Budgerigar songs are characterized by large variations in fundamental frequency (i.e., pitch) and spectral entropy (i.e., the level of "noisiness"). We found a substantial proportion of AAC neurons that were strongly tuned to these parameters. We observed a high degree of heterogeneity across the population, with individual neurons exhibiting maximal responses to different values of these features. We used a principal component analysis to examine population activity and found a strong gradient of fundamental frequency and spectral entropy along the axes corresponding to the first and second principal components, respectively. These results indicate that AAC encodes acoustic features controlling vocalization quality, resembling the representation of vocal articulation and pitch in the human speech motor cortex and potentially contributing to their exceptional vocal mimicry abilities.

Disclosures: Z. Yang: None. **M.A. Long:** None.

Poster

PSTR419. Vocal/Social Communication—Avian II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR419.13/HH19

Topic: F.01. Neuroethology

Support: KAKENHI Grant-in Aid for Early-Career Scientists
NEXT KAKENHI 22H04767

Title: Transient connection from tutor-song-responding neurons in NCM to HVC disappears at the end of the critical period

Authors: *J. A. KOMOROWSKA-MULLER, Y. MOROHASHI, Y. YAZAKI-SUGIYAMA; Neuronal Mechanism for Critical Period Unit, Okinawa Inst. of Sci. and Technol., Tancha, Japan

Abstract: Similarly to human infants, juvenile zebra finches learn to vocalize by memorizing their tutor's song and then matching their own vocalizations to the memorized template via auditory feedback during the developmental critical period. We previously identified neuronal substrate of tutor song memory in the avian analog of secondary auditory cortex, the caudomedial nidopallium (NCM)(Yanagihara and Yazaki-Sugiyama, 2016). Recently, we uncovered a developmentally transient projection from the tutor song-responding cells in NCM to the song premotor area, HVC. We labeled the tutor song-responding cells with GFP using the neuronal activity-dependent AAV (AAV9-cFos-TetON-EGFP) and tutor song playback. The NCM-HVC projection was present in juvenile birds, but not in adult birds suggesting its potential role in song learning (Louder et al., in submission). Here, we further investigated the developmental timeline of NCM-HVC projection and disconnection. We found a decrease in the density of projections to HVC in juveniles at the end of sensorimotor learning (80 days post hatch (dph)) in comparison to the juveniles within sensorimotor learning (60 and 70 dph). In contrast, we found no difference in the number of projections to other brain areas, such as HVC

shelf, AIV, CMM and Area X. Furthermore, we found that the timing of the decrease in NCM-HVC projections coincided with song maturation. Taken together, the dynamic interareal neuronal disconnection between zebra finch auditory and motor cortex is potentially shaping the developmental auditory memory-guided song learning.

Disclosures: **J.A. Komorowska-Muller:** A. Employment/Salary (full or part-time):; Okinawa Institute of Science and Technology. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); KAKENHI Grant-in Aid for Early-Career Scientists. **Y. Morohashi:** A. Employment/Salary (full or part-time):; Okinawa Institute of Science and Technology. **Y. Yazaki-Sugiyama:** A. Employment/Salary (full or part-time):; Okinawa Institute of Science and Technology.

Poster

PSTR419. Vocal/Social Communication—Avian II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR419.14/HH20

Topic: F.01. Neuroethology

Support: MEXT KAKENHI 22H05515

Title: Molecular profiles of auditory tutor song memory neurons, transiently projecting to song premotor area in zebra finches

Authors: Y. MOROHASHI[1], N. TOJI[2], Y. GO[3], K. WADA[2], *Y. YAZAKI-SUGIYAMA[1]; [1]Okinawa Inst. of Sci. and Technol. (OIST) Grad. Univ., Okinawa, Japan; [2]Biol. Sci., Hokkaido Univ., Sapporo, Japan; [3]Grad. Sch. of Information Sci., Univ. of Hyogo, Kobe, Japan

Abstract: Like human infants learning to speak, juvenile male zebra finches learn to sing by forming auditory tutor's song memories and then vocally matching to establish their stereotyped own song in sequentially well-orchestrated auditory then sensorimotor developmental learning periods. However, neural mechanisms supporting auditory memory-guided sensorimotor learning have remained elusive. Recently, we revealed neuronal projections into the song premotor region, HVC from distinct neuronal memory ensembles of a tutor's song in the auditory forebrain area, caudomedial nidopallium (NCM) during (~60 days post hatch (DPH)), but not after (> 100 DPH) the sensorimotor learning period by employing a novel activity-dependent adeno-associated viral expression system in zebra finches, implying roles of transient NCM-HVC projections for developmental song learning and subsequent crystallization. Here, we performed single-nucleus RNA sequencing (snRNA-seq) analysis to elucidate underlying molecular mechanism of transient projections of auditory memory NCM neurons to HVC. Tutor-song responding NCM neurons were labelled by using the AAV vector AAV-cFos-TetON-EYFP-PEST, which express EYFP with an activity dependent cFos promoter combined with a tutor song stimulation, and their nuclei were collected from male zebra finches at different developmental stages. Gene expression profiles were compared between YFP-positive tutor-song

responding neurons and other EYFP-negative neurons in the NCM. Post mRNA-sequencing analysis using Seurat revealed EYFP mRNA positive tutor song responsive neurons within 2/2 neuronal clusters expressing VGLUT2 mRNA and 3~5/8 neuronal clusters expressing GAD1 mRNA both in zebra finches at 60 DPH and > 100 DPH. We further found synaptic adhesion and vesicle trafficking molecules, such as NRCAM, ADGRB3/Bai3, Rab33B, TBC1D22B and DENND2A, from the list of differentially expressed genes (DEGs) in VGLUT2 positive excitatory neurons in zebra finches at 60 DPH. Taken together, these results suggest that expression changes in synaptic connection and membrane trafficking molecules mediate dynamic remodeling of intercortical auditory-motor projections in the developmental sensorimotor learning period.

Disclosures: Y. Morohashi: None. **N. Toji:** None. **Y. Go:** None. **K. Wada:** None. **Y. Yazaki-Sugiyama:** None.

Poster

PSTR419. Vocal/Social Communication—Avian II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR419.15/HH21

Topic: F.01. Neuroethology

Title: A generative model of neuron migration in adult songbird HVC

Authors: *S. ANALOUI[1], N. R. SHVEDOV[2], B. B. SCOTT[1];
[1]Psychological and Brain Sci., Boston Univ., Boston, MA; [2]Boston Univ., Boston Univ. Grad. Program For Neurosci., Boston, MA

Abstract: Songbirds add new neurons to brain circuits, such as the song nucleus HVC, throughout their lifetimes. New neurons are born in the ventricular zone (VZ) and migrate hundreds of microns to their integration targets throughout the forebrain, including HVC, over the span of weeks. Previous in vivo imaging studies have revealed that adult-born neurons in HVC exhibit a form of diffusive migration where cells move in all directions and make frequent turns. Here we design a computational model of neuron migration in the HVC to assess the consequences of this type of motion. Our model assumes an independent stochastic migration process wherein 3D movement is generated in a series of steps each representing 10 minutes. Cells are born in random locations along a 2D plane representing the VZ and have their migration simulated via random draws from probability distributions derived from in vivo tracking data. HVC is represented as a 1 mm diameter sphere intersected by the VZ plane. We ran our simulation for up to 40 days, the maximum duration of migration identified in previous literature (Alvarez-Buylla & Nottebohm 1988). This model successfully recapitulated multiple features of in vivo cell migration with simulated cells having highly similar tortuosities and levels of disorderedness as tracked cells. After 21 days cells migrated an average of 365 μm, covering the distance between the VZ and the center of HVC. After 40 days of migration, simulated cells traveled an average of 530 μm and up to 1.96 mm away from the VZ. These

results suggest that this form of diffusive migration is sufficient to fill the entirety of HVC but insufficient to generate regions that lie farther than ~2 mm from the VZ. These results are consistent with a potential model in which alternative forms of migration exist outside of HVC that enable cells to travel long distances and reach integration sites far from the VZ that diffusive migration alone cannot achieve. Furthermore, this model can be used to make specific quantitative predictions regarding the outcomes of migration of newborn cells that can be tested experimentally.

Disclosures: **S. Analoui:** None. **N.R. Shvedov:** None. **B.B. Scott:** None.

Poster

PSTR419. Vocal/Social Communication—Avian II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR419.16/HH22

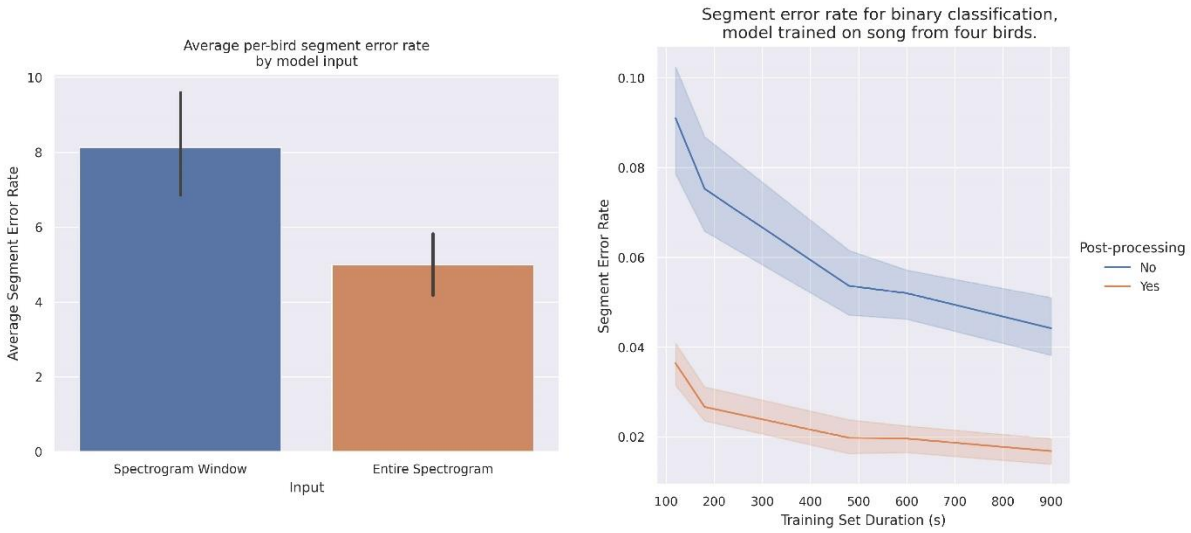
Topic: F.01. Neuroethology

Title: Benchmarking neural network models for acoustic behavior with vak and VocalPy

Authors: *D. A. NICHOLSON[1], Y. COHEN[2];
[1]Emory Univ., Baltimore, MD; [2]Weizmann Inst. of Sci., Weizmann Inst. of Sci., Rehovot, Israel

Abstract: To understand the neural bases of how animals communicate with sound, we need detailed quantification of this behavior. Recently, deep neural network models have emerged as promising tools for quantifying behavior. We previously presented TweetyNet, a neural network that automates segmentation and annotation of birdsong for downstream analyses, such as fitting statistical models of motor learning or song syntax. To enable researchers to easily work with TweetyNet and other deep neural network models for acoustic behavior, we developed a framework in Python, vak (https://github.com/vocalpy/vak). Additionally we have developed a core Python package for this research community, VocalPy (https://github.com/vocalpy/vocalpy) with the goal of making code more readable and reusable. Here we report on further studies of TweetyNet and other neural network models, using these Python libraries. We show that we can reduce the segment error rate of TweetyNet by an average 3.13% on a benchmark dataset of Bengalese finch song (https://nickledave.github.io/bfsongrepo/), without post-processing steps for clean up. (Segment error rate is analogous to the word error rate used in automatic speech recognition.) We achieve this by training TweetyNet on entire bouts of song instead of random time windows. We also extend previous work from others that used TweetyNet to segment birdsong into two classes, syllables and silent gaps, without assigning labels to the different syllables types. In this binary classification setting, models trained on 900 seconds of song from the same benchmark dataset achieve a 1.9% average segment error rate. We compare this result to off-the-shelf algorithms for segmenting audio, using VocalPy. Additionally we will present results from experiments in progress benchmarking other neural network models for audio segmentation, and demonstrating how vak can speed up training using transfer learning methods.

These results show how vak and VocalPy can support the research community investigating how the brain controls acoustic behavior.



Disclosures: D.A. Nicholson: None. **Y. Cohen:** None.

Poster

PSTR419. Vocal/Social Communication—Avian II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR419.17/HH23

Topic: F.01. Neuroethology

Support: National Geographic Society - 51460R-18

Title: High frequency vocalizations of black jacobin hummingbirds show annual shifts suggestive of learning

Authors: *C. OLSON[1], C. V. MELLO[3], C. DUCA[4], M. DA SILVA[5], S. RODRIGUES SILVA[4], M.-Q. TRAN[2];
[1]Dept. of Physiol., [2]Midwestern Univ., Glendale, AZ; [3]Dept. of Behavioral Neurosci., Oregon Hlth. and Sci. Univ. Sch. of Med., Portland, OR; [4]Pontifícia Univ. Católica de Minas Gerais, Belo Horizonte, Brazil; [5]Univ. Federal do Pará, Belém, Brazil

Abstract: Black jacobins are a basal lineage hummingbird species of the Topaz clade from the Brazilian Atlantic Forest. This species coexists with 40 other species/subspecies of hummingbird, as well as a rich community of songbirds and vocalizing mammals. We previously reported on black jacobin's high frequency vocalizations of a mean fundamental frequency of 11.5 kHz, with oscillations up to 14 kHz and no sounds below 10 kHz (Olson et al., Curr Biol, 2016). This vocal range is well above the known limit of avian hearing (up to 8-9 KHz),

challenging a conventional belief that avian communication is limited to low frequencies. Analysis of recent recordings and behavioral observations at our field site (Sta. Teresa, ES, Brazil) in 2019 and 2022, in comparison with the published data from 2015/16, now provide further insights into the behavioral ecology context and variations of this unique avian vocalization. We found that these vocalizations are predominantly directed to other black jacobins at short distances during inflight displays, and not towards other hummingbird species, but black jacobins will also produce them while perched in a non-directed manner. Interestingly, a 3-syllable utterance with a characteristic syllable duration and acoustic structure was the predominant vocalization in the population at our study site in 2015, occurring ~71% of the time. By 2016 we noted an increase in a 2-syllable vocalization type. In 2019 the 3-syllable type occurred in only ~15% of the recordings, and by 2023 they were completely absent, replaced by the 2-syllable song type. In addition to this shift from 3 syllables to 2 syllables, the mean syllable durations increased by 20% from 2015 to 2022. Sample recordings from several birds in captivity in 2022 revealed that that individual birds have a single song type with consistent within-individual acoustic features, allowing for the possibility of individual recognition. We also found conclusive evidence that vocalizations are produced by both sexes and juveniles. These observations support the notion that the black jacobin's high-frequency vocalizations evolved as an exclusive communication channel, and provide strong suggestive evidence that these vocalizations are learned. They also set the stage for more extensive investigation of the acoustic biology of this species, and present a valuable tool for future research on vocal communication in complex environments.

Disclosures: C. Olson: None. **C.V. Mello:** None. **C. Duca:** None. **M. da Silva:** None. **S. Rodrigues Silva:** None. **M. Tran:** None.

Poster

PSTR419. Vocal/Social Communication—Avian II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR419.18/HH24

Topic: F.01. Neuroethology

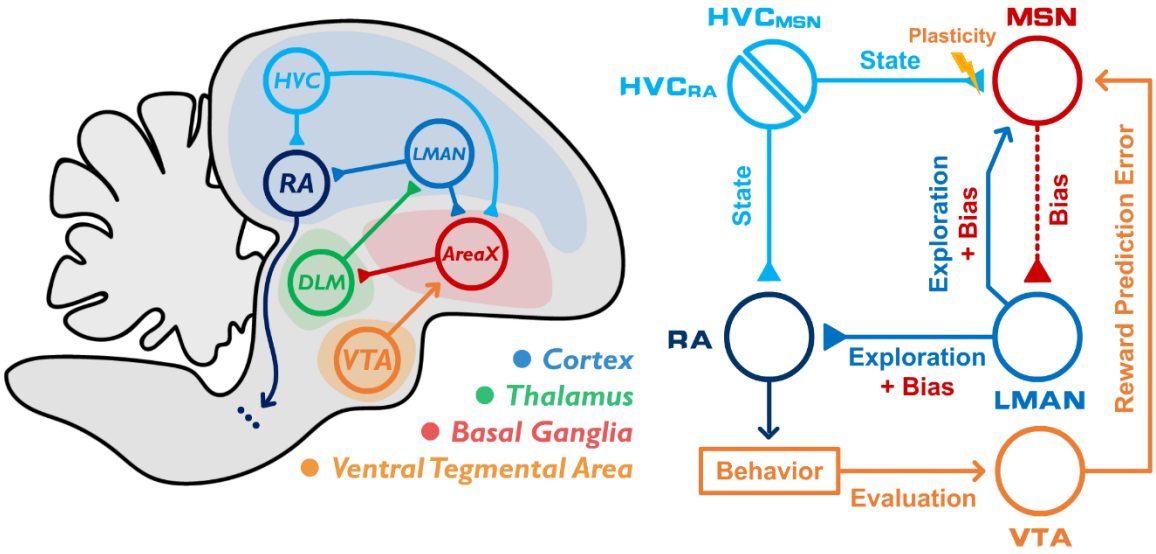
Support: Simons Foundation 542989SPI

Title: A neural circuit model of reinforcement learning in songbird

Authors: *Y. WANG[1], J. KORNFELD[2], M. S. FEE[3], M. S. GOLDMAN[4];
[1]UC Davis, Davis, CA; [2]Max Planck Inst. for Biol. Intelligence, Martinsried, Germany; [3]Brain & Cog Sci. / McGovern Inst., Massachusetts Inst. Tech., Cambridge, MA; [4]Ctr. for Neurosci., Davis, CA

Abstract: Reinforcement learning is widely recognized as a biological learning paradigm, but the underlying neural mechanisms are largely unknown. We propose a neural circuit model in songbird that employs policy-based reinforcement learning to guide song learning at a

millisecond timescale. We map the song learning process to a classical reinforcement learning problem known as the two-arm-bandit problem, where the neural circuit makes a sequence of binary action selections directly controlled by neuronal spikes. Our model learns the behavior policy by combining cortico-striatal activity representing action selection and midbrain dopamine signal representing behavior evaluation. During song learning, dopamine has been found to resemble a reward prediction error (RPE) signal. We demonstrate in our model how the RPE-like dopamine signal can be computed in a simple neural circuit and guides direct policy learning. Besides RPE, we propose that an action prediction error (APE) signal, stored as a molecular trace in the cortico-striatal synapse, also supports reinforcement learning in the brain. APE and RPE can be combined to avoid incorrect learning and enhance learning speed. Meanwhile, the sparseness of exploratory actions, a property often overlooked in most reinforcement learning models, plays an important role under accuracy constraints. Furthermore, the biologically motivated APE and RPE learning rules may inspire novel computational algorithms in reinforcement learning.



Disclosures: **Y. Wang:** None. **J. Kornfeld:** None. **M.S. Fee:** None. **M.S. Goldman:** None.

Poster

PSTR419. Vocal/Social Communication—Avian II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR419.19/HH25

Topic: F.01. Neuroethology

Support: NIH R01DA050374

Title: Weight transfer in the reinforcement learning model of songbird learning

Authors: *K. H. TRAN[1,2], A. KOULAKOV[2];
[1]Neurobio. and Behavior, Stony Brook Univ., Stony Brook, NY; [2]Cold Spring Harbor Lab., Cold Spg Hbr, NY

Abstract: Song learning behavior observed in the songbird system provides a notable example of learning through trial-and-error. We present a computational model of song learning that integrates reinforcement learning (RL) and unsupervised learning (UL) and agrees with known songbird circuitry. The song circuit outputs activity from the nucleus RA, which receives two primary inputs: timing information from area HVC and stochastic activity from the nucleus LMAN. Additionally, song learning relies on Area X, a basal ganglia area that receives dopaminergic inputs from VTA. In our model, song learning begins with the HVC-to-Area X connectivity, employing an RL mechanism that involves node perturbation. This acquired information is then consolidated in the HVC-to-RA weight matrix through a UL mechanism. The transfer of weights from Area X to RA takes place via the thalamus, employing a specific form of spike-timing-dependent plasticity. Thus, we present a computational model in which the optimal policy is initially guided by reinforcement and subsequently transferred to another circuit through Hebbian plasticity.

Disclosures: K.H. Tran: None. **A. Koulakov:** None.

Poster

PSTR419. Vocal/Social Communication—Avian II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR419.20/HH26

Topic: F.01. Neuroethology

Support: NSF award EF-1822476 (DZJ)
NIH T32 Grant

Title: Growth and Splitting of Sequences in Neural Activity in a Computational Model of Songbird HVC

Authors: *D. SEDERMAN, D. Z. JIN;
Penn State Univ., University Park, PA

Abstract: Zebra finches sing a stereotyped sequence of syllables, with each syllable driven by a distinct sequence of bursting neurons in the premotor nucleus HVC. During development, a single ‘protosyllable’ sequence initially forms before a zebra finch produces multiple syllable types (Okubo et al., 2015). This sequence splits into multiple syllable-specific sequences as the juvenile matures and listens to an adult tutor. The mechanism underlying this splitting is not well characterized. Here we simulated the growth and splitting of sequences in an HVC network as a juvenile zebra finch learns its song. Our computational model has a realistic HVC-sized scale

with over 20,000 HVC neurons simulated using a GPU implementation. Model input from nuclei Uva and Nif spontaneously activates a group of HVC "seed neurons". Immature HVC neurons are recruited to form a feedforward chain network starting from the seed neurons through spontaneous activity, burst-timing dependent synaptic plasticity and axon remodeling. As the immature neurons fire more consistently, they mature and burst with precise trial-to-trial timing. Consistent with previous work, a large single sequence forms among HVC neurons (Tupikov and Jin, 2021). However, as the simulation continues, this sequence splits and distinct burst sequences form within HVC. This occurs with unstructured inputs to HVC neurons that lack any timing or sequence information. As development progresses, neurons belonging to each sequence more strongly inhibit one another via feedback inhibition through interneurons in HVC. This results in the sequences that initially fire simultaneously consistently firing sequentially later in the simulation. We propose this as a model for the formation of distinct neural sequences during songbird development that does not require external timing inputs. Unstructured input is sufficient to trigger networking wiring and splitting into neural sequences within HVC.

Disclosures: D. Sederman: None. **D.Z. Jin:** None.

Poster

PSTR419. Vocal/Social Communication—Avian II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR419.21/HH27

Topic: F.01. Neuroethology

Support: NSF 2154646

Title: Songbird motor cortex analogue neurons utilize specialized Nav and Kv3 channels to generate fast spiking: Computational modeling of ultra-narrow and energy-efficient action potentials

Authors: *L. E. S. TAVARES[1], B. ZEMEL[1], H. VON GERSDORFF[1,2];
[1]Vollum Institute, OHSU, Portland, OR; [2]Oregon Hearing Res. Center, OHSU, Portland, OR

Abstract: In songbirds, the robust nucleus of the arcopallium (RA) is a distinctive brain area of the song system dedicated to vocal motor control during singing behavior. Projection neurons in RA (RAPNs) provide the sole descending vocal-motor output to the brainstem and orchestrate moment by moment complex acoustic features of the song. RAPNs exhibit continuous and spontaneous firing of spikes during periods of silence (~40 Hz) and high-frequency burst-pause firing during song production. Being essential for courtship behavior, birdsong imposes significant temporal and energetic constraints on RAPNs. For instance, in whole cell recordings in slices at physiological temperatures (40o Celsius in birds), RAPNs fire ultranarrow action potentials (APs) of 0.16 ms halfwidth and can sustain high frequency firing with little adaptation during current injection. These properties are supported in part by the high expression of fast-

gating, high-threshold Kv3 channels in conjunction with fast-gating Nav1.6, which contribute, respectively, to high depolarization and repolarization rates during AP generation (Zemel et al, 2021, 2023). Based on these observations, we developed a detailed Hodgkin-Huxley conductance-based model of RAPNs in slices at 40oC. In our model, fast transient Na+ and high-threshold K+ gating coordinate to drive ultranarrow and fast APs. We observe that the fast kinetics of activation and inactivation of Na currents and the fast activation and deactivation kinetics of Kv3 channels also helps in diminishing the excess of Na+ influx during APs by reducing the temporal overlap between voltage-gated K+ and Na+ currents. We propose that this property may be used by RAPNs to reduce the metabolic cost of fast spiking. Indeed, the RA of adult male zebra finches is marked by high expression of cytochrome oxidase, an enzyme involved in ATP production in the mitochondria (Adret & Margoliash, 2002). Our RAPN computer model represents a new tool for understanding how RA supports birdsong behavior and provides insights for future experiments.

Disclosures: L.E.S. Tavares: None. **B. Zemel:** None. **H. von Gersdorff:** None.

Poster

PSTR419. Vocal/Social Communication—Avian II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR419.22/HH28

Topic: F.01. Neuroethology

Title: Decision-making mechanism of songs in zebra finches by using song as a reward.

Authors: *M. IWASAKI[1], S. NOGUCHI[3], M. INDA[4], K. HOTTA[3], K. OKA[2,5];
[2]Dept Biosci. & Informatics, [1]Keio Univ., Yokohama-city, Japan; [3]Keio Univercity, Yokohama-city, Japan; [4]Fakultät für Psychologie, Ruhr-Universität Bochum, Fakultät für Psychologie, AE Biopsychologie, Bochum, Germany; [5]Kitasato Univ., Sagamihara-city, Japan

Abstract: The male zebra finches (Taeniopygia guttata) sing courtship songs to the females. The courtship song differs among individuals; females identify each song and make a pair with the male singing their preferred song. The song preferences of females have been investigated in many previous studies (Riebel, 2000; Riebel et al., 2002; Le Maguer et al., 2021), and in operant conditioning behavioral experiments, females were observed to repeatedly listen to their favorite songs (Rodríguez-Saltos et al., 2023). This indicates that listening to songs is rewarding. Previous studies on song discrimination have also shown that when females are asked to discriminate between two songs, they showed as high as 80% of the responses correct (Nagel et al., 2010; Paul et al., 2021). However, the remaining 20% were found to be incorrect, because of hearing and responding to only the beginning part of the song. We hypothesize that songbirds listen to only the first part of a song, and it can lead to misidentification of the song. Based on the above facts, we constructed an experimental system to measure preference by song rewards, and analyzed the order of songs heard with a Markov chain model in female zebra finches. We considered three types of states in the state transition diagram. The single Markov model shows

the bird selects the next song according to the only previous one, the dual model is the type of song selection it had heard on the two previous trials, and the triple model is the type of song selection it had heard on the three previous trials. By comparing the goodness of fit to the results of simulations with these three models to the raw data, we revealed differences among the models. We also examined the possibility that birds' song discrimination may include a prediction by mixing two courtship songs in the measurement of preference at the top of discrimination and by using stimuli that change in steps. If there is the prediction, the bird's behavior should initially be the same as when it heard the original song, but as the song stimulus changes and gradually resembles to the mixed song, it should behave the same as when it heard the mixed song. We tested this hypothesis in a preference experiment.

Disclosures: **M. Iwasaki:** None. **S. Noguchi:** None. **M. Inda:** None. **K. Hotta:** None. **K. Oka:** None.

Poster

PSTR420. Neuroendocrine Physiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR420.01/II1

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH R01HD093907-01A1

Title: Sensorimotor development in rodent neonates exposed to the clinically relevant synthetic progestin, 17-α-hydroxyprogesterone caproate (17-OHPC)

Authors: ***P. L. GRANEY**, J. MILLER, E. SARNO, C. K. WAGNER;
Univ. At Albany, Albany, NY

Abstract: 17-OHPC is commonly prescribed to individuals at risk for recurrent preterm birth. 17-OHPC was detectable within cord plasma of neonates up to 44 days after the maternal treatment, suggesting that the fetus is exposed to 17-OHPC in utero. Yet, little is known regarding the long-term effects of 17-OHPC on neural and behavioral development in children. The timing of 17-OHPC administration coincides with a period of cortical maturation. In rodents, neural maturity during the first postnatal week is comparable with human fetal neural development at term. Thus, administration of 17-OHPC in rodents during the first postnatal week can be used to model the effects of 17-O HPC on cortical development in humans. Progesterone receptor (PR) is expressed in virtually all functional regions of developing cortex including within subplate (SP) of somatosensory and motor cortex (E18-P10). Disruption to thalamocortical innervation of SP of somatosensory cortex is associated with deficits in sensorimotor behavior. PR knock-out mice (PRKO) demonstrated irregular loss of a sensorimotor-dependent behavior, rooting reflex. These data suggest that cortical development and behavioral indicators of normal nervous system development, such as sensorimotor behavior, may be sensitive to neonatal exposure to 17-OHPC. Therefore, it was hypothesized

that 17-OHPC exposure during development alters sensorimotor behavior development. There was a significant main effect of treatment, sex, age, and a day by treatment interaction ($p = 0.003$) on body weight with 17-OHPC exposed rats weighing significantly more than control rats of the same sex. Preliminary data also suggest a significant effect of treatment at postnatal days 3 and 4 on surface righting (P3: $\chi^2 = 30.392$, P4: $\chi^2 = 7.799$) and forelimb grasping (P3: $\chi^2 = 14.545$, P4: $\chi^2 = 9.468$) with a greater proportion of 17-OHPC-exposed rats successfully righting and successfully grasping with the forelimbs. 17-OHPC exposed rats has a shorter latency to surface right at P3. However, on P5 a greater proportion of oil-treated rats demonstrated successful righting ($\chi^2 = 9.013$). There was also a significant effect of treatment on hindlimb grasping at P5 ($\chi^2 = 8.720$), P7 ($\chi^2 = 16.707$), P8 ($\chi^2 = 12.029$), P10 ($\chi^2 = 18.357$), and P11 ($\chi^2 = 7.292$) with a greater proportion of oil-treated rats demonstrating partial or successful hindlimb grasping when compared to 17-OHPC-exposed animals. Results from this study demonstrate a novel effect of neonatal 17-OHPC exposure on behavior dependent on sensorimotor development and suggest that neonatal PR activity may regulate aspects of cortical development necessary for the expression of normal sensorimotor behaviors.

Disclosures: P.L. Graney: None. **J. Miller:** None. **E. Sarno:** None. **C.K. Wagner:** None.

Poster

PSTR420. Neuroendocrine Physiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR420.02/II2

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Hunting a chimera: In pursuit of an elusive Nsmf transcript

Authors: *H. BOW[1], ***H. BOW**[2], D. C. JACOBS[2], J. SHORT[2], S. M. FARMER[2], S. WRAY[2]; [1]NINDS, Washington, DC; [2]NINDS, Bethesda, MD

Abstract: Gonadotropin-releasing hormone (GnRH) is essential for neuroendocrine control of reproduction. During embryogenesis, GnRH neurons migrate from nasal areas to the forebrain along olfactory sensory/vomeronasal axons. This migration is guided by many cues, and mutations in associated genes have been shown to prevent successful migration of GnRH neurons into the forebrain, resulting in reproductive disorders. NMDA receptor synaptonuclear signaling and neuronal migration factor (Nsmf) is one potential migratory factor. Nsmf is expressed by migrating GnRH neurons, and mutations in the Nsmf gene have been implicated in idiopathic hypogonadotropic hypogonadism (IHH). However, since its discovery in 2000, the expression and role of Nsmf have been the subject of conflicting reports. It was initially described as a membrane-bound migration cue but has since been well-documented as a synaptonuclear transcription factor. The latter role is supported by a nuclear localization signal and lack of a transmembrane domain on the Nsmf transcript. Additionally, discrepancies have been reported in knockout mice. One line showed decreased numbers of GnRH cells postnatally, pubertal delay, and subfertility, while another line exhibited normal fertility and no change in

GnRH cell number. Searching mammalian protein databases, we identified a novel chimeric protein that incorporated amino acids from Nsmf and a neighboring gene, ENTP8. We found that this novel chimeric gene product (Nsmf/Entpd8) is present in cDNA made from E11.5 mouse embryo nasal tissue, and we are currently investigating the expression of this transcript in migrating GnRH neurons. This chimeric transcript lacks a nuclear localization signal but has a transmembrane domain that accounts for the membrane localization initially observed. In addition, an alternative transcription start site has been identified. As such, this chimeric transcript may underlie the discrepancies reported in the two knockout mice lines. These results highlight the complexities of unraveling the role of genes in physiological processes and the importance of deciphering the genetic basis of variants in human disorders.

Disclosures: H. Bow: None. **H. Bow:** None. **D.C. Jacobs:** None. **J. Short:** None. **S.M. Farmer:** None. **S. Wray:** None.

Poster

PSTR420. Neuroendocrine Physiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR420.03/II3

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: University of Wisconsin-Madison Grant AAK8133

Title: Head color morph and sex-specific differences in follistatin gene expression in the Gouldian finch brain

Authors: *C. ZHAO[1], F. MADISON[2];
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Abstract: Simple genetic variation and differential gene expression have been tightly linked to differences in behavioral phenotypes, endocrine physiology, and reproduction. The Gouldian finch (*Erythrura gouldiae*) demonstrates a head color polymorphism that is tightly coupled to marked differences in aggression, parental care, immune, and stress responses. Red-headed males are neophobic, aggressive, and exhibit higher levels of testosterone (T) and corticosterone (CORT) in socially competitive environments, while black-headed males are neophilic, passive, but T and CORT remain unaltered. This head color polymorphism has been associated with genetic variation in a small non-coding region upstream of the follistatin (FST) gene. FST is a single-chain gonadal protein that is implicated in a variety of endocrine, physiological, and behavioral processes, including regulation of energy metabolism, gonadal function, stress response and anxiety-related behavior. We hypothesized that the FST gene would be differentially expressed in association with morph and sex-specific differences in the brain between male and female red-headed and black-headed morphs. To test this hypothesis, we examined FST gene expression in brain regions known to be involved in aggression, stress responses, and parental care using RNAscope in situ hybridization assay. We found that FST

mRNA was heterogeneously distributed in the Gouldian finch brain with the most abundant expression in the ventromedial hypothalamic nucleus (VMH) and the lateral part of the hypothalamic paraventricular nucleus (PVNL). Importantly, we observed that FST gene expression differed significantly between morphs and sex. Specifically, mRNA levels of FST were higher in the VMH, PVNL, medial bed nucleus of stria terminalis (BSTM), lateral bed nucleus of the stria terminalis (BSTL) and nucleus of the hippocampal commissure (nHpC) in black-headed males and females than those in red-headed males and females. Furthermore, in all these regions within the same morph type, male finches displayed greater levels of FST mRNA relative to females. Taken together, our findings suggest that head color morph and sex-specific differences in FST gene expression in the Gouldian finch brain may contribute to the morph and sex-specific differences in aggression, stress responses, and parental care.

Disclosures: C. Zhao: None. **F. Madison:** None.

Poster

PSTR420. Neuroendocrine Physiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR420.04/II4

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: UNAM-PAPIIT-GI200121
CONAHCYT CF-2023-G-243
NIMH-MH 002386

Title: Kisspeptin cell-type and fiber projection analysis reveals its potential role on central sensorial processing and behavioral state control

Authors: *L. ZHANG[1], V. S. HERNÁNDEZ[1], M. A. ZETTER[1], L. E. EIDEN[2];
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Abstract: Previous chemoanatomical studies on kisspeptin (KP) distribution in rodent brain have focused on hypothalamic regulation of the hypophysial-gonad axis. Here, we examined the chemoanatomical phenotype and fiber distribution of KP neurons in mouse and rat, comparing male and female, and with and without gonadectomy, in order to extend our current knowledge of kisspeptin chemical neuroanatomy, and potential function, to extra-hypothalamic structures. Serial immunohistochemistry sampling and assessment, dual in situ hybridization (DISH), combining mRNA probes for kisspeptin with VGAT, VGLUT2, neurokinin, dynorphin, and tyrosine hydroxylase were used. Eighty-one among the 107 brain regions observed with KP fiber and terminal presence, were identified as extra-hypothalamic, including telencephalic septal, ventral striatal, amygdaloid nuclei and bed nucleus of stria terminalis; diencephalic habenular complex; mesencephalic superior and inferior colliculi, and periaqueductal grey; metencephalic parabrachial nucleus and locus coeruleus; myelencephalic nucleus tractus solitarii (NTS),

reticular formation and the gelatinous layer of spinal sensorial trigeminal nucleus. All of these regions are closely involved in central sensorial processing and behavioral state control. KP glutamatergic (VGLUT2 mRNA-expressing) neurons are mainly located in arcuate nucleus and scattered in the dorsal hypothalamic region from anterior to posterior hypothalamus; KP GABAergic (VGAT mRNA-expressing) neurons are found in the rostral periventricular region, the medial amygdala and the NTS. Some targets of these KP populations were determined based on correlation of anatomical analysis with sensitivity to gonadectomy. This study provides an anatomical basis for further hypothesis generation and testing on KP's role in a wider scope of brain function beyond endocrine regulation located to the hypothalamus. In particular, it draws attention to the remarkable presence of KP projections in central sensory and behavioral state control structures that suggest additional physiological/regulatory roles for kisspeptin.

Disclosures: L. Zhang: None. **V.S. Hernández:** None. **M.A. Zetter:** None. **L.E. Eiden:** None.

Poster

PSTR420. Neuroendocrine Physiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR420.05/II5

Topic: F.02. Neuroendrocrine Processes and Behavior

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Title: An excitatory pathway from arcuate Kiss1 neurons to the dorsomedial hypothalamus mediates the melanocortin action on energy expenditure in the male mouse

Authors: *R. TALBI[1,2], T. L. STINCIC[3], E. MEDVE[1,2], K. FERRARI[1,2], C. J. HAE[1,2], K. WALEC[1,2], O. K. RØNNEKLEIV[3,4], M. J. KELLY[3,4], V. M. NAVARRO[1,2];
[1]Harvard Med. Sch., Boston, MA; [2]Div. of Endocrinology, Diabetes and Hypertension, Brigham and Women's hospital, Boston, MA; [3]Dept. of Chem. Physiol. and Biochem., Oregon Hlth. & Sci. Univ., Portland, OR; [4]Div. of Neurosci., Oregon Natl. Primate Res. Ctr., Beaverton, OR

Abstract: Hypothalamic Kiss1 neurons are essential regulators of reproduction through the direct release of gonadotropin-releasing hormone (GnRH) secretagogue kisspeptin onto GnRH neurons. Kiss1 neurons also control metabolism, as documented by the anorexigenic effect of

kisspeptin in rodents and the obese phenotype of kisspeptin receptor knockout (Kiss1rKO) mice. In the control of energy balance, arcuate (ARC) proopiomelanocortin (*Pomc*) neurons induce satiety and increase energy expenditure (EE) through the direct action on the melanocortin receptor 4 (MC4R). Since POMC neurons have been described to project to Kiss1 neurons, we set out to characterize the role of Kiss1 neurons in the metabolic action of melanocortins. Using RNAscope, we found that *Mc4r* co-expresses with 84% of $Kiss1^{ARC}$ neurons, suggesting that these neurons are direct targets for melanocortins. Indeed, high frequency optogenetic stimulation of $POMC^{ARC}$ neurons elicited a slow inward (excitatory) current in $Kiss1^{ARC}$ neurons that was attenuated in the presence of a MC4R antagonist in both sexes. To assess the role of $Kiss1^{ARC}$ neurons in the metabolic action of melanocortins, we generated a conditional mouse model with a specific deletion of *Mc4r* from Kiss1 neurons ($Kiss1^{cre}$:$Mc4r^{fl/fl}$ mice). $Kiss1^{cre}$:$Mc4r^{fl/fl}$ male mice, but not females, developed obesity under regular chow and high fat diet. Metabolic assessment revealed that $Kiss1^{cre}$:$Mc4r^{fl/fl}$ mice had reduced EE, without changes in overall food intake or activity. The decrease in EE correlated with significantly lower expression of the uncoupling protein 1 (*Ucp1*) gene in the interscapular pad of brown adipose tissue (BAT), suggesting impaired BAT thermogenesis in mutant mice. To further test the role of $Kiss1^{ARC}$ neurons in EE, we chemogenetically activated these neurons in $Kiss1^{Cre}$ het and Kiss1KO males, which led to increased BAT temperature in both models, suggesting that this is a kisspeptin-independent effect. Moreover, we documented that this action is mediated, at least in part, by the dorsomedial hypothalamus (DMH) based on the following evidence: a) Viral tracing revealed that $Kiss1^{ARC}$ neurons innervate leptin receptor (Lepr) expressing neurons of the DMH ($Lepr^{DMH}$); b) the chemogenetic activation of $Kiss1^{ARC}$ neurons induced cfos expression in $Lepr^{DMH}$ neurons; and c) a retrograde tracing approach revealed that these neurons project to the raphe pallidus nucleus (RPa), which is known to regulate BAT activity.
In summary, we have documented that $Kiss1^{ARC}$ neurons mediate the action of melanocortins on EE in male mice through the activation of the dorsomedial pathway that controls BAT thermogenesis.

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Poster

PSTR420. Neuroendocrine Physiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR420.06/II6

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH R01 DK 114279
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NIH R01 DK120858
Brain & Behavior Research Foundation 2019 Young Investigator Research Award

Title: Lateral septum as a melanocortin downstream site for integrative control feeding behaviors and emotional states

Authors: *Y. XU[1,2], Z. JIANG[3], H. LI[3], J. CAI[3], Y. JIANG[3], J. ORTIZ-GUZMAN[4], Y. XU[4], B. ARENKIEL[4], Q. TONG[3];
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Abstract: The melanocortin pathway is well established to be critical for body weight regulation in both rodents and humans. Despite extensive studies focusing on this pathway, the downstream brain sites and underlying mechanisms that mediating melanocortin action on the regulation of feeding and obesity development are still incompletely understood. By using multidisciplinary approaches, we hereby found that, among the known paraventricular hypothalamic (PVH) neuron groups, those expressing melanocortin receptors 4 (PVH^{Mc4R}) preferably project to the ventral part of lateral septum (LSv), a brain region known to be involved in integrating feeding behaviors and the associated emotional status in the state incorporating a choice to get food while exposure to a stressful context. Photostimulation of PVH^{Mc4R} neuron terminals in the LSv reduces feeding and causes aversion, whereas deletion of Mc4Rs or disruption of glutamate release from LSv-projecting PVH neurons both causes obesity. In addition, specific disruption of AMPA receptor but not the NMDA receptor function in PVH-projected LSv neurons causes obesity, and target deletion of GABA release from PVH-projected LSv neurons leads to the rapid development of diet-induced obesity. Importantly, chronic inhibition of PVH- or PVH^{Mc4R}-projected LSv neurons results in the alleviated anxiety-like behaviors and causes obesity associated with reduced energy expenditure. Altogether, our data indicated that the LSv functions as an important downstream node to mediate melanocortin action on feeding/body weight regulation and the integrated emotional states.

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Poster

PSTR420. Neuroendocrine Physiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR420.07/II7

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: European Research Council Advanced Grant (TOGETHER).
Swedish Research Council Distinguished Professor Program (2021-00671)

Title: Identification of hypothalamic tuberoinfundibular dopamine (TIDA) neurons in the rat by electron microscopy

Authors: *J. FERRARIS[1], V. JUAREZ[2], C. LEIMGRUBER[2], N. SABOUNE[1], J. P. PETITI[2], C. BROBERGER[1];
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Abstract: The neuroendocrine TIDA neurons in the dorsomedial arcuate nucleus (dmArc) of the hypothalamus play a central role in reproduction as an inhibitory gatekeeper of pituitary secretion of the hormone, prolactin. In rats, TIDA neurons exhibit a stereotyped, slow, and synchronized network oscillation (Lyons et al., 2010). Recent work has shown that key features of this oscillation are dictated by strong TIDA-TIDA electrical synapses, the expression of the gap junction protein, Connexin-36 (Cx36) (Stagkourakis et al., 2018) and that the rhythm itself is pivotal to shaping prolactin levels (Stagkourakis et al., 2020). These new insights into TIDA physiology generate new questions about cellular and intercellular structure that we are addressing using electron microscopy (EM) on male Sprague Dawley rats (P21-35). As TIDA neurons form a small, discrete population with a restricted localization, we developed a strategy to dissect the dmArc, obtaining a sample sufficiently small for EM, but preserving anatomical references, such as the third ventricle (3V) and the median eminence. In parallel, by immunofluorescence (IF), we identified the spatial coordinates for TIDA, using tyrosine hydroxylase (TH) as a marker. TIDA neurons, identified by IF were located in a rage of ≈30-60 µm from the 3V wall (*x*-axis), and dorsoventrally (*y*-axis), from ≈180 -590 µm from the 3V ventral limit. TIDA soma length was ≈ 20 µm (n=28 cells, 6 rats).TH- immunogold staining confirmed TIDA cell location and size. TH+ cells exhibited a prominent nucleus (1:3 Nucleus: Cytoplasm ratio), a bulbous and characteristic nucleolus (n=11/12 TH+ cells, n=1/7 TH negative cells, $p<0.05$ χ^2, n= 4 rats) with nuclear-membrane invaginations (9/12 TH+ cells, n= 4 rats). The neurons with these ultrastructural features were Cx36+, present also on other structures than somata, possibly dendrites.Next, samples containing the dmArc were processed for detailed ultrastructural analysis. TIDA-TIDA somata distance (1.8 ± 0.2 µm n= 8 cells, 3 animals) indicate that the proximity may not be sufficient for electrical synapse formation. However, electron-dense gap junctions were observed in appositions between vesicle containing-dendrites segments in the dmArc, and in apposition to TIDA neuron's proximal dendrite, yet to be identified as TIDA- TIDA contact. In conclusion, we developed a reliable method for localization of TIDA neurons for EM imaging. Also, we confirm the presence of Cx36 in dopamine neurons in the dmArc and describe their main ultrastructural characteristics, opening the door to further exploration and understanding of the structural basis of network dynamics in this population.

Disclosures: J. Ferraris: None. **V. Juarez:** None. **C. Leimgruber:** None. **N. Saboune:** None. **J.P. Petiti:** None. **C. Broberger:** None.

Poster

PSTR420. Neuroendocrine Physiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR420.08/II8

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: ERC Grant 101031496

Title: Postnatal development of Tuberoinfundibular Dopamine (TIDA) neurons in the mouse

Authors: *A. LOCARNO, C. BROBERGER;
Dept. of Biochem. and Biophysics, Stockholm Univ., Stockholm, Sweden

Abstract: Neuroendocrine tuberoinfundibular dopamine (TIDA) neurons of the dorsomedial arcuate nucleus (dmArc) control secretion of the pituitary hormone, prolactin (Prl), via inhibitory dopamine D2-type receptors. Prolactin is a key hormone regulating parental physiology and behaviour and shows considerable serum level fluctuations across reproductive states, sex, and species. Importantly, TIDA neuron activity, characterized by stereotyped membrane potential oscillations, also shows notable diversity between these states, in some cases causally implicated in Prl status. Despite this dynamic nature, it remains to be determined if TIDA features are preprogrammed at birth, or undergo maturation during the early postnatal period, and, if so, which factors shape this process. In this study, we combine slice patch clamp electrophysiology, Ca^{2+} imaging, immunohistochemistry, and pharmacology to elucidate the neurodevelopmental changes occurring within the TIDA network during the first postnatal month. To identify TIDA neurons in brain slices, we crossed a dopamine transporter (DAT) Cre driver mouse line with a Cre-dependent tdTomato mouse line ("DAT-tdT mice"). In DAT-tdT mice, the number of fluorescent cells in the Arc was sparse in the early postnatal period (p 5-8; n=6) in both sexes. This is in line with observations in the midbrain and striatum showing that DAT mRNA levels increase between the first and the second postnatal week. From the second postnatal onwards, the expression of tdT in the Arc was more substantial. In this time window, we found TIDA neuron activity to be more autocorrelated in younger mice compared to older ones. This was accompanied by an increase in the frequency of oscillation, measured by brain slice Ca^{2+} imaging through the genetically encoded indicator, GCaMP6s. These changes in the activity pattern of TIDA neurons were paralleled by an age-dependent increase in circulating Prl levels. Overall, our preliminary results show a plastic maturation of the TIDA system during the first postnatal weeks suggesting a possible crosstalk between the TIDA network and the anterior pituitary during this crucial time window.

Disclosures: A. Locarno: None. **C. Broberger:** None.

Poster

PSTR420. Neuroendocrine Physiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR420.09/II9

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSERC Discovery Grant RGPIN-2017-06272
NSERC Canadian Graduate Scholarship - Doctoral Program

Title: Mouse beta klotho receptor expression at glutamatergic neurons in the bed nucleus of the stria terminalis

Authors: *B. S. BONO, M. J. CHEE;
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Abstract: Fibroblast growth factor 21 (FGF21) is a metabolic hormone that stimulates the paraventricular hypothalamic nucleus (PVH) to increase sympathetic tone. FGF21 signalling requires its obligate co-receptor beta-klotho (KLB), which is only sparsely expressed within PVH, thus it is necessary to consider that FGF21 may act via cells upstream of the PVH to drive sympathetic tone. FGF21 actions are thought to be excitatory, thus we hypothesize that FGF21 increases sympathetic output via glutamatergic cells that innervate the PVH. One such glutamatergic candidate is the bed nucleus of the stria terminalis (BST), particularly the anteromedial region, which densely innervates the PVH and whose electrical stimulation increases sympathetic tone. We assessed if the BST may underlie FGF21-mediated actions by determining the spatial distribution of *Klb* mRNA in the BST and whether *Klb* mRNA colocalized with glutamatergic cells marked by mRNA expression of vesicular glutamate transporters (*Vglut1*, *Vglut2*, *Vglut3*). We performed *in situ* hybridization in male and female wildtype brain tissues to determine if *Klb* and/or *Vglut1-3* hybridization that localized to a 4',6-diamidino-2-phenylindole (DAPI)-labelled soma was low (1-3 dots/cell), medium (4-9 dots/cell), or high (10+ dots/cell) and mapped their distribution onto *Allen Reference Atlas* brain templates. *Klb* hybridization was abundant throughout the BST but was highly concentrated within the anteromedial BST. In contrast, *Vglut1-3* hybridization was differentially expressed in the BST, as *Vglut2* mRNA was concentrated in the posterior portion and *Vglut3* mRNA was concentrated in the anterior portion. Notably, more than half of all *Vglut3*-positive cells expressed *Klb* mRNA and were most prevalent within the anteromedial BST. Only 37% of *Vglut2* BST cells expressed *Klb* mRNA, which were predominantly found within the principle nucleus of the BST. Finally, *Vglut1* hybridization within the BST was mostly low-expressing and only a few *Vglut1*-positive cells contained *Klb* mRNA. Taken together, *Klb* hybridization was abundant within the anterior BST at glutamatergic neurons marked by the expression of *Vglut3* mRNA. Future work will determine if activation of *Vglut3* BST cells innervating the PVH can increase sympathetic tone and whether FGF21 can regulate BST output.

Disclosures: B.S. Bono: None. **M.J. Chee:** None.

Poster

PSTR420. Neuroendocrine Physiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

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Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Canadian Institute for Health Research PJT-178289
Canada Foundation for Innovation 35188

Title: Disinhibition-mediated activation of neuroendocrine stress effector neurons

Authors: *A. ICHIYAMA[1], S. MESTERN[1], G. BENIGNO[2,5], K. SCOTT[3], B. L. ALLMAN[3], L. MULLER[1,5], W. INOUE[4,6];
[2]Mathematics, [3]Anat. and Cell Biol., [4]Physiol. and Pharmacol., [1]Univ. of Western Ontario, London, ON, Canada; [5]Brain and Mind Inst., London, ON, Canada; [6]Robarts Res. Inst., London, ON, Canada

Abstract: The stress response necessitates an immediate boost in vital physiological functions from their homeostatic operation to elevated emergency response. However, neural mechanisms underlying this state-dependent change remain largely unknown. Using a combination of *in vivo* and *ex vivo* electrophysiology with computational modeling, we report that corticotropin releasing hormone (CRH) neurons in the paraventricular nucleus of the hypothalamus (PVN), the effector neurons of hormonal stress response, rapidly transition between distinct activity states through recurrent inhibition. Specifically, *in vivo* optrode recordings in anesthetized mice show that under non-stress conditions, CRHPVN neurons often fire with rhythmic brief bursts (RB), which, somewhat counterintuitively, constrains firing rate due to long (~2 s) inter-burst intervals. In the same anesthetized mice, sciatic nerve stimulation, a pain-mimicking stressful stimuli, rapidly switched RB to continuous single spiking (SS), permitting a large increase in firing rate. A spiking network model shows that recurrent inhibition can control this activity-state switch, and consequently the gain of spiking responses to excitatory inputs. In biological CRHPVN neurons *ex vivo*, the injection of whole-cell currents derived from our computational model recreates the in vivo-like switch between RB and SS, providing direct evidence that physiologically relevant network inputs enable state-dependent computation in single neurons. Moreover, *in vivo* neuropharmacology showed that local antagonism of GABAA receptors, but not activation of glutamate receptors, produced model-predicted increases in continuous single spiking underlying stress-induced high activity state. Finally, in awake head-fixed mice, we demonstrate the presence of similar firing dynamics in CRHPVN neurons and correlated firing activities between single units. Together, we present a network mechanism for state-dependent activity dynamics in CRHPVN neurons.

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Poster

PSTR420. Neuroendocrine Physiology

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Program #/Poster #: PSTR420.11/II11

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: PICT 2019-3054
PICT 2017-3196

Title: Agouti-related protein-expressing neurons mediate enhanced saccharine intake in calorie restricted male mice

Authors: *D. A. CASSANO[1], F. BARRILE[1], M. REYNALDO[1], N. FERREIRA[2], M. CURE[1], M. CORNEJO[1], G. GARCÍA ROMERO[1], R. RORATO[2], H. SCHIOTH[3], M. PERELLO[1,3]; [1]IMBICE, La Plata, Argentina; [2]Dept. of Biophysics, Paulista Med. School, Federal Univ. of Sao Paulo, São Paulo, Brazil; [3]Dept. of Surgical Sciences, Functional Pharmacol. and Neuroscience, Univ. of Uppsala, Uppsala, Sweden

Abstract: Animals under calorie restriction (CR) display an enhancement of their reward-related behaviors towards palatable stimuli, and the molecular basis underlying such adaptations remain uncertain. Agouti-related protein-expressing neurons (AgRP neurons) are located in the arcuate nucleus (ARH), which is nearby the fenestrated capillaries of the median eminence and, consequently, able to sense circulating factors. AgRP neurons project to many brain centres, including the paraventricular hypothalamus (PVN), the lateral hypothalamic area (LHA), the paraventricular thalamus (PVT) and the bed nucleus of the stria terminalis (BNST), and they are activated under energy deficit condition, such as fasting. Several evidences have established a connection between AgRP neurons and reward-related behaviours, including food foraging and locomotor activity. Here, we studied if AgRP neurons orchestrate the enhancement of reward-related behaviours observed in mice subjected to CR. We used an experimental protocol in which male mice were fed with the 40% of their daily *ad libitum* food intake for 5 days. Additionally, they were daily exposed to a solution of the non-caloric sweetener saccharine for 4 hours before each meal. We found that C57BL6 (WT) mice under CR showed an increase of saccharine intake and an induction of the neuronal marker activation c-Fos expression in several brain regions, including the ARH-AgRP neurons, LHA, PVN, PVT and BNST. Furthermore, using an animal model in which the ARH was ablated, we found that the ARH integrity was required for CR-induced enhancement of saccharine intake and for the induction of c-Fos in most ARH-targets. By utilizing loxP-Cre technology in mice, we selectively expressed inhibitory DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) in the AgRP neurons, and we observed that CR-induced enhancement of saccharine intake was reduced when AgRP neurons were selectively inhibited. Moreover, employing the same technology but using excitatory DREADDs selectively in AgRP neurons, we found that the pharmacogenetic activation of AgRP neurons alone was sufficient to induce saccharine intake in *ad libitum* fed mice. Thus, we conclude that AgRP neurons' activation is required for the enhancement of saccharine intake in CR, and that AgRP neurons' activation is sufficient to induce saccharine intake in ad libitum fed mice. Of note, current studies were performed in male mice and it remains to be investigated if these findings can be extended to female mice.

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Poster

PSTR420. Neuroendocrine Physiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR420.12/II12

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSFC Grant 82172605

Title: The D2R-SSTR5 heterodimer in pituitary neuroendocrine tumors and its functional crosstalk.

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Abstract: Pituitary neuroendocrine tumors (PitNETs) are common intracranial tumors, among which prolactinoma (PRLoma) is the most common functional pituitary adenoma. Dopamine receptor agonist is the first choice for the clinical treatment of PRLoma, and the target is the dopamine type 2 receptor (D2R), which is a typical GPCR have been well studied, especially in the psychiatry field. The D2R agonist cabergoline and bromocriptine are very efficient for PRLoma but 10-25% of patients develop drug resistance. It's urgent to solve this problem. Heterodimerization is a way to regulate and integrate the GPCR downstream signal pathway. But little is known about how dimerization regulates the D2R signal in PRLoma.It was reported that the SSTR5 can form heterodimer with D2R in HEK293. In this study, initially, we assessed the expression levels of D2R and SSTR5 in a tissue chip comprising 70 PitNET samples using immunohistochemistry (IHC). Notably, we observed high expression of both GPCRs in 23 samples.Subsequently, we employed the proximity ligation assay (PLA) to detect the formation of SSTR5-D2R heterodimers in situ. The presence of these heterodimers suggests a physiological role within pituitary tumor.Further investigations were conducted using a NanoBIT-based assay to evaluate the functional crosstalk of D2R and SSTR5. We discovered that the presence of SSTR5 enhances the activation of G_o signaling pathway while suppressing beta-arrestin recruitment of D2R, which is typically induced by the clinically used agonist cabergoline. These findings provide insights into the modulatory effect of SSTR5 on D2R signaling. Moreover, our study revealed that SSTR5 can reduce the internalization of D2R induced by cabergoline, thereby promoting the stability of D2R on the cell membrane. The work mentioned above has provided valuable insights into the potential role of SSTR5 in regulating the downstream signaling and membrane stabilization of D2R. Furthermore, the detection of D2R-SSTR5 heterodimer in PitNET samples suggests that SSTR5 may play a regulatory role in the D2R signaling pathway within pituitary tumors through heterodimerization with D2R. By elucidating the interplay between SSTR5 and D2R, this research offers valuable information that can guide the design of future therapeutic interventions for PitNET.

Disclosures: **C. Li:** None. **Y. Liu:** None. **Y. Feng:** None. **Z. Wu:** None. **L. Xue:** None.

Poster

PSTR421. Neural Control of GI and Urogenital Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR421.01/II13

Topic: F.06. Autonomic Regulation

Support: NIH Grant OT2OD030535

Title: Compartmental Modeling of the Vagus Nerve Pathway that Connects the Nucleus Tractus Solitarii to the Stomach with Potential Application to Treat Gastrointestinal Disorders

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Abstract: Vagus nerve stimulation (VNS) involves delivering electrical impulses to the vagus nerve. Sensory and motor aspects of gastric physiology are controlled by the vagus nerve. There are two methods of vagus nerve stimulation: invasive and non-invasive stimulation. Invasive VNS requires a surgical implantation procedure of a small pulse generator. Non-invasive stimulation is simpler as surgical device implantation is not required. Transcutaneous auricular vagus nerve stimulation (taVNS) is a non-invasive approach to vagus nerve stimulation. This stimulation targets the vagus nerve through the auricular branch of the vagus nerve that is accessible through the ear. The nucleus tractus solitarii (NTS) in the brainstem, targeted by taVNS, also regulates gastric motility and emptying via efference from the dorsal motor nucleus of the vagus. Therefore, taVNS can be used to treat gastric disorders like functional dyspepsia by controlling the peristaltic movement in the stomach (Sclocco et al., 2022). In prior work (SFN 2021), a compartmental model (CM) of the stomach was derived to simulate gastric motility. The CM modeling approach is computationally cheap as compared to complex finite element methods (FEM) models since the compartments only contain Ordinary Differential Equations (ODE). However, to date, no model of the vagus nerve has been established that links the NTS to the gut. In this study, a CM model will be derived to model the vagus nerve pathway of the NTS to the stomach, so the stomach motility can be modulated by VNS. In the vagus nerve pathway, three groups of neurons have been reported in the literature: myelinated afferents forming two distinct groups (A-type and Ah-type) with different functional profiles, and unmyelinated afferents forming a separate group (C-type). The myelinated neurons showed higher conduction velocity than unmyelinated neurons. The action potential of A-type, Ah-type and C-type neurons are simulated using Hodgkin-Huxley gated channel equations. The propagation of the action potential along the vagus nerve pathway is simulated using cable theory equation where the spatial variable of the equation is compartmentalized. The system is solved with ODE solvers in MATLAB. The model is compared with experimental data (1) Action potential shape and amplitude (Schild et al., 1994) (2) Action potential propagation velocity (Arcilla et al., 2020). By combining the vagus nerve and stomach motility compartmental model, a model is developed

where the stomach motility can be controlled using taVNS. This study will ultimately facilitate the development of closed-loop controllers as neurostimulation-based therapy for treating GI disorders.

Disclosures: **S. Fernandes:** None. **R. Sclocco:** None. **M. Kothare:** None. **B. Mahmoudi:** None.

Poster

PSTR421. Neural Control of GI and Urogenital Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR421.02/II14

Topic: F.06. Autonomic Regulation

Support: NIH SPARC OT2OD028191

Title: Perineal and rectal nerve recruitment order varies during pudendal neurostimulator implant surgery

Authors: ***P.-J. CHEN**[1,2], A. C. LAGUNAS[1,2], V. SORIANO[2,3], P. GUPTA[4], T. M. BRUNS[1,2]; [1]Biomed. Engin., [2]Biointerfaces Inst., [3]Neurosci. Program, [4]Urology, Univ. of Michigan, Ann Arbor, MI

Abstract: Pudendal nerve stimulation (PNS) is an off-label use of sacral neuromodulation (SNM) for pelvic pain and bladder symptoms, including urinary retention, overactive bladder, and incontinence. These symptoms bother millions of Americans, especially elderly women. This study collected data from the PNS implant surgery in 13 female cases to investigate the nerve recruitment order.

The pudendal nerve has three primary branches: genital nerve (GEN), perineal nerve (PER), and inferior rectal nerve (REC). GEN is sensory only, and PER and REC have mixed sensory and motor functions. PER innervates muscles in the urethra, perineum, and pelvic floor, and REC innervates the external anal sphincter (EAS) and levator ani. The standard PNS implant surgery uses EAS electromyography to guide placement of the 4-electrode lead, omitting PER responses. We placed a multi-sensor pressure catheter in the urethra during PNS implant surgery to monitor PER activation patterns. Eleven participants had PNS-driven urethral responses. Six of these participants had urethral responses at the threshold for EAS activation, suggesting that the PER can be recruited before the REC. Across participants, there were 45 electrodes which evoked EAS responses (86.5%) and 35 electrodes that drove urethral pressure increases (67.3%). Seventeen electrodes had PER responses before REC responses, and one electrode only had a PER response. These results suggest that urethral responses are substantial during PNS.

The multi-sensor catheter also allowed us to distinguish PNS-driven responses between the proximal and distal urethra. Among participants with urethra responses, four had distal urethral responses first, four had proximal urethral responses first, and three had concurrent proximal and distal urethra pressure onset during PNS.

The varied perineal and rectal nerve recruitment indicates that each surgery and participant are unique. The activation patterns may provide insights for personalized programming. Within our study group, a majority of participants (11/13, 84.6%) have had successful outcomes with their PNS implant. The two explanted participants had urethral responses and improved bladder symptoms, but did not have sufficient pelvic pain relief. Our analysis of urethral recruitment extends the current perspective on PNS and may contribute to expanded use of PNS for bladder and pelvic dysfunctions.

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Poster

PSTR421. Neural Control of GI and Urogenital Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR421.03/II15

Topic: F.06. Autonomic Regulation

Support: NIH Grant 5R01DK120108
NIH Grant 1R01DK124580

Title: The role of TRPV1 in repeated variate stress (RVS)-induced bladder dysfunction in adult female mice

Authors: *A. SIDWELL, C. MCCLINTOCK, B. M. GIRARD, S. CAMPBELL, M. A. VIZZARD;
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Abstract: Psychological stress is associated with urinary bladder dysfunction (e.g., increased voiding frequency, urgency and pelvic pain); however, the mechanisms underlying the effects of stress on urinary bladder function are unknown. Transient receptor potential (TRP) channels (vanilloid family) may be potential targets for intervention due to their distribution in the lower urinary tract (LUT) and role in pain. Specifically, the TRPV1 channel has been implicated in LUT disorders, but its role in stress-induced bladder dysfunction remains unclear. As recently shown, a 2-week RVS paradigm in adult female mice negatively impacts bladder function. Using continuous infusion, open-outlet cystometry in conscious mice, RVS significantly ($p \leq 0.05$) decreased infused volume (IV) and intermicturition interval (IMI). Bladder pressures (threshold, average, minimum, and maximum pressures) were unchanged with RVS. Anxiety-like behavior, as measured by an open field test, showed a significant ($p \leq 0.05$) decrease in time spent in the open area of the arena in RVS mice compared to control. TRPV1 mRNA levels were upregulated in spinal cord levels L6 and S1 following RVS. Here, we used a specific TRPV1 receptor antagonist, capsazepine (CPZ) (50 μM), to determine the role of TRPV1 in RVS-induced bladder dysfunction. Intravesical infusion of CPZ during cystometry significantly ($p \leq 0.05$) improved bladder function in RVS mice. Specifically, the IMI and maximum IV before voiding

significantly ($p \leq 0.05$) increased. CPZ infusion did not affect bladder function in control mice. Fecal pellets, collected on each day of RVS, were used to measure corticosterone expression as a quantifiable measure of HPA axis activation. RVS mice exhibited significantly ($p \leq 0.05$) greater corticosterone expression compared to controls, suggesting that mice did not habituate to stressors. In conclusion, CPZ (50 μM) improves bladder function following 2-week RVS suggesting the involvement of TRPV1 in RVS-induced bladder dysfunction. Current studies are quantifying TRPV1 protein expression in the lumbosacral spinal cord and associated dorsal root ganglia following RVS and CPZ. Future studies will examine changes in anxiety-like behaviors and corticosterone levels following CPZ administration.

Disclosures: A. Sidwell: None. **C. McClintock:** None. **B.M. Girard:** None. **S. Campbell:** None. **M.A. Vizzard:** None.

Poster

PSTR421. Neural Control of GI and Urogenital Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR421.04/II16

Topic: F.06. Autonomic Regulation

Support: NIH Grant T32DK007314
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NIH Grant R01DK021697
NIH Grant R01DK45586
JPB Foundation

Title: The vagus nerve controls the circadian clock-independent liver transcriptome and relays hepatic signals to regulate rhythmic feeding behaviors

Authors: *L. N. WOODIE[1], M. MIDHA[1], A. DE ARAUJO[3], L. C. MELINK[1], A. J. ALBERTO[1], G. DE LARTIGUE[3], M. R. HAYES[2], M. A. LAZAR[1];
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Abstract: Many mammalian behaviors and metabolic processes exhibit circadian rhythmicity. Synchrony among these is critical for organismal health as desyncrhony has been shown to exacerbate obesity and metabolic disease. How synchrony/desynchrony are transmitted and detected within an organism is a major unanswered question in the field of chronobiology. Parasympathetic innervation via the vagus nerve has been shown to transmit metabolic signals between the brain and liver, but its role in relaying circadian signals has not been established. We hypothesized that signaling between the brain and liver via the hepatic vagus (HV) nerve regulates chrono-metabolic homeostasis and gene expression. To test this, we surgically interrupted the HV (HVx) in mice and collected their tissues at multiple times for transcriptomic analysis. We found that HV efferents regulate the rhythmic expression of the clock-independent

transcriptome in the liver as HVx rendered a large portion of the rhythmic hepatic transcriptome arrhythmic without impacting the endogenous liver clock. HVx did not alter feeding on its own, but remarkably protected against aberrant feeding behaviors observed in mice with disrupted hepatocyte clocks. This was due to altered signals sent via HV afferents that changed the rhythmic expression of orexigenic genes in the arcuate nucleus (Arc). These trancriptomic changes were then attenuated by HVx. In sum, HV signaling serves to maintain chrono-metabolic synchrony by orchestrating clock-independent rhythms between the brain and liver and this homeostatic mechanism becomes deleterious under metabolic stress. Since obesity and metabolic disease are known to be exacerbated by chrono-disruption, it is likely that parasympathetic relay between the brain and liver plays an important role in normal metabolic physiology, as well as the disturbances caused by overnutrition and circadian disruption.

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Poster

PSTR421. Neural Control of GI and Urogenital Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR421.05/II17

Topic: F.06. Autonomic Regulation

Support: JSPS KAKENHI Grant JP22K06489

Title: The modulation of sympathetic activity by GABA in the sympathetic ganglion.

Authors: *H. KANDA[1,2], H. YAMANAKA[3,2], Y. DAI[4,2], K. NOGUCHI[2];
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Abstract: The sympathetic activity is controlled primarily in the hypothalamus. While the central regulatory mechanisms of sympathetic activity have been well studied, the regulatory mechanisms in the peripheral nervous system remain unclear. The celiac ganglion (CG) is the site of neurotransmission between pre-sympathetic nerve terminals and post-sympathetic ganglionic neurons. The presence of small neural circuits in the CG has been suggested before, but the details of these circuits are still unclear. Here, we determined the intra-ganglionic regulation of sympathetic activity in the peripheral nervous system. In addition, we determined the role of Gamma-aminobutyric acid (GABA) on synaptic activity in the CG. To evaluate the synaptic activities in the CG, we established a rat whole-mount CG preparation, and applied *in situ* patch-clamp technique combined with high-speed pressure-clamp device. The synaptic activities were successfully detected from CG neurons under the voltage-clamp configuration. The results of perforated patch-clamp showed that the CG neurons have relatively higher concentration of intracellular chloride than that in CNS neurons. The bath application of GABA induced the GABAA-mediated inward currents under the voltage-clamp configuration, and

depolarized the membrane potential under the current-clamp mode. On the other hand, GABA suppressed the cell excitability above the chloride reversal potential of -45mV. Immunohistochemistry revealed that the GABAergic nerve terminals were scattered within the CG. We performed the comprehensive analysis of GABAA receptor by real time PCR, and the alpha1 and alpha2 subunits were expressed in the CG. Collectively, we successfully established the *in situ* whole-cell patch-clamp recording on the CG neurons, and directly measured the peripheral synaptic activity by analyzing synaptic transmission in the peripheral nervous system. We revealed that GABA has a bidirectional action for regulating cell excitability on post-sympathetic neurons. Our findings may provide new insights into the mechanism of regulation for sympathetic nerve activities in the peripheral nervous system.

Disclosures: H. Kanda: None. **H. Yamanaka:** None. **Y. Dai:** None. **K. Noguchi:** None.

Poster

PSTR421. Neural Control of GI and Urogenital Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR421.06/II18

Topic: F.06. Autonomic Regulation

Support: CONAHCYT (840925)
VIEP-BUAP (100-310-955-UALVIEP-19/2)
BUAP NSS525517-VIEP2021

Title: Subdiaphragmatic organization of the right and left vagus nerves, a region of neural connections with the enteric system, ganglia, and ovaries

Authors: *M. RIVERA CASTRO[1,2], C. PASTELÍN[3], J. BRAVO BENÍTEZ[2], C. MORÁN[2]; [1]Univ. Veracruzana, Puebla, Mexico; [2]Inst. de Ciencias, Benemérita Univ. Autónoma de Puebla, Puebla, Mexico; [3]Facultad de Medicina Veterinaria y Zootecnia, Benemérita Univ. Autónoma de Puebla, PUEBLA, Mexico

Abstract: The vagus nerve in the subdiaphragmatic region has been described as an important way in the control of different autonomic functions in the body. In the female rat, the vagal communication in the ovaries is associated with the control of the estrous cycle, ovulation, and physiological response, however, is not clear the neural pathway through the vagus nerve is connected. In this study, we proposed to determine the neuroanatomical pathway that links the subdiaphragmatic vagus with the ovaries and describe the organs, ganglia and branches involved. Adult female rats (Long Evans CII-ZV strain) were used during the estrous cycle (240 -350 g) and were divided into three groups, 1) gross anatomy, 2) neuroanatomy by acetylcholinesterasetechniqueand 3) immunofluorescence analysis for tyrosine hydroxylase (TH), choline acetyltransferase (ChAT), and tryptophan hydroxylase 2 (TPH) antibodiesin the right vagal plexus. The results obtained indicates that right vagus nerve (RVN) travels parallel and caudal to the esophagus, where three nerve branches were identifiedthat form a plexus

described as aRVP, examining it, we found that it is formed by some neurons, axons that cross it and glial cells. The left vagus nerve (LVN) travels over the esophagus, bifurcates before its insertion into the stomach and arrives to the celiac plexus.The immunoanalysis of RVP showed it was reactive for TH mainly, accumulating the enzyme in vesicles but without finding positive neuronsto TH.Nevertheless,were found some serotoninergic and cholinergic neurons but not vesicles in the RVP. This neuroanatomical and biochemical description of the RVN and LVN in the female rat, suggests the RVP is formed by presynaptic catecholaminergic terminals and cholinergic and serotoninergic neurons. With this, we suggest that in the rat, vagalinnervation in the subdiaphragmatic region plays a role in the physiological response of the abdominal organs including the ovaries.

Disclosures: **M. Rivera Castro:** None. **C. Pastelín:** None. **J. Bravo Benítez:** None. **C. Morán:** None.

Poster

PSTR421. Neural Control of GI and Urogenital Systems

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Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR421.07/II19

Topic: F.06. Autonomic Regulation

Support: NIH Grant F31DK130229

Title: Sympathetic innervation in islet plasticity during pregnancy

Authors: *J. S. YAMAMOTO[1], R. KURUVILLA[2];
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Abstract: Pancreatic islets of Langerhans are essential for glucose homeostasis, and the loss of insulin-producing beta-cells in islets or their dysfunction causes diabetes. Pregnancy is a unique physiological condition when islets show striking morphological and functional plasticity during adulthood. During pregnancy, there is an increase in islet mass and proliferation of insulin producing beta-cells, as well as enhanced insulin secretion to compensate for increased insulin resistance. Failure of normal islet adaptive responses to an increased metabolic demand during pregnancy has been proposed to contribute to gestational diabetes. Thus, it is critical to understand the molecular mechanisms that underlie islet plasticity during pregnancy. Pancreatic islets receive neuronal input from sympathetic nerves, which is important to control hormone secretion and maintain glucose homeostasis. Whether sympathetic nerves contribute to islet plasticity during pregnancy remains unknown.Using iDISCO-based tissue clearing and 3D imaging, I found that sympathetic nerves undergo structural changes as the islet mass expands during pregnancy in mice. To assess if sympathetic innervation contributes to islet plasticity during pregnancy, I chemically ablated nerves using 6-OHDA injections. After confirming loss of innervation, I observed that denervation results in increased islet area in pregnant mice compared to pregnant mice with intact nerves. Pregnant mice injected with 6-OHDA also exhibit

slightly increased insulin secretion in response to a glucose challenge, and improved glucose tolerance at gestational day 16, compared to vehicle-treated pregnant mice. Islet plasticity during pregnancy is a tightly regulated process, where negative regulatory pathways are likely to be as important as positive regulatory pathways to prevent un-restricted beta-cell proliferation, islet expansion, and insulin secretion. My results suggest a previously uncharacterized role for sympathetic nerves in acting as a 'brake' to prevent excessive islet growth and insulin secretion during pregnancy.

Disclosures: J.S. Yamamoto: None. **R. Kuruvilla:** None.

Poster

PSTR421. Neural Control of GI and Urogenital Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR421.08/II20

Topic: F.06. Autonomic Regulation

Support: CONACYT/JMBB772933
VIEP/1724/2022

Title: Analysis of adrenergic and cholinergic system in the functional structures of the ovary in the rat senescence process

Authors: *J. BRAVO[1], M. RIVERA CASTRO[2], I. SARMIENTO GARCIA[3], A. HERNÁNDEZ SILVA[3], V. BOHORQUEZ[3], C. MORAN[4];
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Abstract: In the rat ovary, has been demonstrated the presence and distribution of autonomic nerves, these arrive to the ovary by the ovarian plexus nerve and the superior ovarian nerve. This neural pathway is related to the release of norepinephrine from nerve endings that inhibit testosterone production via R1A (α1-adrenergic receptor), located on testosterone-producing cells in the ovary. Also, was found acetylcholine (ACh), which participates in the regulation of cellular proliferation in the follicles and corpus lutea. The presence of ACh receptors in the rat ovary, such as M1R (μ1-muscarinic Receptor) has been found in young rats, however, ovarian innervation increases their activity at the end of the lifespan. The aim was to analyze the presence of M1R and R1A and their relationship with steroid hormone receptors such as 17β estradiol receptor and α1-progesterone receptor. In this study adult and senescent female rats were used and assigned to the following groups: young adult rats aged 3-5 months (3M, n=4), middle-aged rats aged 12 months (12M, n=4), and senescence rats (15M; n=4). The ovaries were removed and fixed, then were cut at 10 μm sections and were analyzed to the primary antibodies for the 17β-estradiol receptor, α1-adrenergic receptor, μ1-muscarinic receptor, and progesterone receptor. The results obtained from the analysis of the immunoreactivity in the ovarian stroma

and cells surrounding functional structures of the ovary such as the corpus luteum, ovarian cysts, and follicles. The results obtained for the combination **R1A/ER** receptors were a significant difference between the three ages in the quantitative analysis of the number of immunoreactive cells showed (3M: 181.3±18.3; 12M: 1677± 0.89; 15M: 884.5± 0.91 SEM) finding the major difference for the group of 12M. Also, in the measure of the area of the cells was found a difference too (3M: 65.88±0.75; 12M: 61.74±0.27; 15M: 52.99±0.38 SEM). In the case of the number of cells that expressed for the combination of **M1R/PR** the results showed a significant difference between three different ages (3M: 1565.3±21.3; 12M: 1766± 23.89; 15M: 371.4± 0.4 SEM) and this difference was also significative for the area of the cells (3M: 67.99±0.41; 12M: 75.08±0.34; 15M: 78.81±0.78 SEM). In this combination, many cells reactive were at 12M too. The stroma cells and the cell surrounding functional structures of the ovary such as the follicles, the corpus luteum, and cysts express α1-adrenergic and μ1-muscarinic receptors, which confirms the influence of the autonomic nervous system. However, in the 12M age animals, the adrenergic system is the most active in the senescence process in the rat ovary.

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Poster

PSTR421. Neural Control of GI and Urogenital Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR421.09/II21

Topic: F.06. Autonomic Regulation

Support: OT2OD030524
R01DK133605

Title: Understanding micturition reflex control by manipulating bladder volume through time in rat

Authors: *D. J. JASKOWAK[1], Z. C. DANZIGER[2];
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Abstract: It remains unclear exactly what sensory criteria the nervous system uses to trigger the micturition reflex for voiding. Bladder afferent activity is tightly correlated with global bladder tension; however, hypotheses of a possible dependence on bladder volume have also been proposed. In typical cystometry fluid volume is added to the bladder at a constant rate until a void occurs. Introducing infusion nonlinearities may help distinguish which aspects of the bladder sensorium are most responsible for activating the micturition reflex. Here we explored whether the volume-time integral (TI) or a sudden change in bladder volume increased the likelihood of evoking the micturition reflex, in urethane anesthetized female Sprague-Dawley rats. To achieve this, we filled the bladder via transurethral catheter at typical cystometric rates but superimposed temporary large or small volume fluid boluses for either long or short

durations (before removing the bolus volume from the bladder) to control volume-TI and volume derivative independently. We quantified the presence of reflex bladder contractions after each bolus with pressure-TI. Fluid boluses were added when the bladder reached 70 or 80% of the rat's bladder capacity (BC) estimate so that the total volume in the bladder after the addition of the bolus was between 80 and 100% BC. When fluid boluses were added at 70% BC, the evoked pressure-TI was less than when fluid boluses were added at 80% BC, indicating that background bladder volume increases the chance of activating the micturition reflex (e.g., to enter micturition mode). Adding fluid boluses of 20% BC (large bolus) at 80% BC increased the evoked pressure-TI compared to when the bolus was 10% BC (small bolus). The same effect was not observed when the fluid boluses were added at 70% BC. Volume-TI was varied by increasing the time between infusion and withdrawal of the fluid bolus such that one of the 20% BC boluses had an equivalent volume-TI to a 10% BC bolus. When the fluid boluses were added at 80% BC, larger volume-TI increased the evoked pressure-TI and EUS EMG-TI. Group differences were assessed with a three-way ANOVA. Taken together these data suggest that the nervous system integrates volume to activate the micturition reflex.

Disclosures: D.J. Jaskowak: None. **Z.C. Danziger:** None.

Poster

PSTR421. Neural Control of GI and Urogenital Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR421.10/II22

Topic: F.06. Autonomic Regulation

Support: Postdoctoral fellow to NMA 2415269.
CONAHCYT: 591688 to NMA

Title: Location of Sensory neurons of the clitoris in the rat

Authors: P. GÓMEZ HERNÁNDEZ[1], *N. MIRTO AGUILAR[2,3], A. ALBARADO-IBAÑEZ[3], C. MORAN[3];
[1]Facultad de Ciencias Biológicas, Benemérita Univ. Autónoma de Puebla, Puebla, Mexico; [2]Benemérita Univ. Autónoma de Puebla, Xalapa-Enriquez, Mexico; [3]Ctr. de Investigación en Fisicoquímica de Materiales, Benemérita Univ. Autónoma de Puebla, Puebla, Mexico

Abstract: The clitoris is an external sexual organ located anterior to the urethral wall. In rats, the nerve innervating the external genitalia is the dorsal nerve of the clitoris, but we lack a thorough description of the sensory neuron distribution of the organ. This study aims to determine the location and number of sensory neurons in the clitoris in rats. The rats were deeply anesthetized with ketamine and xylazine, then placed in a supine position. Under a stereoscopic microscope, a retrograde axonal transport fluorescent dye (True blue, TB; 6-7 µl) was injected into three different regions of the clitoris. At each injection site, the needle was kept in place for 20 s to ensure the diffusion of TB into the tissue. After seven days of survival, the animals were

anesthetized with sodium pentobarbital and transcardially perfused with a saline solution followed by 10% formalin. Thereafter, the dorsal root ganglia (DRGs), from L-4 to S-2 were bilaterally excised. Tissues were post-fixed for 12 h and cryoprotected. Then ganglia were sectioned longitudinally at a thickness of 14 μm. The slides were examined with a fluorescence microscope, and the cells labeled with TB were counted. Only labeled cells with clearly visible nuclei were counted. Cell counts were corrected by the method of Abercrombie. TB-positive neurons projecting to the clitoris were localized to L6 (94%) and S1 (6%) DRGs. No neurons were in the L4, L5, and S2 DRGs. The mean number of total TB-positive neurons from the clitoris was 565±206.4. The Somata area of L6 primary afferents ranged from 1287 to 107 μm^2 and the mean of the area was 432.1±210.4 μm^2. While the somata of the neurons in S1 ranged from 637.2 to 100 μm^2 and the mean of the area was 245.2±112.2 μm^2. In another way, we analyzed the intraganglionic distribution of the TB labeled in both DRGs, and the neurons were localized in the rostromedial and caudomedial areas of the L6 DRG. However, in S1 DRG was not observed a specific pattern of neuronal distribution. In conclusion, our results show that the clitoris of the rat receives innervations exclusively from L6-S1 DRGs. Furthermore, both the urethra and urinary bladder sensory innervation are also mainly projecting from L6 DRG. Thus, suggesting that this ganglion may be the main afferent supplier of the rat urogenital tract. Knowledge of the clitoral innervation is important for the understanding of the female genital function and proximity to the urethra, which could directly affect the urinary function and/or urogenital dysfunction.

Disclosures: P. Gómez Hernández: None. **N. Mirto aguilar:** None. **A. Albarado-Ibañez:** None. **C. Moran:** None.

Poster

PSTR421. Neural Control of GI and Urogenital Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR421.11/II23

Topic: F.06. Autonomic Regulation

Support: NIH Grant R01 NS126816
NIH Grant R21 DA055166
Startup funding from UT Dallas and UT Board of Regents

Title: Transcriptomics of lateralized vagal sensory pathways

Authors: *H. F. WELCH, I. SANKARANARAYANAN, K. MAZHAR, T. J. PRICE, C. A. THORN;
Univ. of Texas at Dallas, Richardson, TX

Abstract: The vagus nerves mediate bidirectional communication between the gut and brain, modulating appetite, satiety, and food-seeking behaviors. However, recent studies have unveiled a remarkable lateralization of vagal signaling. Electrical activation of the right vagus nerve or

optogenetic stimulation of right nodose ganglion neurons, but not left, have been found to enhance neural activity in midbrain dopaminergic nuclei and drive reward-seeking behaviors. Expanding upon recent genetic sequencing research into the nodose ganglion's diversity of vagal sensory neuron subtypes, our study aims to investigate how gene expression differences between the right and left nodose ganglia may contribute to the distinct lateralization of reward-related signaling in the vagus nerve. Right and left nodose ganglia of young adult (8-week-old) male (n=18) and female (n=18) rats were dissected, and single-cell RNA sequencing (scRNA-seq) was performed. Our analyses focus on investigating the differential gene expression patterns of mechanosensory and chemosensory neuronal subtypes in the right versus left nodose ganglia. By classifying nodose ganglia neurons into putative subtypes based on their ability to sense mechanical or chemical stimuli, we aim to identify potential sensory pathways that contribute to the occurrence of lateralized vagal reward-related signaling. Preliminary analyses suggest that chemosensing subtypes may be more likely to contribute to the distinct encoding of these reward signals by the right versus left vagus nerves. Ultimately, gaining insights into the distinct molecular expression of neurons within the right and left nodose ganglia will advance our understanding of the mechanisms involved in appetitive signaling between the visceral organs and the brain.

Disclosures: H.F. Welch: None. **I. Sankaranarayanan:** None. **K. Mazhar:** None. **T.J. Price:** None. **C.A. Thorn:** None.

Poster

PSTR421. Neural Control of GI and Urogenital Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR421.12/II24

Topic: F.06. Autonomic Regulation

Support: Faculty of Health, Medicine and Life Sciences of Maastricht University

Title: Assessment of viscerosensory processing in the human brain stem using 7T fMRI

Authors: *M. DE RIJK[1,2], S. PINCKAERS[1], A. KNOPS[1], J. JANSSEN[2], A. KLIJNSMA[1], G. VAN KOEVERINGE[1,2], J. VAN DEN HURK[1,3];
[1]Urology, Univ. of Maastricht, Maastricht, Netherlands; [2]Urology, Maastricht Univ. Med. Center+, Maastricht, Netherlands; [3]Scannexus Ultra-High Field Imaging Ctr., Maastricht, Netherlands

Abstract: The brain stem plays an essential role in the processing of visceroceptive information. In particular, the periaqueductal gray (PAG) and pontine micturition (Barrington's nucleus) and storage center are brainstem areas indicated to play an essential role in lower urinary tract (LUT) control. Human and animal studies have indicated that the PAG is organized in distinct columns which are involved in sympathetic or parasympathetic control of the LUT. Earlier work indicates that *in vivo* parcellation of the PAG into symmetrical clusters can be accomplished using 7T

fMRI. Here, we used fMRI to investigate changes in functional connectivity patterns between PAG clusters and voxels in the pons associated with changes in bladder sensations. We acquired fMRI data during a bladder filling protocol. First, we ran a resting-state fMRI scan while participants had an empty bladder and experienced no desire to void, followed by a full bladder resting-state fMRI while participants experienced a strong desire to void. A connectivity matrix of the PAG was created based on the full bladder data, and parcellated using the Louvain module detection algorithm. For each resulting cluster, functional connectivity was assessed with each voxel in the pons for both empty and full fMRI scans. Based on these data we computed the difference in PAG-pons functional connectivity between empty and full bladder scans for each PAG cluster per participant. We then assessed which PAG clusters show opposite connectivity changes with pontine areas. For each pair-wise PAG cluster comparison, we multiplied the pontine connectivity maps, yielding an *opposite-response (OR) map*, in which negative voxels point towards opposite connectivity directions of the PAG cluster pair. We assessed these OR-maps on pontine transversal sections covering the levels of Barrington's nucleus. The group level maps clearly show the neuroanatomical outline of the area including Barrington's nucleus. This indicates that the approach outlined here can be used to assess interaction between brain stem nuclei associated with visceroceptive processing at unprecedented resolution. The opposing interactions between different PAG clusters resulting from our parcellation procedure with the pons indicates that the observed pontine clusters may be involved in opposing functional processes associated with changes in bladder sensations. Furthermore, it may be stipulated these functional connectivity patterns indicate that parcellations of the PAG using the Louvain module detection algorithm results in meaningful clustering of the PAG into clusters that show distinct functional connectivity patterns with other brain areas.

Disclosures: **M. de Rijk:** None. **S. Pinckaers:** None. **A. Knops:** None. **J. Janssen:** None. **A. Klijnsma:** None. **G. van Koeveringe:** 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.; Boston Scientific, Astellas, Medtronic, Solace Therapeutics, Minze Health. F. Consulting Fees (e.g., advisory boards); Astellas, Boston Scientific, Solace Therapeutics, Medtronic, Laborie, Astra Zeneca. **J. van den Hurk:** None.

Poster

PSTR421. Neural Control of GI and Urogenital Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR421.13/II25

Topic: F.06. Autonomic Regulation

Support: NIH Grant P20-DK119789
NIH Grant R01-DK125708

Title: Exploring the sexually dimorphic role of proenkephalin-expressing neurons in lower urinary tract control

Authors: *N. KLYMKO[1], A. M. SARTORI[1,2], M. LEON[1], M. L. ZEIDEL[1,2], A. M. J. VERSTEGEN[1,2];
[1]Med. Res., Beth Israel Deaconess Med. Ctr., Boston, MA; [2]Harvard Med. Sch., Boston, MA

Abstract: *Background:* The understanding of the neural control of the lower urinary tract (LUT) remains limited. Barrington's nucleus (Bar) in the brainstem has been implicated in initiating voiding and consists of distinct neuron subpopulations. Through single-nuclei RNA-sequencing, our group identified a novel subpopulation within Bar characterized by the expression of the Proenkephalin (Penk) gene. In this study, we investigate the Penk-expressing neuron population and its role in LUT control. *Methods:* We utilized transgenic mice expressing Cre-recombinase specifically in Penk-expressing neurons. Stereotaxic injections of Cre-dependent viral tracers, Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), Diphtheria Toxin A (DTA), or Channelrhodopsin 2 (ChR2), were performed. Voiding function and voluntarily controlled scent marking behavior were assessed using non-invasive micturition video thermography. Additionally, bladder pressure and external urethral sphincter (EUS) electromyography recordings were obtained. Appropriate controls were used, and post hoc analysis of the central nervous system confirmed injection sites and descending projections. *Results:* Penk-expressing neurons in Bar project to the lumbar-sacral level of the spinal cord, where interneurons and motor neurons that innervate the bladder and EUS reside. Activation of Bar^{Penk} neurons significantly increased voiding frequency in male mice; however, similar activation in females did not produce the same effect. Furthermore, selective ablation of Penk neurons in males revealed their pivotal role in a specific micturition behavior, scent marking. Utilizing modified rabies virus tracing, we identified a number of forebrain structures providing input to these neurons, and we continue to explore molecular and connectivity differences between sexes. *Conclusions:* Penk-expressing neurons residing in Barrington's nucleus project to the lumbosacral segment of the spinal cord, innervating specific downstream neurons responsible for distinct aspects of LUT function. This study uncovers sex-dependent differences in the functional roles of these neurons and highlights the intricate molecular and neural connectivity profiles linked to the control of the lower urinary tract.

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Poster

PSTR421. Neural Control of GI and Urogenital Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR421.14/II26

Topic: F.08. Food and Water Intake and Energy Balance

Support: startup funds from UVU

Title: Analysis of Estrus Cycles in POMC-deficient Mice

Authors: *R. RICKS, B. MCHOES, Z. THOMPSON;
Utah Valley Univ., Orem, UT

Abstract: The pro-opiomelanocortin (POMC) gene, prominently expressed in the hypothalamus and pituitary, is pivotal in vital physiological processes. The gene product is a protein cleaved into various peptides which have important functions in regulating feeding behavior, body weight, and melanin synthesis. Mutations in the POMC gene yield either short versions of the POMC protein or no protein at all. As a result of this deficiency, individuals exhibit severe early-onset obesity, among other symptoms. Similarly to humans, mouse models with a POMC deficiency demonstrate pronounced early-onset obesity, hyperphagia, and infertility. Our research aims to elucidate the underlying cause(s) of infertility in POMC-deficient mice. As part of this work, we are analyzing the estrus cycle in POMC-deficient mice compared to wild-type mice to determine if POMC-deficient mice exhibit normal estrus cycles. Using a pipette, we collect samples of vaginal cells using a 0.9% saline wash, and the sample is examined under a microscope by a researcher blind to genotype. The ratio of three different types of cells: cornified cells, nucleated cells, and leukocytes, is used to determine which of the four stages of the estrus cycle a mouse is in each day. Female mice typically complete a cycle every 3-5 days, going through the stages of proestrus, estrus, metestrus, and diestrus generally in order. After investigating a month's worth of samples, we will determine whether POMC-deficient mice exhibit normal estrus cycles or not. Preliminary analyses indicate that POMC-deficient mice do not exhibit normal cycles, but we are collecting more data to better address this question. Anticipated outcomes from this study hold promise for a more comprehensive understanding of the mechanisms underlying infertility in POMC-deficient mice, subsequently offering insight into potential therapeutic approaches for humans who may also experience infertility due to POMC deficiency.

Disclosures: R. Ricks: None. **B. McHoes:** None. **Z. Thompson:** None.

Poster

PSTR421. Neural Control of GI and Urogenital Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR421.15/II27

Topic: F.06. Autonomic Regulation

Title: Amygdala Control of the Emotional Motor System

Authors: *G. HOLSTEGE[1], *G. HOLSTEGE[1], H. H. SUBRAMANIAN[2];
[1]Groningen Academic Hosp., Den Haag, Netherlands; [2]Boston Scientific, Valencia, CA

Abstract: Amygdala Control of the Emotional Motor System [1]Gert Holstege and [2]Hari H. Subramanian[1]The University of Groningen, The Netherlands, [2]Boston Scientific Neuromodulation, CA, USA The amygdala regulates fear and anxiety. Expression of fear and anxiety is associated with changes in the emotional motor system control of breathing,

cardiovascular and pelvic organ function. The amygdala via its strong projections to the periaqueductal gray (PAG) could function as one of the emotional motor pathways for the modulation of fear and anxiety. In that case, stimulation of the amygdala should have a direct causal influence on breathing as well as on cardiovascular and pelvic organ functions. This was investigated by stereotaxic glutamate stimulation of different portions of the amygdala in both cats and rats. Using this technique it was possible to find out whether within the amygdala a topographical organization of emotional functions exist. **Results from the cat studies****Respiration:** In the cat, stimulation of the lateral amygdala generated hyperpnea and tachypnea, while stimulation of the central amygdala induced dyspnea, bradypnea, inspiratory apneusis and double diaphragm breathing patterns. Stimulation in the amygdala never generated apneas.**Cardiovascular effects:** Stimulation in both the lateral and central amygdala induced hyper- as well as hypotension. **Micturition:** In the cat stimulation of central amygdala generated micturition, but stimulation in the lateral amygdala did not. **Results from the rat studies**Focal stimulation of the central amygdala in the rat induced both tachypnea/hypertension as well as apnea/hypotension. Topical stimulation in the lateral portion of the central amygdala produced breath-hold. Focal glutamate stimulation in the lateral amygdala did not result in any cardiorespiratory effect. In the rat micturition was never generated in either the lateral or the central amygdala. **Conclusion**The lateral and the central amygdalar areas produce distinct modulations of the emotional motor system depending on its association with stress, fear and anxiety. Differences between the cat and rat exist in terms of modulation of the emotional motor system. Acknowledgement and DeclarationThe animal studies were undertaken by GH and HHS wholly at The University of Queensland between 2013-2017 under UQ ethics approval. HHS and GH designed the project, performed the experiments, analysed primary data and made figure illustrations. GH curated and approved the final data/figure representation in this presentation/poster. None of work described here were undertaken at either of the current work designations of Hari Subramanian or Gert Holstege.

Disclosures: G. Holstege: None. **G. Holstege:** None. **H.H. Subramanian:** None.

Poster

PSTR422. Ingestion and Energy Balance: Non-Peptidergic Regulators

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR422.01/II28

Topic: F.08. Food and Water Intake and Energy Balance

Title: The impact of a high-fat, high-sugar diet on dopamine release, direct pathway medium spiny neuron activity, and satiation during naturalistic feeding

Authors: *C. E. MURPHY[1,2], D. MOURRA[3,1,2], J. A. BEELER[2,1,4];
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Abstract: Obesity remains a global public health crisis. High rates of obesity are often attributed to the influence of the Western diet, characterized by processed foods that are highly palatable and energy-dense, containing substantial sugar and fat content. The hedonic properties of these foods are hypothesized to lead to compulsive eating and overconsumption behaviors present in obesity and have been suggested to 'hijack the reward system', likened to addiction. However, how highly palatable foods alter dopamine (DA) and direct pathway medium spiny neuron (dMSN) activity during a naturalistic free-feeding paradigm is unclear. We aimed to assess whether acute access to a diet containing high levels of fat and sugar alters dopaminergic signaling and satiety processes. We used automatic feeders (FED3) to track the *ad libitum* feeding patterns of lean D1-Cre expressing mice and provided the option to consume either standard, grain-based pellets or high-fat and high-sugar pellets across 5 days. We used dual-color fiber photometry with a red-shifted fluorescent DA sensor (RdLight1) and a Cre recombinase-dependent, green calcium indicator (GCaMP6f) virally expressed to measure simultaneous changes in DA and dMSN activity across a meal. We hypothesized that mice with access to the HFHS diet will exhibit increased pellet intake and prolonged consumption, leading to larger meal sizes which reflect a delay in the satiation process. We also predicted that phasic DA release would be increased in response to the HFHS diet, along with increased activity of dMSNs during food approach. We found that mice with access to a HFHS diet consumed more HFHS pellets than grain pellets and ate more meals when compared to mice fed only a grain-based diet. These mice had significantly increased dMSN activity during food approach but not consumption. DA attenuated similarly in both diets, suggesting that HFHS diet did not delay satiation. These findings may suggest that HFHS pellets increase motivation to approach food, rather than prolonging satiation, consistent with incentive theories of motivation.

Disclosures: C.E. Murphy: None. **D. Mourra:** None. **J.A. Beeler:** None.

Poster

PSTR422. Ingestion and Energy Balance: Non-Peptidergic Regulators

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR422.02/JJ1

Topic: F.08. Food and Water Intake and Energy Balance

Title: Natural striatal signaling dynamics during food approach at different timescales

Authors: *D. MOURRA[1,2,3], C. E. MURPHY[2,3], E. HOURANY[3], J. A. BEELER[3,2,4];
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Abstract: Reduced dopamine (DA) signaling has been hypothesized to induce compulsive overeating in obesity. However, DA signaling facilitates food pursuit by acting on direct and indirect pathways. Why individuals compulsively overeat despite decreased DA, and the role of DA in mediating satiety signals, is unclear. In this study, we aimed to investigate the role of DA

and pathway-specific medium spiny neuron (MSN) activity in the nucleus accumbens (Core; NAcc vs. Shell; NAcSH) in regulating satiation (stopping a meal). We predicted that reduced food approach would correlate with reduced DA release and dMSN activity during satiation. We used fiber photometry with a red-shifted fluorescent DA sensor (RdLight1) and a Cre recombinase-dependent, green calcium indicator (GCaMP6f)) to test this. Both fluorescent sensors were injected into mice expressing dopamine D1 receptor (D1-Cre) or adenosine A2a receptor (Adora-Cre) (markers of direct and indirect pathway MSNs, dMSNs, and iMSNs, respectively). Using a free-feeding paradigm, we used dual color fiber photometry to measure real-time changes in DA and MSN activity in mice during natural food approach and consumption. This approach allows for the simultaneous recordings of both DA and pathway-specific MSN activity across varying degrees of satiation, from hungry to sated, and is designed so that mice directly control their eating patterns. We found that DA and d/iMSNs activity is increased during food approach in natural feeding, but only iMSNs were significantly decreased during consumption. Furthermore, on a minute-to-minute timescale, we found that DA and dMSNs activity attenuated as mice approached satiation in the NAcc, but not in the NAcSH. We find that DA release changes with hunger and satiety on different timescales ranging from seconds to minutes to hours, reflecting varying stages of food motivation across several hours of free-feeding. These studies are the first to directly measure DA and d/iMSN activity from initiation of active cycle free-feeding to satiation. These findings expand our understanding of dynamic changes in striatal signaling during the progression of satiation.

Disclosures: D. Mourra: None. **C.E. Murphy:** None. **E. Hourany:** None. **J.A. Beeler:** None.

Poster

PSTR422. Ingestion and Energy Balance: Non-Peptidergic Regulators

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR422.03/JJ2

Topic: F.08. Food and Water Intake and Energy Balance

Title: Identification of a novel feeding-sensitive subtype of arcuate neurons

Authors: *R. ONOHARIGHO[1], J. CAMPBELL[2];
[2]Biol., [1]Univ. of Virginia, Charlottesville, VA

Abstract: Abstract body:Neurons in the arcuate nucleus (ARC) sense changes in caloric supply and demand toadaptively regulate feeding and metabolism. Previous research indicates that apopulation of inhibitory ARC neurons, which express vesicular inhibitory amino acidtransporter gene (*Slc32a1*) and leptin receptor gene (*Lepr*) control metabolism but notfeeding; however, the identity of these ARC neurons remains otherwise unknown. Wehypothesize that these neurons are a molecularly distinct subtype marked byexpression of the phosphoinositide interacting regulator of transient receptor potentialgene (*Pirt*), and these neurons are called Pirt^{ARC} neurons. To determine whether Pirt^{ARC}neurons are metabolically responsive, we assessed their expression of the immediate-early gene and neuronal activity marker, *Fos*, after feeding, fasting and post-

fastrefeeding in mice using RNA fluorescence *in situ* hybridization (FISH). Male and femaleC57BL/6J mice at 13 to 14 weeks of age were singly housed and fed *ad libitum*, fastedfor 18 hours, or fasted for 18 hours and then refed for 2 hours (n=4 mice per condition).We performed RNA FISH for *Pirt*, *Fos*, and *Agrp* mRNA in the ARC and assessed theirco-localization across the feeding conditions. Consistent with our previous studies, wefind that the *Agrp* and *Pirt* genes are not co-expressed, indicating that AgRP neuronsand $Pirt^{ARC}$ neurons are distinct populations in the ARC. Moreover, we find that AgRPneurons highly co-express with *Fos* in the fasted state, confirming previous findings thatAgRP neurons are particularly active in the fasted state. By contrast, our results showthat the percentage of $Pirt^{ARC}$ neurons expressing *Fos* is significantly lower in fastedmice than in *ad libitum* fed mice ($p=0.0135$; one-way ANOVA followed by Tukey'smultiple comparisons test), though neither group differed significantly from post-fast re-fed mice ($p>0.05$). Thus, $Pirt^{ARC}$ neurons appear to be more active in fed mice than infasted mice. Our results therefore identify $Pirt^{ARC}$ neurons as a novel feeding-sensitivesubtype of ARC neurons.

Disclosures: R. Onoharigho: None. **J. Campbell:** None.

Poster

PSTR422. Ingestion and Energy Balance: Non-Peptidergic Regulators

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR422.04/JJ3

Topic: F.08. Food and Water Intake and Energy Balance

Title: Biological sex differentially modulates food reward in male and female mice

Authors: *A. DOFAT[1], K. RUNYON[4], M. TSYGLAKOVA[2], A. HARTLE[5], K. MARCHALKO[3], K. JACOBS[6], R. JACOB[2], G. HODES[2], W. HOWE[2];
[1]Sch. of Neurosci., [3]TBMH, [2]Virginia Tech., Blacksburg, VA; [4]Virginia Tech. Neurosci. PhD Program, Blacksburg, VA; [5]Virginia Tech. Neurosci. PhD Program, christiansburg, VA; [6]Keyrstin Jacobs, Blacksburg, VA

Abstract: Recent studies have shown that the macronutrient profile of a food has a major influence on how the brain encodes its reward value, independent of calorie content and self-reported liking. The ability of macronutrients to modulate food reward is hypothesized to stem from post-ingestive signals triggered in the gut that are then relayed to midbrain dopamine (DA) systems. A long-standing literature also highlights that food reward and preference may be influenced by biological sex, potentially via the influence of steroid hormone receptors positioned along the gut brain-axis. Here, we have designed a set of studies to test the broad hypothesis that the rewarding qualities of macronutrients in food vary between male and female mice in a dopamine-dependent manner. First, we gave male (n=9) and female (n=9) mice free access to foods made of fat, carbohydrates, or a combination of fat and carbohydrates ("combo"). We found that whereas male mice overwhelmingly consumed combo, females rapidly develop an enduring preference for fat. Similarly, fat was found to induce a place preference over combo

in female mice (n=7), whereas the reverse pattern was seen in males (n=8). We next explored the link between these differences in preference and DA release. In male and female mice, food preference corresponded with the density of DA release in the nucleus accumbens (n=7; 4 female; 3 male), but not the dorsal striatum (n=6; 4 male, 2 female), as measured using fiber photometry and the DA sensor dLight1.1. Finally, because male and female mice are differentially sensitive to the effects of chronic stress, we assessed the impact of 6 days of variable stress on food reward in male and female mice (n=40; 18 male, 22 female). Again, we found that male mice a preferentially consumed the combo stimulus, though stress further biased this preference (n=8 control; n=10 stress). In contrast, whereas female control mice gravitated toward the more caloric dense fat (n=12), those exposed to stress developed a strong preference for combo (n=10). Combined, our results suggest that the macronutrient content of foods modifies its attractiveness, and that biological sex can have a major impact on the neural encoding of food reward. Our on-going experiments using intragastric infusions suggest that different macronutrients and combinations recruit different ensembles of midbrain neurons. Follow-up studies will test the hypothesis that such post-ingestive signals triggered by food in the gut recruit molecularly distinct midbrain ensembles in male and female mice.

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Poster

PSTR422. Ingestion and Energy Balance: Non-Peptidergic Regulators

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR422.05/JJ4

Topic: F.08. Food and Water Intake and Energy Balance

Support: NIH 1R01DK133823-01

Title: Meso-striatal dopamine systems differentially encode food preference and content

Authors: *A. HARTLE[1], C. SALLEE[1], S. BHATTACHARYYA[2], S. SENGUPTA[2], A. DIFELICEANTONIO[3,4], M. HOWE[1];
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Abstract: Fats and carbohydrates have been shown to recruit separate ascending pathways from the gut that converge onto midbrain dopamine (DA) cells. Recent human research suggests that these post-ingestive pathways play a causal role in food reward. Specifically, foods made of combinations of fats and carbohydrates are over-valued relative to equally liked foods of the same calorie content composed of just one macronutrient. We hypothesized that this effect of macronutrient combinations on food valuation is mediated through differential post-ingestive

effects on striatal DA release. To test this hypothesis, we first assessed preference for foods made of different macronutrients in male and female mice. We found that when foods made of fat, carbohydrate, and fat/carbohydrate combined (combo) were simultaneously available, mice consumed far more combination compared to either of the individual macronutrients (n=14). To test if post-ingestive signals have a causal influence such food preferences, next we manipulated DA release during the post-ingestive window using optogenetics (n=8). We found that stimulating DA cell bodies in the substantia nigra during the post-ingestive window was sufficient to shift future preference. To link these findings to striatal DA release, we used fiber photometry and the fluorescent DA sensor dLight1.1 to measure DA binding in dorsal (DS) and ventral striatum (VS) as mice consumed foods made of fat, carbohydrate, or combo. Interestingly, phasic DA release events in the VS were highest as mice consumed the combination food compared to either individual macronutrient alone (n=7). In contrast DS DA release events were highest while mice consumed the more calorically dense fat (n=7). These data suggest that food preference and content are differentially encoded by DA release in the VS and DS, respectively. On-going efforts are designed to identify the signature of post-ingestive signals on striatal DA release recorded during these free feeding tests. To this end, we are currently using intragastric infusions to bypass the influence of oro-sensory signals on DA release during food intake. In addition, we are developing a novel approach to control for movement-related DA dynamics in the post-ingestive signal by modeling the cross-correlation between movement (velocity, acceleration) and fluorescence in the VS and DS. Our initial analyses identify region and sex-specific signatures of movement-related DA release in the DS and VS that will be integrated into our larger model of how post-ingestive DA dynamics influence canonical “reward”-related DA signals.

Disclosures: **A. Hartle:** None. **C. Sallee:** None. **S. Bhattacharyya:** None. **S. Sengupta:** None. **A. DiFeliceantonio:** None. **M. Howe:** F. Consulting Fees (e.g., advisory boards); Takeda.

Poster

PSTR422. Ingestion and Energy Balance: Non-Peptidergic Regulators

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR422.06/JJ5

Topic: F.08. Food and Water Intake and Energy Balance

Title: Ampk signaling mediates the cortical regulation of over-consumption of high-fat diet

Authors: J. XIANG[1], M. SHI[1], ***Z.-X. XU**[1,2];
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Abstract: Abstract: The growing prevalence of obesity and accompanying metabolic disorders underscores the importance of understanding the mechanisms that control overeating behaviors, especially in relation to an energy-dense diet. In this study, we investigate the complex interplay

between cortical circuit and molecular mechanism that drive context-dependent overeating. Employing food-induced conditioned place preference behavioral paradigms, coupled with in vivo calcium imaging, we probed the dynamic cortical neuronal activity in response to energy-dense food stimuli. Our results elucidate the role of cortical neurons in integrating homeostatic signals as well as hedonic cues that significantly promote context-dependent overeating of energy-dense food. Our study further uncovers the molecular mechanisms underlying overeating, highlighting the critical role of AMPK signaling in the overconsumption of high-fat diets. From a circuit perspective, we found that cortical projections to the lateral hypothalamus play an important role in mediating the overconsumption of high-fat diets. Taken together, our study emphasizes the need to comprehend the cortical regulation of context-dependent overconsumption of high-fat diets. Such understanding will inform the development of targeted interventions to mitigate obesity.

Disclosures: **J. Xiang:** None. **M. Shi:** None. **Z. Xu:** None.

Poster

PSTR422. Ingestion and Energy Balance: Non-Peptidergic Regulators

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR422.07/JJ6

Topic: F.08. Food and Water Intake and Energy Balance

Support: NSERC Discovery Grant RGPIN-2017-06272
NSERC CGS M (KSB)

Title: Zona incerta neurons release GABA and dopamine in the superior colliculus

Authors: K. S. BAKER[1], ***P. A. MILLER**[1], D. FUSCA[2,3], H. FENSELAU[4], P. KLOPPENBURG[2,3], M. J. CHEE[1];
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Abstract: The zona incerta (ZI) is a narrow but long hypothalamic region that is seen rostrocaudally throughout the entire hypothalamus. This structure is predominantly GABAergic, but there is a small, spatially-distinct subset of ZI GABA neurons that coexpress tyrosine hydroxylase (TH), and we recently showed that these TH-ZI neurons produce dopamine. The ZI is implicated in fear, feeding, sleep, hunting, and motor control behaviors. The densest projections from TH-ZI neurons arrive at brain regions involved in motor coordination, including the midbrain motor regions, like the superior colliculus (SC). Here, we probed the functional contribution of TH-ZI neurons to regulate SC cells by GABA and/or dopamine release. We first injected a cre-dependent virus expressing channelrhodopsin-mCherry into the ZI of male or female *Th-cre* mice and determined if TH-ZI neurons can release GABA and/or dopamine in the motor-related layers of the SC (SCm), especially along the midline where mCherry-labelled fibers were most prominent. Photostimulation (470 nm, 5 ms) of channelrhodopsin-expressing

Th-cre fibers produced an optogenetically-evoked inhibitory postsynaptic current (oIPSC) in one-third of SCm cells recorded (n = 29/87 cells). These oIPSCs were abolished by bicuculline treatment but persisted in the presence of tetrodotoxin thus indicating that TH-ZI neurons innervated SCm cells by monosynaptic GABA release. To determine if TH-ZI neurons also released dopamine in the SCm, we transduced *Th-cre* neurons with a cre-dependent AAV encoding the red-shifted opsin ChrimsonR, and we additionally injected an AAV under control of the CAG promoter to express the dopamine sensor dLight1.1 and track dopamine content in the SCm. Photostimulating ChrimsonR-expressing TH-ZI neurons with 620 nm (1 ms) red light pulses produced time-locked firing of up to 20 Hz at the soma. We thus photostimulated ChrimsonR-expressing *Th-cre* fibers in the SCm with 20 Hz pulses within a 0.5 Hz pulse train delivered over 5 min and captured dLight fluorescent images at 0.2 Hz (excitation 470 nm; emission: 500–550nm). dLight fluorescence peaked within 5 minutes of TH-fiber photostimulation (0.15 ± 0.04%; n = 7; N = 4) and persisted for > 5 minutes before gradually returning to baseline levels over 20 min. SCm cells were dopamine-sensitive, as bath application of 10 μM dopamine directly hyperpolarized (−9.3 ± 1.8 mV) 42% (n = 20/48 cells) and depolarized (+7.1 ± 0.6 mV) 25% (n = 16/48 cells) of SCm cells by activating D2 and D1 receptors, respectively. Taken together, our findings revealed that TH-ZI cells released both GABA and dopamine to elicit acute or prolonged regulation of downstream targets such as in the SCm.

Disclosures: K.S. Baker: None. **P.A. Miller:** None. **D. Fusca:** None. **H. Fenselau:** None. **P. Kloppenburg:** None. **M.J. Chee:** None.

Poster

PSTR422. Ingestion and Energy Balance: Non-Peptidergic Regulators

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR422.08/JJ7

Topic: F.08. Food and Water Intake and Energy Balance

Title: Impact of Intrauterine Growth Restriction on Feeding Behavior and Nucleus Accumbens Dopamine Receptor Expression in Wistar Rats

Authors: *J. J. ISIDOR[1], M. NAVARRO MEZA, Sr.[3], E. BARRIOS DE TOMASI[2]; [1]Inst. de Neurociencias, Univ. de Guadalajara, Guadalajara, Mexico; [2]INSTITUTO DE NEUROCIENCIAS, Univ. de Guadalajara, GUADALAJARA, Mexico; [3]Lab. de Nutrición y Memoria, Ctr. Universitario Del Sur/ Univ. De Guad, Ciudad Guzman, Mexico

Abstract: Clinical research suggests that intrauterine growth restriction (IUGR) may lead to addiction-like behavior in offspring and persistent changes in preference for palatable foods due to fetal neural modulation. IUGR in rats from maternal malnourishment resulted in abnormal Dopamine DR2 receptor (DR2D) expression, possibly causing sex-specific programming of the mesolimbic dopamine system and impacting feeding behavior. However, the direct relationship between DR2 dopamine receptors and IUGR and the sex-specific effects on DR2D expression

remains unclear. This study compared food preferences, the role of the mesolimbic dopaminergic system, and the sex-specific effects of IUGR on feeding behavior and DR2D expression. Time-mated pregnant Wistar rats (n=13) were randomly assigned to a control group (standard chow ad libitum) or a 50% food restriction (FR) group. Pups were sexed, weighed, and the litter was standardized within 24 hours of birth. During lactation, mothers had ad-libitum diets with reduced interaction for lower stress. On the 22nd day of lactation, pups were weighed, separated by sex (male=52; female=52), and randomly divided into four groups per sex based on the adult ad-libitum access diets, palatable or control: Adlib/Chow (n=13), FR/Chow (n=13), FR/Palatable (n=13), and Adlib/Palatable (n=13). From postnatal day (PD) 66 to day 126, palatable or control diets were provided *ad-libitum*. On day 126, euthanasia was performed, preserving brain tissue at -40°C. Western blot analysis will assess DR2D expression in the nucleus accumbens. Food consumption, weight gain, anthropometric measurements, and D2 receptors will be compared. Exploratory two-way ANOVA results suggest IUGR rats had a stronger preference and higher caloric intake of palatable foods. FR/palatable females showed greater preference than controls. No weight differences were seen from day PD-43 to PD-80, but as approaching PD-126 both sexes under palatable food, exhibited weight gain differences compared to control, but males on palatable diets gained more. Our behavioral data suggest IUGR rats' preference for palatable foods may be linked to mesolimbic reward pathway alterations, possibly in a sex-specific manner which will be correlated with the D2RD quantification.

Disclosures: J.J. Isidor: None. **M. Navarro Meza:** None. **E. Barrios De Tomasi:** None.

Poster

PSTR422. Ingestion and Energy Balance: Non-Peptidergic Regulators

Location: WCC Halls A-C

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Program #/Poster #: PSTR422.09/JJ8

Topic: F.08. Food and Water Intake and Energy Balance

Support: NIH R15 DK119897-01A1

Title: Fasting and Diet Type Influence Food Intake Independent of Insulin Infusion in the Nucleus Accumbens

Authors: *H. EMMONS, S. FORDAHL;
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Abstract: Title: Fasting and Diet Type Influence Food Intake Independent of Insulin Infusion in the Nucleus Accumbens
AuthorsH. A. Emmons[1], S. C. Fordahl[1]
DisclosuresH.A. Emmons: None. S.C. Fordahl: None.
Abstract Introduction: The obesity epidemic is projected to affect 50% of the U.S. population by 2030. Changes in neurotransmission have been identified in obese individuals; however, the underlying mechanism(s) for disruption of dopaminergic neurons is unknown, and whether it

impacts satiety signals is unclear. The purpose of this study is to investigate if disrupted insulin signaling in the nucleus accumbens (NAc) alters a dopaminergic satiety circuit in mice fed a high-fat diet. We hypothesized that infusing insulin into the NAc would significantly reduce high sugar food intake and feeding duration in low-fat fed animals with dampened response in high-fat fed counterparts. One proposed mechanism is reduced insulin signaling in a NAc to hypothalamus circuit that aids in satiety. Dysregulated dopamine may interfere with homeostatic caloric intake.Methods: C57 male (n=42) and female (n=40) mice were separated into low-fat and high-fat groups (10% or 60% total kcals from fat, respectively). Insulin (0.2 μL of 5μU in 10% vol/vol aCSF) or artificial cerebrospinal fluid (aCSF) were infused into the NAc at 0.1 μL/min for 2 minutes. Mice then received 30-minute access to a high sugar food pellet with 45% of kcals from fat in either a fasted or sated metabolic state. Results: A three-way ANOVA was used to analyze differences between diet group, metabolic state, and treatment infused. We identified a main effect of diet group (LF vs HF) with the LF groups consuming significantly more of the high sugar food pellet under fasted (males: $p=0.0006$; females: $p=0.0002$) and sated (males: $p=0.0322$; females: $p<0.0001$) conditions. Additionally, we identified a main effect of insulin decreasing high sugar food intake for females under sated ($p=0.0359$) conditions.Conclusions: Insulin infused into the NAc did not significantly alter food intake in either sated or fasted conditions of LF or HF-fed mice. This indicates that under our conditions, insulin does not influence satiety via the NAc. Interestingly, HF-fed males and females consumed significantly less food in both sated and fasted conditions compared to controls. These data suggest a potential avoidance or reduced preference for their non-standard high sucrose treat; however, further experiments are needed to directly examine food preference. Overall, we show that diet composition (specifically percentage of fat intake) plays a role in food intake, with current metabolic state (in conjunction with sex) affecting insulin's actions.

Disclosures: H. Emmons: None. **S. Fordahl:** None.

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PSTR422. Ingestion and Energy Balance: Non-Peptidergic Regulators

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR422.10/JJ9

Topic: F.08. Food and Water Intake and Energy Balance

Support: NIDA Grant P50DA037844

Title: Genome-wide association study in heterogeneous stock rats identified QTLs relating to fluid intake.

Authors: *L. HANNAN[1], D. BRAKEY[2], O. POLESSKAYA[4], Q. E. CARROLL[2], T. SANCHES[4], K. L. VOLCKO[6], A. CHITRE[4], M. POSTOLACHE[2], C. P. KING[1], J. SANTOLLO[7], H. BIMSCHLEGER[4], J. GAO[4], K.-M. NGUYEN[4], R. CHENG[4], L. C. SOLBERG WOODS[8], A. A. PALMER[4,5], P. J. MEYER[1], D. DANIELS[2,3];
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York, Univ. at Buffalo, Buffalo, NY; [4]Dept. of Psychiatry, [5]Inst. for Genomic Med., Univ. of California - San Diego, La Jolla, CA; [6]Behavioral and Translational Neurosci., Univ. of Tromsø – The Arctic Univ. of Norway, Tromsø, Norway; [7]Dept. of Biol., Univ. of Kentucky, Lexington, KY; [8]Dept. of Intrnl. Med., Wake Forest Univ., Winston-Salem, NC

Abstract: Fluid homeostasis and food intake are crucial life-sustaining functions maintained under behavioral control. Although many endogenous mechanisms that regulate fluid homeostasis are largely conserved across individuals, there are significant behavioral variations influenced by genetic and environmental factors. To characterize the genetic basis of these differences, we performed a genome-wide association study (GWAS) on genetically diverse N/NIH Heterogeneous Stock (HS) rats (n=826), focusing on free-intake of food and water over a 24-hour period. Rats were housed in hanging wire cages, where total food intake was measured and microstructure of water intake was measured by contact lickometers. Water intake data were collected on a variety of measures including total intake, total number of licking bursts (i.e., two or more licks with an interlick interval of less than one second), and mean interlick interval (ILI). These measures were found to have moderate single-nucleotide polymorphism-based heritability (SNP h^2 = .18-.425). GWAS identified four significant quantitative trait loci (QTLs) for this set of traits. Two of these QTLs were associated with burst number and size on the same section of chromosome 1, a region including the gene *Stx11* which encodes a SNARE protein. Burst size was also associated with an expression-QTL (eQTL) and coding variant identifying *Fuca2* in the forebrain. These results show that individual variation in a highly conserved motivated behavior is genetically influenced, and that HS rats are useful for identification of genetic variants contributing to these individual differences.

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Poster

PSTR422. Ingestion and Energy Balance: Non-Peptidergic Regulators

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR422.11/JJ10

Topic: F.08. Food and Water Intake and Energy Balance

Support: NIH to D.A. R01DK126740

Title: Agrp neuron opioidergic signaling promotes satiety

Authors: *H. KIM[1], N. S. ATASOY[2], C. LAULE[3], C. DONG[5], I. AKLAN[4], J. RYSTED[1], K. H. FLIPPO[3], D. DAVIS[1], B. YILMAZ[6], L. TIAN[7], Y. YAVUZ[6], D. ATASOY[3]; [2]Uiowa, [3]Univ. of Iowa, [1]Univ. of Iowa, Iowa City, IA; [4]Univ. of Iowa, Univ. of Iowa,

Coralville, IA; [5]Univ. of California, Davis, UC Davis, Davis, CA; [6]Yeditepe Univ., Istanbul, Turkey; [7]Univ. of California, Davis, Univ. of California, Davis, Davis, CA

Abstract: Central opioid signaling promotes hedonic feeding through mesolimbic pathways. While endogenous opioids and their cognate receptors are widely expressed in hypothalamic feeding circuits, their role in homeostatic hunger is unresolved. In this study, we sought to understand the role of mediobasal hypothalamic opioids in appetite. We hypothesized that opioids decrease appetite through suppression of neurons that encode hunger. To assess the role of opioids on hypothalamic neurons we performed fiber photometry with a novel fluorescent opioid sensor, deltaLight, in combination with electrophysiology. We found that feeding rapidly increases mediobasal hypothalamic opioid release which directly inhibits AgRP neurons through the mu-opioid receptor (MOR). Moreover, MOR antagonists blunt AgRP inhibition caused by food and satiety hormones. Lastly, we found that selective deletion of MOR in AgRP neurons increases palatable food preference. Our findings suggest that AgRP MOR signaling may sculpt diet choice mediated by post-ingestive signals.

Disclosures: H. Kim: None. **N.S. Atasoy:** None. **C. Laule:** None. **C. Dong:** None. **I. Aklan:** None. **J. Rysted:** None. **K.H. Flippo:** None. **D. Davis:** None. **B. Yilmaz:** None. **L. Tian:** None. **Y. Yavuz:** None. **D. Atasoy:** None.

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PSTR422. Ingestion and Energy Balance: Non-Peptidergic Regulators

Location: WCC Halls A-C

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Program #/Poster #: PSTR422.12/JJ11

Topic: F.08. Food and Water Intake and Energy Balance

Support: R01 DA054091-02
T32DA007281

Title: High-resolution neurochemical characterization of the paraventricular nucleus of the thalamus

Authors: *B. A. RAMOS[1], S. B. FLAGEL[2], R. T. KENNEDY[3];
[1]Neurosci. Grad. Program, [2]Psychiatry, [3]Chem., Univ. of Michigan, Ann Arbor, MI

Abstract: Psychiatric disorders are associated with neurochemical imbalances in the brain that promote maladaptive behaviors. To-date, little research has focused on characterizing neurochemical signatures within a given brain region. The paraventricular nucleus of the thalamus (PVT) has emerged as a key neural node in the control of motivated behavior and is well-placed to integrate input pertaining to the internal state of the body as well as cognitive and emotional states. Of particular interest is the role of the PVT within the subcortical hypothalamic-thalamic-striatal motivation circuit. The PVT is composed of anterior and posterior subregions, known to play a role in arousal and valence, respectively. Our research aims to characterize the neurotransmitters, their metabolites, and energy molecule concentrations

that contribute to the role of the posterior PVT (pPVT) in this regard. While the PVT is known to be neurochemically heterogeneous, it has not yet been well characterized, likely because of its neuroanatomical location and difficulty in detecting neurochemical signals with high resolution due to various physicochemical properties and low concentration. Here we employ a liquid chromatography mass spectrometry (LC-MS) method that utilizes benzoyl chloride (BzCl) labeling to account for these difficulties. Specifically, we combined a neurochemical profiling approach with microdialysis sampling in outbred rats to characterize a panel of 24 neurochemicals in the PVT. These include dopamine, norepinephrine, serotonin, histamine, glutamate, GABA, acetylcholine, choline, adenosine, DOPAC, 3MT, HVA, normetanephrine, taurine, serine, aspartate, glycine, glutamine, epinephrine, cysteine, VMA, DOPEG, MOPEG, and glucose. We detected all the compounds from a panel of 24 neurochemicals. Glutamine was relatively high in dialysate (31000 nM) at baseline conditions, which is more than a 10-fold increase relative to the other dialysate concentrations quantified. The next tier of abundant analytes included glucose (2700 nM), taurine (1700 nM), choline (1500 nM), and GABA (160 nM), respectively. With knowledge of baseline concentrations, we can begin to study how experimental manipulations alter these concentrations. This neurochemical profile of the PVT will be further examined with respect to behavioral phenotypes of relevance to psychiatric disorders.

Disclosures: B.A. Ramos: None. **S.B. Flagel:** None. **R.T. Kennedy:** None.

Poster

PSTR422. Ingestion and Energy Balance: Non-Peptidergic Regulators

Location: WCC Halls A-C

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Topic: F.08. Food and Water Intake and Energy Balance

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Quebec Research Funds Fellowship 315201 to LDS
Alzheimer's Association Research Fellowship to Promote Diversity
AARFD-22-972811 to LDS

Title: Western diet consumption impairs hippocampal encoding of memory and energy valence

Authors: *A. M. R. HAYES[1], L. TIERNO LAUER[1], L. DECARIE-SPAIN[2], M. E. KLUG[1], L. TSAN[2], A. KAO[1], S. SUN[3], J. REA[1], K. SUBRAMANIAN[1], C. GU[1], K. N. DONOHUE[1], A. A. FODOR[3], S. E. KANOSKI[2];
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Abstract: Western diet (WD) consumption during early life periods is associated with impaired hippocampus (HPC)-dependent learning and memory function. Here, we developed an early life

WD model in rats to investigate the neurobiological mechanisms mediating these diet-induced effects. Male Sprague Dawley rats received either a highly palatable cafeteria-style WD (*ad libitum* access to various high-fat and/or high-sugar foods; CAF; n=12) or standard healthy chow (CTL; n=12) during the juvenile and adolescent stages (postnatal days 26-56). Behavioral and metabolic assessments were performed both before and after a 30-day healthy diet intervention period beginning at early adulthood. Results revealed HPC-dependent contextual episodic memory impairments in CAF rats that persisted despite the healthy diet intervention. These impairments were observed in the absence of effects on body weight, body composition, glucose tolerance, or anxiety-like behavior (CAF n=12; CTL n=12). Given that dysregulated HPC acetylcholine (ACh) signaling is associated with memory impairments in humans and animal models, we examined markers of ACh function via immunoblotting and found lower protein levels of vesicular ACh transporter (VAChT) in the dorsal hippocampus (HPCd) of CAF (n=8) vs. CTL (n=8) rats, indicating chronically reduced ACh tone. Using intensity-based ACh sensing fluorescent reporter (iAChSnFr) *in vivo* fiber photometry targeting the HPCd, we next observed that ACh binding during object-contextual novelty recognition was highly predictive of memory performance and was disrupted in CAF (n=6) vs. CTL (n=8) rats. The functional relevance of dysregulated HPC ACh signaling to CAF-associated memory impairments was further supported by neuropharmacological results revealing that nicotinic ACh receptor alpha 7 agonist infusion in the HPCd during training rescued memory deficits in CAF rats (n=8 agonist, n=9 vehicle). In addition to exhibiting memory impairments, CAF rats also demonstrated increased average meal size consumption following the healthy diet intervention period (CAF n=7; CTL n=6). To explore whether this outcome was associated with aberrant HPC ACh signaling, iAChSnFr photometry was performed in the HPCd in rats consuming a large meal following a fast. Relative to the fasted pre-meal state, CTL rats (n=10) showed increased ACh binding in the HPCd upon meal termination, whereas pre- vs. post-meal ACh binding levels did not differ in CAF rats (n=6). Overall, these findings reveal a functional connection linking early life Western diet intake and ACh signaling with hippocampal-dependent memory and encoding of meal-related satiation signals in the HPCd.

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Poster

PSTR422. Ingestion and Energy Balance: Non-Peptidergic Regulators

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR422.14/JJ13

Topic: F.08. Food and Water Intake and Energy Balance

Title: Computational normative frameworkto temporally dissociate homeostatic hypothalamic neuronal roles

Authors: *H. CHOI[1], K. KIM[2], Y. LEE[2], Y.-B. KIM[2], H. SONG[1], J.-W. YOON[3], J. PARK[2], S.-H. JUNG[1], H. KIM[3];
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Abstract: Title : **Computational Framework to Temporally Dissociate Homeostatic Hypothalamic Neuronal Roles**
Physiological needs evoke motivational drives to produce specific natural behaviors for survival. However, it has been challenging to differentiate between need and motivation in classic experimental paradigms due to the temporally intertwined dynamics of need and motivation. In this study, we developed simple and intuitive experimental paradigms that enabled us to distinguish distinct roles of the subpopulations of neurons in the hypothalamus. Based on classic homeostatic theories, we derived normative computational models of neural activity and behaviors for need-encoding and motivation-encoding neurons during predicted gain and loss events. We then recorded neural activity using in vivo calcium imaging in Agouti-related peptide (AgRP) and LHLepR neurons and also monitored eating behaviors after optogenetic manipulations of the two subpopulations, respectively. In our experimental paradigm, AgRP and LHLepR neural activity was consistent with theory-driven-neural-activity-models for need and motivation respectively. Furthermore, the temporal dynamics of behavioral activity evoked by optogenetic stimulation of AgRP and LHLepR neurons was consistent with the theory-driven-behavior-activity-model for need and motivation respectively. Thus, our study provides a parsimonious understanding of how distinct hypothalamic neurons encode need and motivation separately to produce adaptive behaviors for maintaining homeostasis.

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PSTR422. Ingestion and Energy Balance: Non-Peptidergic Regulators

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Program #/Poster #: PSTR422.15/JJ14

Topic: F.08. Food and Water Intake and Energy Balance

Support: SEP-CINVESTAV-CA-27

Title: Changes in ACTH and serum glucose in 21-day-old rat pups rearing by rats underfed during gestation and lactation.

Authors: *M. MENDOZA[1], I. JIMENEZ-ESTRADA[2], C. G. TORIZ[3];
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Abstract: The purpose of the present study was to evaluate the metabolic impact in the offspring of mothers with different nutrition conditions during pregnancy and lactation. A 30% reduction in the protein-caloric supply of female adult rats before and during pregnancy, and along the lactation period. At birth the pups were placed with a foster mother and four groups were done: 1) pups from *ad libitum* fed mother during pregnancy and lactation (C-C), 2) pups from underfed mother during pregnancy and lactation (U-U), 3) pups from well fed mother but lactated by a underfed foster mother (C-U), 4) pups from a underfed mother lactated by a well fed foster mother (U-C). At the end of the lactation period the pups were weighted and obtained their body length, blood serum, and liver. Serum levels of ACTH and glucose were determined. The body mass index showed that U-C pups have the highest values than the other pup groups. Serum ACTH concentrations were statistically higher in U-U and U-C pups than C-C and C-U pups. Serum glucose levels showed differences between the four groups: the higher levels were observed in U-C pups followed by C-C pups, then C-U pups, and the lowest level were found in U-U pups. The heaviest liver was from U-C pups. These results showed that serum ACTH concentrations are impacted by the gestation under nutrition condition independently of the abundance of meal during the lactation period. The lower serum glucose levels were obtained in pups lactated by underfed foster mothers. However, the data obtained from pups gestated by underfed mothers and with full meals after birth suggest that these rats present a pre-diabetic metabolic pattern. We need to measure other metabolic hormones to reinforce this interpretation.

Disclosures: M. Mendoza: None. **I. Jimenez-Estrada:** None. **C.G. Toriz:** None.

Poster

PSTR422. Ingestion and Energy Balance: Non-Peptidergic Regulators

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR422.16/JJ15

Topic: F.08. Food and Water Intake and Energy Balance

Title: Dopaminergic modulation of parabrachial nucleus activity and feeding behavior

Authors: *G. GABRIELSON, D. LAFFERTY, D. KERSPERN, A. LUTAS;
NIDDK, NIH, Bethesda, MD

Abstract: The acquisition and consumption of food, or feeding behavior, is a multi-system process all organisms undergo to maintain energy balance. The brain is involved in the appetitive, consummatory, and terminating phases of feeding behavior. The parabrachial nucleus (PBN) is an important node in the neural circuit that controls feeding. Recent evidence implicated dopamine in the direct modulation of PBN neuron activity and feeding behavior. Dopamine, produced in the substantia nigra and ventral tegmental area (VTA), signals through G protein-coupled receptors, which include two major types: D1 and D2. Both D1 and D2 receptor expression is found within the PBN. Our project looks to better understand how dopamine signaling in PBN modulates neuronal activity and the cessation of feeding. Using 8-10 week old male and female C57BL/J mice, we expressed channelrhodopsins via adeno-associated viral

gene delivery and implant optic fibers above the PBN. Following 4 weeks of recovery and viral expression, we test the influence of optogenetic manipulations on feeding. In Slc6a3-Cre mice(n = 6) injected with a Cre-dependent, red-shifted opsin (Chrimson) in the VTA to target expression in dopamine neurons, photostimulation of VTA axons in the PBN decreased consumption of both standard and more palatable high-fat diet. In D1-Cre mice (n = 6) injected with Chrimson in the PBN to target D1-expressing neurons, photostimulation drove even more robust decreases in standard and high-fat diet feeding. In D2-Cre mice (n = 6) injected with Chrimson in the PBN to target D2-expressing neurons, photostimulation lead to complete suppression of feeding and pronounced motor phenotypes. Utilizing combined optogenetics and fiber photometry in D1-Cre mice (n = 3) injected with Cre-dependent calcium sensor(GCaMP6s) in the PBN and Chrimson in VTA axons in PBN, we see an increase in neural activity during feeding aligning with feeding and the ability of photostimulation to drive responses in D1-expressing PBN neurons. However, we see that systemic injection of a D1receptor antagonist, which was capable of impairing feeding behavior, did not block photostimulation-evoked PBN D1 neuron activity, indicating that dopamine signaling did not mediate the increased activity. Fiber photometry recordings of the D2-expressing PBN neurons(n = 2 mice) also showed feeding evoked increases in neural activity. Systemic injections of aD2 agonist, decreased D2 PBN neuronal activity and suppressed feeding behavior. On-going fiber photometry, optogenetic stimulation, and acute brain slice two-photon imaging will continue to address the role of D1 and D2 receptor signaling on PBN activity and feeding behavior.

Disclosures: **G. Gabrielson:** None. **D. Lafferty:** None. **D. Kerspern:** None. **A. Lutas:** None.

Poster

PSTR422. Ingestion and Energy Balance: Non-Peptidergic Regulators

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR422.17/JJ16

Topic: F.08. Food and Water Intake and Energy Balance

Support: CONAHCyT CF-2023-I-355

Title: Effect of the inhibition of anaplastic lymphoma kinase receptor on the type 2 dopamine receptor internalization of the nucleus accumbens of cafeteria-diet fed mice.

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Abstract: Obesity is a complex disease with a multifactorial etiology and is one of the major health problems worldwide. The palatability, availability, and energy density of foods play an important role on its development. The type 2 dopamine receptor (D2R) is an inhibitory G-protein coupled receptor that is desensitized and internalized upon repeated stimulation by its ligand, dopamine. Overconsumption of palatable foods is involved in the internalization of D2R in the nucleus accumbens (NAc) by continuous dopamine release, which impairs hedonic feeding regulation. The tyrosine kinase receptor anaplastic lymphoma kinase (ALK), previously known for its role in cancer development, is involved in D2R internalization and when inhibited, it is prevented. The present study aims to evaluate the effect of ASP3026, an ALK inhibitor on accumbal D2R internalization along with feeding behavior of cafeteria-diet fed (CAFD) mice. Adult male Balb-C mice were offered a standard diet (SD) or CAFD (n=12/group) for 10 days. On days 8, 9 and 10, half of the animals of each group was subcutaneously (s.c.) administered with 30 mg/kg of ASP3026 (CAFD/ASP n=6; SD/ASP n=6) and half with vehicle (DMSO 10% and corn oil 90%) (CAFD/VEH n=6; SD/VEH n=6) 7 hours before the beginning of the dark phase. Daily food intake was registered, animals were sacrificed on day 10 and brains were extracted and frozen at -70°C. To determine the internalization of the D2R in the NAc, we performed a western blot of the membrane and cytosolic phases measuring the relative levels of D2R/B-actin. Mice in the CAFD/ASP group decreased their CAFD intake by 24.3 ± 0.9% (mean values, SE) vs CAFD/VEH during the 3 consecutive days after the s.c ASP3026 injection. ASP3026 also reduced the intake of standard diet in SD/ASP group by 20 ±1.35% vs. SD/VEH. This decrease is probably explained by the intrinsically rewarding characteristics of food. Our results support that the decrease in CAFD intake of ASP3026 injected mice was associated with accumbal DR2 internalization.

Disclosures: E. Granados: None. **C. Garcia-Luna:** None. **E. Alvarez:** None. **P. Soberanes-Chavez:** None. **P. De Gortari:** None.

Poster

PSTR422. Ingestion and Energy Balance: Non-Peptidergic Regulators

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR422.18/JJ17

Topic: F.08. Food and Water Intake and Energy Balance

Support: EA was supported by a Humboldt Research Fellowship for Postdoctoral Researchers from the Alexander von Humboldt Foundation.

Title: Are trpv1 receptors in the supraoptic and paraventricular nucleus obligatory for osmoregulation?

Authors: *E. ALCIN, H. MOERZ, R.-D. TREEDE, W. GREFFRATH;
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Abstract: Introduction: The hypothalamic supraoptic (SON) and paraventricular (PVN) magnocellular cells (MCs) play a crucial role in the regulation of water and salt balance by vasopressin (VP) in an osmolality-dependent manner. MCs react precisely to hypertonic stimulation with membrane depolarization. Some in vitro electrophysiological studies propose a product of TRPV1 gene commits to osmosensory transduction in hypothalamic neurons to regulate VP secretion. In contrast, in an in vivo study, it has been shown that TRPV1 channels are not obligatory for VP secretion and thirst stimulated by hypernatremia. We aimed to test the role of hyperosmolarity (Mannitol 40 mOSM), Capsaicin 10 µM, Glutamate 200 µM or high K^+ 140 mM on dissociated MCs from SON and PVN and on brain slices . **Methods:** Adult male Sprague-Dawley (SD) and TRPV1 KO rats were used in all experiments. **Calcium imaging:** For measurements of [$Ca2^+$]i, dissociated MCs were loaded with either 1 µM Fura-2 AM or 1 µM Rhod-2 for 60 min in Tyrode's solution. After washing with Tyrode's solution, cells were mounted on the stage of an inverted microscope in an open bath chamber and superfused by Tyrode's solution (1-3 ml/min) either at room temperature (RT) or 37 ºC. **Preparation and maintenance of brain slices:** 200-300 µm coronal slices containing the SON were collected and maintained in an incubation chamber at RT for at least 2 h in aCSF. Slices were placed in the recording wells with a 6×10 multi-electrode recording array. Throughout all procedure, aCSF was saturated with 95% O_2, 5% CO_2 at 32 ºC. **Results:** Dissociated MCs from SD rats; none of MCs reacted to Capsaicin, but Mannitol (23,5±2,9 %, n=16), Glutamate (136,2±13,4 %, n=33) and high K^+ (171,9±18 %, n=48) increased Ca^{2+} influx at RT. Mannitol (66,3±11,2 %, n=6) Glutamate (48,3±10,6 %, n=6) and high K^+ (101,4±11,8 %, n=14) increased Ca^{2+} influx in dissociated MCs from KO rats at RT. Dissociated 10 MCs from SD rats; Mannitol (101,8±18,1 %), Capsaicin (65,7±3,4 %) Glutamate (94,2±10,9 %,) and high K^+ (96,9±9,2 %) increased mean intensity value at 37 ºC. Additionally, Mannitol, Capsaicin and Glutamate increased spikes activity in the SON compared to aCSF treatment (number of spikes per min; 127, 127, 232 and 106). **Conclusion:** None of MCs from SD or KO rats reacted to Capsaicin but Mannitol and Glutamate at RT. Capsaicin, Mannitol and Glutamate had an effect on MCs from SD rats at 37 ºC and these chemicals also increased spikes activity in the SON. We have shown that regulatory role of TRPV1 at normal body temperature. TRPV1 channels together with other factors like glutamate receptors can contribute to network activity in SON and hence participate in regulation of water and salt balance.

Disclosures: E. Alcin: None. **H. Moerz:** None. **R. Treede:** None. **W. Greffrath:** None.

Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR423.01/JJ18

Topic: G.01. Fear and Aversive Learning and Memory

Support: ERC 716761
SNSF 170654

Title: Fear extinction relies on ventral hippocampal safety codes shaped by the amygdala

Authors: *R. NGUYEN, K. KOUKOUTSELOS, T. FORRO, S. CIOCCHI;
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Abstract: Extinction memory retrieval is influenced by spatial contextual information that determines responding to conditioned stimuli (CS). Yet, it is poorly understood if contextual representations are imbued with emotional values to support memory selection. Here, we performed activity-dependent engram-tagging, and in vivo single-unit electrophysiological recordings from the ventral hippocampus (vH) while optogenetically manipulating basolateral amygdala (BLA) inputs during the formation of cued fear extinction memory. During fear extinction when CS acquire safety properties, we found that CS-related activity in the vH reactivated during sleep consolidation and was strengthened upon memory retrieval. Moreover, fear extinction memory was facilitated when the extinction context exhibited precise coding of its affective zones. Finally, these activity patterns along with the retrieval of the fear extinction memory were dependent on glutamatergic transmission from the BLA during extinction learning. Thus, fear extinction memory relies on the formation of contextual and stimulus safety representations in the vH instructed by the BLA.

Disclosures: **R. Nguyen:** None. **K. Koukoutselos:** None. **T. Forro:** None. **S. Ciocchi:** None.

Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR423.02/JJ19

Topic: G.01. Fear and Aversive Learning and Memory

Support: NSF Grant 2014862
Connecticut Institute for the Brain and Cognitive Sciences

Title: Multiplexed analysis of interneuron heterogeneity in the basolateral amygdala of male and cycling female rats.

Authors: *G. RAIMONDI[1], R. TRIPP[1], L. OSTROFF[2];
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Abstract: The basolateral amygdala (BLA) has a central role in encoding anxiety- and fear-related memories, and inhibitory interneurons are known to be critical for these processes. Approximately 15% of neurons in the BLA are GABAergic interneurons, and these fall into an assortment of functional classes that express distinct combinations of calcium-binding proteins and neuropeptides. The distribution of molecular markers in BLA interneurons has been extensively characterized in male rats, and although some studies report sex differences in the expression patterns of individual calcium-binding proteins in the BLA, no comprehensive

mapping of interneuron markers has been performed in female subjects. Women are at higher risk of anxiety disorders than men during the reproductive years, and fear- and anxiety-related behaviors in rodent models are known to vary across the estrous cycle, which could arise from ovarian hormone effects on inhibitory circuitry. Indeed, there are some reports of sex and estrous cycle differences in parvalbumin (PV) expression in the rat BLA and in estrogen receptor expression in BLA interneurons. Here, we perform single-cell multiplexed imaging of a variety of interneuron markers and hormone receptors in the BLA of male and female rats across the estrous cycle. Using serial ultrathin (50 nanometer) sections, we analyze protein and RNA expression in individual excitatory and inhibitory neurons, and report relative abundance, distribution, and colocalization patterns of calcium binding proteins, sex hormone receptors, and structural proteins in amygdala subnuclei. Our study is the first multiplexed analysis in ultrathin sections comparing broad and fine anatomical features in the brain with an attention to sex and estrous cycle. These findings may also provide insight into mechanisms of amygdala inhibitory circuits and neuroanatomical explanations of sex differences in fear- and anxiety-related behaviors.

Disclosures: G. Raimondi: None. **R. Tripp:** None. **L. Ostroff:** None.

Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

Location: WCC Halls A-C

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Program #/Poster #: PSTR423.03/JJ20

Topic: G.01. Fear and Aversive Learning and Memory

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China Postdoctoral Science Foundation (2021M702137)
Natural Science Foundation of Chongqing (cstc2021jcyj-msxmX1176)

Title: Distinct GABAergic engram neurons in the basolateral amygdala control fear memory extinction

Authors: *X. ZHANG;
Shanghai Jiao Tong Univ., Shanghai City, China

Abstract: Title: Distinct GABAergic engram neurons in the basolateral amygdala control fear memory extinction Xu Zhang[1, 2], Ying Zhou[3], Hechen Bao[4], Xuelian Fan[1], Xiujuan Yang[1],

Caiqin Li[1], Yuting Li[1, 2], Xiangyu Yang[1, 2], Yifang Kuang[1, 2], Zhaohui Lan[1, 2], Jiarun Yang[1, 2], Miou Zhou[5], Guang He[1], Eiki Takahashi[1, 6], Weidong Li[1, 2, *1]Bio-X Institutes, Key Laboratory for the Genetics of Development and Neuropsychiatric Disorders (Ministry of Education), Institute of Psychology and Behavioral Science, Brain Health Center in Global Institute of Future Technology, Shanghai Jiao Tong University, Shanghai 200240, China.[2]WLA Laboratories, World Laureates Association, Shanghai 201203, China.[3]Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, GA, USA.[4]Department of Pharmacology, University of North Carolina, Chapel Hill, NC 27599, USA.[5]Graduate College of Biomedical Sciences, Western University of Health Sciences, Pomona, CA, USA.[6]Department of Biomedicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan.*Correspondence Author.
AbstractThe basolateral amygdala (BLA) is a key structure processing the threaten and emotional information, and plays a key role in controlling the fear memory. Previous research has suggested that the extinction procedure generates a new memory that coexists with the original fear memory, and that interneurons play an important role in extinction. However, the mechanisms that control the competing extinction and fear memories in BLA are not yet fully understood. In this study, we used a chemogenetic approach to investigate these mechanisms in mice that were genetically modified to improve the tagging specificity of active neurons. We found that silencing extinction-tagged BLA neurons, or stimulating fear acquisition-tagged BLA neurons, led to a relapse of fear memory. Additionally, we observed that silencing BLA GABAergic
neurons, or extinction-tagged BLA GABAergic neurons, restored fear expression after extinction, while inhibiting acquisition-tagged BLA GABAergic neurons did not impair fear memory retrieval. Our results indicate that specific inhibitory GABAergic BLA engrams are established during fear extinction, which interfere with existing fear memory-related neural circuits and suppress conditional fear memory. These findings provide new insights into the neural mechanisms underlying fear extinction and suggest that BLA GABAergic neurons are potential targets for therapeutic interventions in cognitive disorders such as post-traumatic stress disorder (PTSD).

Disclosures: X. Zhang: None.

Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

Location: WCC Halls A-C

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Program #/Poster #: PSTR423.04/JJ21

Topic: G.01. Fear and Aversive Learning and Memory

Support: IRTA UNSI

Title: Dynorphin in vmPFC facilitates optimal approach/avoidance conflict resolution

Authors: *H. BRAVO[1], A. LIMOGES[3], C. T. LAGAMMA[4], H. A. TEJEDA[2];
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Abstract: Survival depends on threat detection and the selection of appropriate defensive behavioral strategies to minimize aversive and maximize positive outcomes. This skill is especially critical when motivations to approach rewards and avoid threats co-exist. The Prefrontal cortical (PFC) circuits provide top-down control of threat reactivity. Specifically, the ventromedial PFC (vmPFC) plays a role in suppressing conditioned fear responses after extinction. Furthermore, dynorphin (Dyn) is widely expressed in the limbic circuits, including vmPFC. These findings highlight Dyn as a potential candidate for the adaptive selection of defensive behaviors critical in conflict conditions. To assess the role of Dyn in vmPFC in conflict resolution, we developed a novel approach/avoidance task in which mice are presented with the choice of seeking rewards vs. avoiding a footshock by stepping onto a safe platform. Mice first learn that sucrose availability is signaled by a 30s light cue, whereas a 30s tone co-terminates with a 2s footshock. These two stimuli are also presented simultaneously in some trials, generating a conflict between approaching the food or avoiding the shock. An essential feature of this task is that mice can develop an optimal strategy to retrieve rewards and avoid shocks during conflict trials by nose-poking early and avoiding later when the shock becomes imminent. Here we report that Conditional knockdown of Dyn in vmPFC (with PDyn-shRNA) impaired adaptive conflict resolution by favoring the maladaptive selection of passive over active avoidance. PDyn-shRNA mice also showed delayed learning of avoidance (increased shocks) and a suboptimal conflict strategy that prioritized passive avoidance at the expense of rewards. Fiber photometry recordings of Dyn neurons in vmPFC revealed increased activity in conflict trials where mice retrieved rewards in contrast with trials where they displayed passive avoidance at the expense of rewards. We did not observe this contrast during the reward phase of training in agreement with our previous experiment, where conditional knockdown of Dyn did not affect reward-seeking in the absence of conflict. Together these findings suggest that DYN signaling by vmPFC contributes to adaptive conflict resolution by suppressing passive and allowing/promoting active defensive behaviors.

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Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

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Program #/Poster #: PSTR423.05/JJ22

Topic: G.01. Fear and Aversive Learning and Memory

Support: K99NS119783 (CEV)
R35NS116854 (IMR)

Title: Innate fear responses to distal and proximal predator threats

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Abstract: To survive predation, animals must be able to detect and appropriately respond to predator threats in their environment. Such defensive behaviors are innate: suggesting dedicated neural circuits for the detection, sensorimotor integration, and execution of appropriate behaviors. Ethologically, distal threats (i.e., sweeping visual stimuli) trigger 'passive' avoidance strategies, such as freezing to avoid predator detection. Conversely, more proximal threats (i.e., looming visual stimuli) trigger 'active' avoidance strategies, such as fleeing. The goal of the present study is to examine innate fear responses when the behavioral strategy is determined by extrinsic factors. Specifically, we investigate how environmental and physiological conditions modulate innate freezing responses in mice, with a particular emphasis on understanding the conditions that alter behavioral responses to repeated stimuli. We demonstrate that in both male and female mice looming and sweeping visual stimuli elicit freezing in the absence of a nest to flee towards. However, looming stimuli triggered comparatively prolonged freezing bouts. Furthermore, when looming visual stimuli were repeated at intervals as short as 5 minutes, freezing responses decreased across trials in response to both looming and sweeping stimuli, suggestive of fear suppression. To begin testing whether the fear suppression represents safety learning, we tested animals at intervals of 1 hour and 24 hours, as well as testing fear recall at 1 month. Finally, to correlate fear behavior across paradigms with other physiological measures of stress, we measured circulating corticosterone levels after fear exposure. Together, our results indicate that when mice engage in passive avoidance strategies triggered by distal or proximal threats, the vigor of the response scales with threat proximity. Further, our results demonstrate that freezing responses are modified by repeated threat exposure.

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Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

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Program #/Poster #: PSTR423.06/JJ23

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH grant MH125615

Title: Context-dependent Neural Representations of Visual Stimuli in Primary Visual Cortex

Authors: *L. CUI[1], A. KEIL[2], M. DING[2];
[1]Univ. of Florida, Gainesville, FL; [2]Univ. Florida, Univ. Florida, Gainesville, FL

Abstract: It has been proposed that the cortical representations of visual stimuli are altered by experience. We tested this idea by recording fMRI data from 18 participants performing a Pavlovian fear conditioning paradigm. The paradigm consisted of three sessions. In the initial habituation session, two Gabor patches (45 and 135 degrees) were repeatedly presented in random order for a total of 120 trials. In the acquisition session, which followed the habituation session, the 45-degree Gabor patch (CS+) was paired with a loud scream (US) whereas the 135-degree Gabor patch (CS-) was never paired with the US. In the final extinction session, the CS+ and CS- was again repeatedly presented in random order (120 trials; no US). Applying the MVPA decoding method to fMRI data from the primary visual cortex in a sliding trial window fashion, we observed the following. First, the accuracy of decoding between the two Gabor patches was at the chance level (50%) early in habituation, and became progressively higher as the participant experienced more repetitions of the two stimuli, reaching ~60% at the end of habituation. Second, for extinction, the decoding accuracy started at ~60% and became progressively lower, reaching the chance level at the end of extinction. Third, MVPA classifiers trained on data from the end of habituation could not decode data from the beginning of extinction and vice versa. These results are consistent with the idea that the neural representations of visual stimuli undergo fundamental changes through learning: in habituation, repeated presentations without associative learning sharpened the sensory and perceptual distinctiveness of the two Gabor patches, whereas in extinction, the initially high representational distinctness of CS+ and CS-, reflecting the motivational quality of the CS+ and CS- (threat vs safety) acquired through associative learning, became diminished with extinction learning through potentially an active de-sharpening process.

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Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR423.07/JJ24

Topic: G.01. Fear and Aversive Learning and Memory

Title: Protein phosphatases 1 and 2b mediate safety signal learning produced decreases in Hermissenda Type B cell excitability.

Authors: *J. FARLEY[1], J. B. ANDERSON[2], J. S. CAVALLO[3];
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Abstract: Explicitly Unpaired presentations (EU) of light (L) and rotation (R) produce behavioral changes (e.g., increased phototaxis) in the sea snail *Hermissenda crassicornis* that satisfy criteria for conditioned inhibition (CI)/safety signal learning. This is accompanied by decreased excitability of type B photoreceptors (B cells) due to increases in several somatic potassium (K^+) currents. We've previously found that protein phosphatase 1 (PP1) inhibitors

(e.g., Calyculin A, I-2): 1) depolarize Untrained B cells and reduce somatic K^+ currents, partially mimicking effects of L-R pairings, 2) attenuate excitability (spike frequency) decreases produced by extinction training (EXT), a form of learning that overlaps with EU. Conversely, injection of catalytically-active PP1 (caPP1) into Untrained B cells mimicked the excitability decreases produced by EXT and occluded further reductions in spiking by additional EXT. Thus, we hypothesize that PP1 may also contribute to EU-produced B cell excitability decreases. To test this, we measured B cell photoresponses from Untrained and EU animals (24-48 hr post training) after injection with caPP1. In Untrained B cells, caPP1 produced clear reductions (~ 20 and 25%, respectively) in steady state generator potentials (SSGPs) and light-evoked spike frequencies across five 30-sec test lights, mimicking EU. EU occluded these caPP1 effects. caPP1 also increased (~22 and 27%, respectively) both I_A and $I_{K\text{-}Ca}$ components of K^+ current in Untrained B cells that mediate EU-decreases in excitability. In contrast, caPP1 failed to increase these K^+ currents in EU B cells. We next injected Untrained and EU B cells with the core peptide fragment of the PP1 regulatory G subunit ($GM_{63\text{-}75}$), a competitive inhibitor of PP1. Iontophoresis of 25uM $GM_{63\text{-}75}$ increased B cell SSGPs from Untrained (19.5%; *n.s.*) and EU animals (52.7%; $p < 0.02$). While EU B cells showed a ~40% reduction in SSGP ($p < 0.001$), SSGPs of $GM_{63\text{-}75}$-treated EU B cells failed to differ from Untrained B cells. Thus, $GM_{63\text{-}75}$ treatment abolished the EU B cell excitability decrease. We also tested the involvement of calcineurin/PP2B (CaN) in EU-produced decreased excitability. Cyclosporin A (CsA; 20 nM, CaN inhibitor) had no obvious effect on either Untrained or EU B cell excitability. However, caPP2B injection reduced excitability of Untrained B cells. In contrast, caPP2B failed to affect EU B cell excitability. Our results suggest that: 1) upregulated PP1 activity is critical for EU decreases in B cell excitability, 2) PP1 is constitutively active in Untrained B cells, 3) (transient) activation of CaN by EU disinhibits PP1, 4) decreasing excitability through increases in K^+ currents.

Disclosures: **J. Farley:** None. **J.B. Anderson:** None. **J.S. Cavallo:** None.

Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

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Program #/Poster #: PSTR423.08/JJ25

Topic: G.01. Fear and Aversive Learning and Memory

Support: VA Merit Grant 1I01BX005367
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Title: Deleterious cFos Activation in mPFC Associated with Fear Extinction in a Rodent Model of PTSD/AUD

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Abstract: Alcohol use disorder (AUD) and post-traumatic stress disorder (PTSD) are two of the most common mental health disorders and are highly comorbid. Additionally, maladaptive memories are implicated in disorders such as AUD and PTSD. Fear-/alcohol- related cues become disproportionally salient over non-fear-/ non-alcohol-related cues. This enhanced salience contributes to difficulty extinguishing fear/alcohol associations for people with comorbid PTSD/AUD. A number of recent studies have suggested that specific subregions of the prefrontal cortex mediate these fear/drug related behaviors. Specifically, lesion studies have demonstrated that the prelimbic (PrL) cortex is necessary for the expression of conditioned fear while the infralimbic (IfL) cortex is critical for the expression of extinction behavior. To gain a better understanding of the potential impact co-occurring PTSD/AUD has on neuronal activation in the IfL and PrL cortices we measured c-fos expression following the final day of fear extinction learning using a rodent model of PTSD/AUD.Wistar rats were grouped based on their exposure to the PTSD/AUD paradigm and fear conditioning (FC) task. The PTSD/AUD paradigm consisted of animals being exposed to 2h restraint stress (RS) followed by 2-weeks of chronic intermittent ethanol vapor exposure (CIE). Upon completion of CIE animals were given 10-days to recover from withdrawal symptoms prior to the commencement of the FC task. FC was used to assess future stress sensitivity by examining the acquisition of fear learning and extinction of fear behaviors. On the final day of extinction training animals were euthanized via transcardial perfusion and brain tissue was harvested to analyze changes in c-fos expression. Brain tissue containing IfL and PrL were stained with anti-cFos and Neurotrace Nissl and imaged to assess cell colocalization in each brain region.Our results indicated that animals exposed to RS+CIE had significantly less colocalized cells in the IfL than CTRL animals, suggesting a reduction of cFos activity in these neurons. Additionally, RS+CIE animals showed significantly more PrL colocalization than the CTRL animals, indicating that these animals had an increase in neuronal activation in this region. The current study provides fundamental knowledge on the possible cause in the difficulty for individuals with comorbid PTSD/AUD to extinguish fear memories.

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Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR423.09/KK1

Topic: G.01. Fear and Aversive Learning and Memory

Support: Joint Contract Research (Toyo RESIN)

Title: Modulation of Dorsolateral Prefrontal Cortex Activity through Auricular Skin Stimulation

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Abstract: **Introduction**Currently, there are two external approaches to modulating autonomic nervous activity: auricular electrical stimulation therapy and superficial skin stimulation using microcone patches. The cardiac sympathetic reflex is a physiological response associated with stress and emotion, and it is believed that exerting control over autonomic responses to external stimuli can influence the activity of the ventromedial prefrontal cortex. The purpose of this study was to examine how auricular skin stimulation with microcone patches consequently affects activity in the dorsolateral prefrontal cortex.
Methods We utilized the short text comprehension task (Takahashi, 2023) and executed a mixed model ANOVA with two within-subject factors. The independent variables were the condition of the task (rest for 2 minutes and task for 3 minutes), and the presence or absence of auricular skin stimulation through microcone patches. The dependent variables included the autonomic nervous response and the blood oxygen saturation levels in the bilateral prefrontal cortex. This study has undergone ethical review and has been approved by our institution's ethics committee.
ResultsMixed model ANOVA showed a significant impact of micro-cone auricular skin stimulation on the activity in the left (1ch, $p<.001$, $f=0.30$) and right dorsolateral prefrontal cortex (3ch, $p<.05$, $f=0.17$), indicating increased activity during stimulation. Conversely, activity decreased around the bilateral frontal poles (2ch, $p<.05$, $f=0.18$; 4ch, $p<.001$, $f=0.26$) with stimulation. Effect sizes were moderate to large. Regarding autonomic activity, there was a notable trend for sympathetic tone (LF/HF) based on task presence ($p=.057$, $f=0.17$), though the effect size was small. Additionally, while not significant, there was a trend towards a lower pnn50 (%), an index of vagal tension, during stimulation ($f=0.10$).
Consideration In this study, it is suggested that microcone-induced skin stimulation could influence the activity of the dorsolateral prefrontal cortex. Stimulation appears to suppress medial prefrontal functions related to autonomic activity, potentially permitting the dorsolateral prefrontal cortex to direct cognitive resources to its inherent activities. Given the study's low task difficulty and potential individual differences in response to autonomic nerve activity, future research should aim to clarify the underlying mechanism and consider
varying task difficulties.

Disclosures: **A. Kawasaki:** None. **T. Kawada:** None. **S. Yamazaki:** None.

Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

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Program #/Poster #: PSTR423.10/KK2

Topic: G.01. Fear and Aversive Learning and Memory

Support: DFG 31680338
SFB1280 Extinction Learning

Title: Polygenic risk for internalizing disorders predicts safety-cue learning during differential fear conditioning mediated by cerebello-frontal structural connectivity

Authors: *J. E. SCHNEIDER PENATE[1], C. A. GOMES[1,2], H. ENGLER[2], S. ELSENBRUCH[1], C. J. MERZ[1], O. T. WOLF[1], E. GENC[3], T. SPISAK[2], O. GÜNTÜRKÜN[1], H. QUICK[2], D. TIMMANN[2], N. AXMACHER[1], R. KUMSTA[1,4];
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Abstract: Interindividual variation in fear learning efficiency is partly attributable to the individual genetic make-up. In turn, genetic factors might partly be linked to mental health risk through effects on fear learning. Genetic variants may impact the structural and functional connectivity of the human fear extinction network such that genetically driven alterations of network properties give in part rise to the observed individual rates of learning. Here, we aim to identify genetic risk indices that predict physiological measures of fear learning mediated by connectivity patterns in key regions within the extinction network.For this purpose, we computed polygenic risk scores (PRS) for major depressive disorder, post-traumatic stress disorder, anxiety disorders, cross-disorder risk, and neuroticism for one hundred and seventy-two human subjects that volunteered in several differential fear conditioning experiments. Using diffusion weighted imaging and resting state functional magnetic resonance imaging, we computed structural and functional connectivity measures for all possible pairs out of five key brain regions within the extinction network: amygdala, hippocampus, dorsal anterior cingulate cortex (dACC), ventromedial prefrontal cortex (vmPFC), and the cerebellar nuclei.Learning measures derived from modelled non-linear slopes of skin conductance responses to either conditioned stimuli (CS+) that predict the occurrence of an aversive unconditioned stimulus or stimuli that do not (CS-) during acquisition and extinction training of the experiments were regressed on the PRS. Exploratory mediation analysis as per Serang et al. (2017) was performed to identify relevant sets of region pairs and connectivity metrics that may mediate the association between genetic risk and fear learning.Structural connectivity between the cerebellar nuclei and the vmPFC was found to mediate the relationship between CS- learning and PRS for anxiety disorders, neuroticism, PTSD, and most consistently, for major depressive disorder during acquisition. No other predictor or mediator had a substantial predictive impact on learning for either stimulus type or experimental phase.Our results suggest that genetic predisposition for internalizing disorders explain a fraction of the observed variability in the efficiency of safety-cue learning. They further support the notion that the human cerebellum, a brain region frequently neglected in the field, is indeed involved in fear learning.

Disclosures: J.E. Schneider Penate: None. **C.A. Gomes:** None. **H. Engler:** None. **S. Elsenbruch:** None. **C.J. Merz:** None. **O.T. Wolf:** None. **E. Genc:** None. **T. Spisak:** None. **O. Güntürkün:** None. **H. Quick:** None. **D. Timmann:** None. **N. Axmacher:** None. **R. Kumsta:** None.

Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR423.11/KK3

Topic: G.01. Fear and Aversive Learning and Memory

Title: Alto-100 improves pattern separation in mice through a neurogenesis-dependent mechanism

Authors: *H. CHUNG[1], K. TEGANG[1], A. ETKIN[2], K. RESSLER[3], J. HARTMANN[3], C. STUCKE[4], A. SAVITZ[2], R. HEN[1], W.-L. CHANG[1];
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Abstract: Background: Neurogenesis, the formation of new neurons, continues to occur in the dentate gyrus of the hippocampus throughout adulthood in both mice and humans. ALTO-100 is a novel neurogenic drug that is thought to act at BDNF. Previous work from our group has demonstrated that ALTO-100 improves pattern separation in adult mice without altering fear conditioning. Here, we test whether this effect of chronic treatment with compound ALTO-100 is neurogenesis-dependent by ablating neurogenesis with X-irradiation.Methods: Young adult male c57Bl/6J mice were used for all experiments. X-irradiation was administered to hippocampal areas while mice were anesthetized. Sham mice were also anesthetized, but no X-irradiation was administered. Irradiated and sham mice were allowed to recover for 8 weeks prior to initiation of drug treatment, 30mg/kg of ALTO-100 administered daily by oral gavage. After 21 days of treatment with the active compound or the control vehicle, the mice underwent a series of behavioral tests, including the open field test, a contextual fear conditioning task, followed by 7 days of a contextual fear discrimination task. Successful fear discrimination was achieved when animals distinguished between the conditioned fear context “A” and a similar context “B” that was not paired with foot shock. After the conclusion of pattern separation, the mice were perfused and their brains were collected for histological analyses (N =6-7 mice/group).Results: Each of the four groups of mice displayed elevated levels of freezing after the initial contextual fear conditioning, with no difference between groups. Vehicle-treated sham mice showed a significant effect of context, but no significant context x day interaction. Sham-ALTO-100 mice also showed a significant effect of context, but also a context x day interaction, with significant discrimination between on the 6^{th} day of exposure to both contexts. X-irradiated vehicle-treated mice were not able to significantly discriminate between contexts, and X-irradiated ALTO-100-treated mice also were not able to significantly discriminate between the contexts. Data from the Open Field Test will be presented, along with data from immunohistology staining for markers of neurogenesis.Conclusion: We demonstrated that chronic pharmacological treatment with the compound ALTO-100 reliably improves context discrimination in non-irradiated mice, as we have previously shown.
Furthermore, this effect appears to be neurogenesis-dependent, as X-irradiation and ablation of neurogenesis was able to block the effects of ALTO-100 on pattern separation.

Disclosures: **H. Chung:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Received drugs free of charge from Alto Neuroscience. **K. Tegang:** None. **A. Etkin:** A. Employment/Salary (full or part-time):; Employed by Alto Neuroscience. **K.**

Ressler: None. **J. Hartmann:** None. **C. Stucke:** None. **A. Savitz:** A. Employment/Salary (full or part-time):; Employed by Alto Neuroscience. **R. Hen:** None. **W. Chang:** None.

Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR423.12/KK4

Topic: G.01. Fear and Aversive Learning and Memory

Support: startup fund from the Medical College of Georgia at Augusta University

Title: Glucocorticoid-induced fear-like memory is associated with an increase in h current in the hippocampal CA1 neurons

Authors: J. KIM, K. POKHAREL, S. MICHAEL, A. LEE, ***C. KIM**;
Neurosci. & Regenerative Med., Augusta Univ., Augusta, GA

Abstract: Excessive or inappropriate fear reactions define maladaptive fear memory. Individuals experiencing maladaptive fear memory frequently have vivid and intrusive recollections of traumatic memories. As a result, they exhibit intense fear responses, even in situations that are objectively safe, a phenomenon known as emotional hypermnesia. In addition, they may struggle to retrieve complete narrative memories of the traumatic event, as certain contextual details may be missing (i.e., contextual amnesia). Glucocorticoids can generate abnormal fear-like memory, including emotional hypermnesia and contextual amnesia. However, there is limited understanding of the cellular mechanisms responsible for the development of glucocorticoid-induced maladaptive fear-like memory. Here, we employed a combination of contextual fear conditioning and post-training corticosterone (CORT) injection to produce the maladaptive fear-like memory in adult male mice. Corticosterone is a type of stress hormone known as a glucocorticoid in rodents. Hyperpolarization-activated cyclic nucleotide gated nonselective cation 1 (HCN1) channels are highly expressed in the hippocampus. We have previously reported that CORT reduces the neuronal excitability of dorsal CA1 neurons, which is mediated by HCN channels. In light of this, we inquired about the potential roles in HCN channels during the formation of fear-like memory induced by CORT. We demonstrated that contextual fear conditioning, when immediately followed by CORT (i.p. 2 mg/kg), resulted in emotional hypermnesia, as seen by an increased fear response to a salient but unrelated cue (i.e., tone) connected to traumatic stress. Furthermore, CORT-injected mice exposed to a cue (i.e., context) associated with traumatic stress exhibited impaired fear extinction over time. Following memory tests, dorsal and ventral hippocampal slices were prepared. In mice treated with CORT, the neurons in the dorsal and ventral CA1 regions exhibited diminished input resistance and reduced firing of action potentials both at rest and at -65 mV, without any changes in the resting membrane potential. This decrease in neuronal excitability in the dorsal and ventral CA1 regions was correlated with an elevation in h current (I_h). Based on our findings, it is plausible to propose

that an increase in I_h within the hippocampal CA1 neurons might underlie the development of fear-like memory induced by CORT.

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Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

Location: WCC Halls A-C

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Topic: G.01. Fear and Aversive Learning and Memory

Support: LaCaixa Foundation grant LCF/PR/HP17/52190001
Centro2020 grant CENTRO-01-0246-FEDER-000010

Title: Adenosine A_{2A}receptors control the extinction of fear memories

Authors: S. G. FERREIRA[1], S. L. REIS[2], V. LOURENÇO[2], A. R. TOMÉ[2], A. SIMÕES[2], P. M. CANAS[2], ***R. A. CUNHA**[2,3];
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Abstract: Psychological trauma-related disorders involve pathological fear responses and impaired fear extinction. Polymorphisms of adenosine A_{2A} receptors (A_{2A}R) are associated with panic disorders (*Mol Psychiatry* 3:81,1998; *Neuropsychopharmacol* 29:558,2004) and A_{2A}R control the acquisition and recall of fear memories, through modulation of information processing in the basolateral amygdala (BLA) and ventral hippocampus (VH) (*Neuropsychopharmacol* 41:2862,2016; *Biol Psychiatry* 75:855,2014). However, it is not known if A_{2A}R also control fear extinction, which was now tested. Adult male mice were contextual fear conditioned (FC) and some were then re-exposed to the same context for 3 days without paired shocks (extinction-EXT) and other not (FC-CTR) to evaluate their freezing behaviour; additionally, synaptic plasticity was assessed in CA1 synapses of VH slices and in cortico-amygdalar synapses in BLA-containing slices. The role of A_{2A}R was investigated by injecting a selective antagonist, SCH58261 (0.1 mg/kg, i.p.), 1 hour before each extinction trial. To test the specific contribution of A_{2A}R in VH or BLA, guide cannulas were implanted and either SCH58261 (50 nM) or light stimulation (15 days after transfection with AAV5-CaMKIIα-optoA_{2A}R-mCherry, to allow the optogenetic activation of the transducing system operated by A_{2A}R) was bilaterally applied 1 hour before each extinction trial. FC-CTR mice maintained their freezing behaviour for 4 days, whereas EXT mice progressively decreased freezing. A_{2A}R blockade with SCH58261 i.p. accelerated fear extinction from day 2 onwards, but did not modify passive fear memory extinction, locomotion (open field) or anxiety (elevated plus maze) in FC-CTR mice. Two days after FC, FC-CTR mice displayed a decreased post-tetanic stimulation (5 trains of 100 Hz for 1s separated by 5s) in the BLA, which was normalised by SCH58261 i.p.;

EXT also increased LTD magnitude in BLA, which was larger with SCH58261. FC increased LTP in the VH after 2 days compared to EXT mice, an effect reduced by $A_{2A}R$ blockade, without alterations of depotentiation. In accordance with these alterations of synaptic plasticity, the selective blockade of either BLA-$A_{2A}R$ or VH-$A_{2A}R$ via SCH58261 direct administration in the BLA or VH was sufficient to accelerate fear extinction. Conversely, light stimulation of opto$A_{2A}R$-expressing mice in the VH attenuated fear extinction and increased VH-LTP. These results evidence a role for $A_{2A}R$ on fear extinction, namely in the BLA and in the VH, reinforcing the interest in targeting $A_{2A}R$ to manage fear-related disorders.

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Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR423.14/KK6

Topic: G.01. Fear and Aversive Learning and Memory

Support: ERATO; JPMJER1801
Institute of AI and Beyond of the University of Tokyo
JSPS Grants-in-Aid for Scientific Research (18H05525).

Title: Axo-axonic cells in the basolateral amygdala regulate fear conditioning.

Authors: *M. NAKASHIMA[1], R. HAYASHI[2], S. MORIKAWA[2,3], Y. IKEGAYA[2,4];
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Abstract: The activity and plasticity of excitatory neurons are regulated by local inhibitory neurons in a spatiotemporally specific manner. Axo-axonic cells (AACs) are a unique type of inhibitory neurons that predominantly form synapse onto the axon initial segment of pyramidal neurons. While their anatomical features have been identified, the functional roles of AACs remain unclear. In this study, using a specific labeling approach for AACs in the basolateral amygdala (BLA), we show that AACs play a crucial role in memory acquisition. By performing in vivo calcium imaging and functional silencing of AACs in the BLA, we demonstrated that AACs were activated by salient stimuli, such as foot shock or reward, and provided a mandatory heterogeneous inhibitory signals for associative learning. Furthermore, we found that AACs preferentially received long-range inputs from the basal forebrain and medial geniculate nucleus. These findings suggest a central role for AACs in the representation of salient stimuli, which is essential for the induction of adaptive behavior.

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Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

Location: WCC Halls A-C

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Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH R01 NS106915
VA I01 BX003893-01A1

Title: Emotional stress reduces endocannabinoid signaling in cerebellar cortex

Authors: *M. SAYED, J. PATEL, M. FAROUK, S. LIU;
Cell Biol. & Anat., LSU Hlth. Sci. Ctr., New Orleans, LA

Abstract: The cerebellum is critical for emotional regulation. We have recently shown that fear conditioning reduces endocannabinoid signaling in the cerebellum and this is required for fear memory formation. Since emotional stress can modify subsequent fear memory, we tested whether predator odor stress could alter endocannabinoid signaling in the cerebellar cortex and thereby enhance the formation of fear memory. Male C57BL/6 mice were exposed to fox urine for 5 minutes and the cerebellum was isolated three hours later. Since endocannabinoid (eCB) signaling suppresses neurotransmitter release, we tested the effect of an endocannabinoid receptor (CB1R) antagonist on spontaneous GABA release, assessed by miniature IPSCs (mIPSCs) recorded in stellate cells. Bath application of a CB1R antagonist NESS0327 increases the frequency of mIPSCs in naive mice, indicating the presence of tonic eCB. However, NESS0327 failed to alter mIPSC frequency in mice that were exposed to predator odor, suggesting that predator odor stress reduced tonic endocannabinoid signaling in the cerebellar cortex. The decrease in eCB signaling could result from a loss of endocannabinoid receptor signaling. To test this, we applied a CB1R agonist, WIN55212-2 and found that WIN55212-2 reduced the frequency of mIPSCs in molecular layer interneurons from stressed mice by around 30%, comparable to that in naïve animals. Thus, fox urine exposure did not change the CB1R signaling, and may therefore alter eCB levels. 2-AG, the major eCB in the cerebellum, is produced by diacylglycerol lipase (DAGL) and degraded by monoacylglycerol lipase (MAGL). We quantified MAGL activity and found that predator odor stress did not alter the activity of MAGL, compared to naive mice. We next tested whether stress reduced DAGL levels in cerebellar cortex using western blotting. Our preliminary results show that fox urine exposure reduced the DAGL expression by 40% relative to naive mice. Our results suggest that predator odor stress reduces endocannabinoid signaling in cerebellar cortex by decreasing 2-AG production via DAGL. (Supported NIH R01 NS106915, VA I01 BX003893-01A1).

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Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

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Program #/Poster #: PSTR423.16/KK8

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH MH110425

Title: Neural Correlates of Safety Signaling and Conditioned Inhibition of Fear in the Ventromedial Prefrontal Cortex

Authors: *M. A. VERBRUGGE;
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Abstract: Authors: M Verbrugge[1,3], DP Lyvers[1,3], CC Lapish[2,3], S Sangha[1,31]Department of Psychiatry, [2]Department of Anatomy, Cell Biology & Physiology, [3]Stark Neuroscience Research Institute, Indiana University School of Medicine, Indianapolis, IN
Neural Correlates of Safety Signaling and Conditioned Inhibition of Fear in the Ventromedial Prefrontal Cortex
The ability to accurately discern between safe and threatening situations is challenged in Post-Traumatic Stress Disorder (PTSD), often leading to maladaptive fear. Many PTSD individuals fail to show conditioned inhibition of fear, the downregulation of fear during the simultaneous presentation of a learned fear and safety cue (fear+safety cue). Our lab has a well-validated safety-fear-reward cue discrimination task, which was adapted for this study to produce a greater range in fear suppression in male and female Long Evans rats, such that approximately half of males and females show “good” fear suppression, while the other half do not. During this behavior we collected longitudinal single cell calcium activity within the prelimbic (PL) and infralimbic (IL) regions of the ventromedial prefrontal cortex (vmPFC) in freely behaving male and female Long Evans rats using miniscopes and GCaMP6m expressed via AAV1.GCaMP6m.WPRE.SV40 from Inscopix. These vmPFC regions play key roles in modulating fear, anxiety, and stress behaviors. We have shown in our safety task that the PL is necessary for fear expression, while the IL is necessary for conditioned inhibition of fear. Our data in the IL indicate that there is an ensemble of neurons highly correlated in its activity to the fear+safety and fear cues that is separate from an ensemble of neurons highly correlated in its activity to the fear+safety and reward cues. Preliminary results show that we can also follow these same cells for at least 12 days. Current analyses are examining cue-evoked calcium transients against expressed safety, fear and reward behaviors across the entirety of the 15 day task.
Key Words: discriminative conditioning, safety learning, conditioned inhibition of fear, prefrontal cortex, calcium imaging

Disclosures: M.A. Verbrugge: None.

Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

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JSPS KAKENHI 22H04998
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Title: Reduced top-down influence in the primate fronto-cingulo-striatal network underlying negative bias in conflict decision-making

Authors: *S. AMEMORI[1], A. M. GRAYBIEL[2], K. AMEMORI[3];
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Abstract: Major depressive disorder (MDD) affects a significant portion of the global population. The fronto-cingulo-striatal (FCS) network, including the dorsolateral prefrontal cortex (dlPFC), subgenual anterior cingulate cortex (sgACC), pregenual anterior cingulate cortex (pACC) and the striatum, is implicated in MDD. MDD patients often exhibit a negative processing bias, characterized by negative reactions to emotional stimuli. The approach-avoidance (Ap-Av) decision-making task is used to investigate this bias. Neuroimaging studies have revealed abnormalities in dlPFC activity during the Ap-Av task in MDD patients. Moreover, deep brain stimulation of the sgACC reduced MDD symptoms. In non-human primates, beta-band oscillatory activity in the striatum has been found to represent decision-related variables, and the pattern of beta oscillations has been shown to parallel stimulation-induced negative bias, suggesting a role for FCS network interactions and neuronal synchronization in negative bias. Thus, we hypothesized that beta-band activity and synchronization, particularly involving sgACC, contribute to negative bias processing. We trained two monkeys in an Ap-Av task in which they had to choose between receiving a reward and experiencing an air puff to the face. During task performance, we stimulated the sgACC and recorded simultaneously local field potentials in FCS network. Microstimulation of certain regions within the sgACC (10/38 sites tested) resulted in a significant increase in avoidance choices, indicating the involvement of the sgACC in negative bias. Notably, these negative effective sites were significantly surrounded by neurons that were activated by avoidance decision (n = 31) and airpuff delivery (n = 129). Next we analyzed task-related LFPs (n = 3942/4745) during a decision period in FCS network and extracted beta responses. Through multidimensional scaling, we categorized beta responses that represent approach and avoidance choices into positive (*P*, n = 911) and negative (*N*, n = 591) groups. Effective microstimulation

reduced the number of responses in *P* group, suggesting a bias towards negative outcomes. Furthermore, we performed granger causality analysis on alpha (5-13 Hz) and beta (13-30 Hz) responses in FCS network to assess regional interactions. Effective sessions showed reduced directional influences, particularly from dlPFC to pACC and striatum. These results suggest that the top-down signal from the dlPFC to the limbic system was mediated by the beta synchrony, and that the blunting of top-down processing may underlie the negative bias observed in decision-making, which is a hallmark symptom of MDD in humans.

Disclosures: S. Amemori: None. **A.M. Graybiel:** None. **K. Amemori:** None.

Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

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Program #/Poster #: PSTR423.18/KK10

Topic: G.01. Fear and Aversive Learning and Memory

Title: Model-based extinction of the fear of heights by active flight experience in VR

Authors: *M. FUJINO[1,2], M. HARUNO[2,1];
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Abstract: Many studies have shown “model-free” fear extinction in which a cue associated with a negative outcome is repeatedly exposed to participants without the negative outcome. The participants gradually construct a new association that this context is safe. In traditional exposure therapies for acrophobia, participants asked to experience insecure situations repeatedly, such as escalators. Such safety learning is based on “model-free” learning.
By contrast, “model-based” reinforcement learning explicitly considers transitions among contexts (state-transition probability) to predict future outcomes. It still remains unclear whether there exists a “model-based” extinction fear extinction which uses state transition information to predict a negative outcome.
Therefore, in this study, we tested this hypothesis using fear response to heights in VR. Specifically, we tested whether participants’ fear response to height reduces after the active VR flight experience. This is because after the VR flight experience, participants could then infer that they would be safe even if they fall. In other words, the participants will simulate the state transitions of falling -> flying -> safety by constructing a new safe state achieved by the flying action. At the beginning of the experiment, subjects felt a strong fear of heights. However, once they had the flight experience, they could predict that falling would not result in injury.
In total, 59 adults with fear of heights were randomly assigned to the flight group or a control group. Subjects in the flight group flew over the city in VR for more than 7 minutes. Subjects of the control group watched the flight video, which differed from the flight group only in that they could not control the course of the flight. Before and after tasks, participants in both groups walked on a plank in VR (plank task). Electrodermal activity (EDA) and photoplethysmography

(PPG) were recorded as physiological indicators of the fear response.The analysis data consisted of 16 subjects in the flight group and 15 subjects in the control group. Subjects with low EDA response were excluded. We compared the mean EDA for 30 seconds before and after the first and second plank tasks between groups.
In our experiment, as we expected, the flight group showed a significant decrease in mean SCR at high altitude ($t(21)=4.381$, $p=0.0001$). In contrast, there was no significant difference in the control group ($t(20)=1.194$, $p=0.123$).
These results revealed that an active flight experience using VR can reduce the automatic fear response to heights, suggesting the existence of "model-based" extinction of fear, which may lead to a novel treatment for phobias.

Disclosures: **M. Fujino:** None. **M. Haruno:** None.

Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR423.19/KK12

Topic: G.01. Fear and Aversive Learning and Memory

Support: Original Technology Program through the National Research Foundation of Korea funded by the Ministry of Science and ICT (No. 2021M3F3A2A01037811)
Korea Institute of Science and Technology Institutional Program (project no., 2E32211)

Title: Computational modeling revealing the role of spatiotemporally modulated serotonin in fear conditioning and extinction

Authors: *A. BADRIPOUR, T. KIM;
Korea Inst. of Sci. and Technol. (KIST), Seoul, Korea, Republic of

Abstract: Fear conditioning (FC) has been frequently used to study emotional learning and memory. The brain-wide distribution of involved neural circuits and diverse influence from neuromodulatory transmitters (NTs) make FC complex. Several computational approaches have tried to explain the complicated dynamics of involving circuits, including the role of NTs. Prior FC experiments showed phasic serotonin release during aversive stimuli like foot shock and serotonin-selective reuptake inhibitor has greatly helped depression and post-traumatic stress disorder which FC and extinction partially characterize, suggesting a substantial role of serotonin during FC. However, in previous computational models emulating FC and extinction, the dynamics of serotonergic involvement have been overlooked. Therefore, we built the computational model that captures behavioral responses and neural circuit dynamics under multiple scenarios of differentially modulated spatiotemporal serotonin levels that were categorized based on previously reported experimental evidence. First, our punishment-driven

reinforcement learning model demonstrated that serotonin controls the discounting factor and learning rate, as previously reported in reward-based reinforcement learning. Higher serotonin levels raised the discounting factor, reflecting the agent's prolonged expectation of punishment, while the reduction of serotonin level downregulated the learning rate, resulting in the reduced conditioned response during learning. Second, our rate-based circuit model incorporating the amygdala, medial prefrontal cortex (mPFC), and hippocampus (HPC) regenerated the time course of FC and extinction. In this model, phasic serotonin accompanied by unconditioned stimulus enhanced fear learning and regulated the time course of fear extinction via disinhibition of input pathway converging onto basal amygdala from mPFC and HPC, indicating the hub microcircuit for FC and extinction. Thus, further development of our model may contribute to a comprehensive understanding of fear conditioning and extinction, enabling the development of more precise intervention strategies for neuropsychiatric disorders.

Disclosures: A. Badripour: None. **T. Kim:** None.

Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

Location: WCC Halls A-C

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Program #/Poster #: PSTR423.20/KK13

Topic: G.01. Fear and Aversive Learning and Memory

Support: 5R01MH116445
5R01MH105414
5R21MH114170

Title: Hippocampal somatostatin interneurons mediate retrieval of conflicting threat and safety memories

Authors: *A. F. LACAGNINA, T. N. DONG, R. IYER, S. KHAN, M. MOHAMED, R. CLEM;
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Abstract: Fearful experiences create enduring negative associations with the surrounding context. Emotional responses to these contextual cues normally subside in the absence of threat, a process known as extinction. Fear and extinction memories appear to be represented by competing neural ensembles; however, very little is known about the mechanisms governing the switching between these conflicting memories. Understanding neural circuits responsible for the gating of emotional memory expression can provide crucial insights for developing novel therapeutics for treating disorders of pathological fear. We identified activity of somatostatin interneurons (SST-INs) in the ventral hippocampal CA1 area (vCA1) as uniquely correlated with extinction retrieval. An extinction-specific recruitment of vCA1 SST-INs was confirmed using an intersectional genetic tagging strategy. Optogenetically silencing vCA1 SST-INs impaired extinction retrieval, while stimulating these cells, either broadly or in an activity-dependent

manner, prevented fear relapse. In contrast, manipulations of vCA1 parvalbumin-expressing INs did not affect expression of contextual fear or extinction. We confirmed that fear and extinction retrieval reactivate orthogonal vCA1 excitatory ensembles and, additionally, silencing excitatory projections from vCA1 to the prefrontal cortex specifically impairs extinction retrieval. Our results suggest that retrieval of conflicting memories is mediated by vCA1 SST-INs. We hypothesize the vCA1 SST-INs gate the activity of orthogonal excitatory ensembles to suppress the activity of the ensemble associated with the initial contextual memory and promote the retrieval of the extinction-related ensemble.

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Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR423.21/KK14

Topic: G.01. Fear and Aversive Learning and Memory

Title: A midbrain-extended amygdala pathway controls contextual fear memory formation.

Authors: K. MÜLLER[1], B. BRUZSIK[2], L. ROVIRA-ESTEBAN[3], E. PARADISO[4], R. KARLÓCAI[5], G. A. NAGY[6], O. PAPP[3], Z. FEKETE[3], F. FERRAGUTI[7], E. MIKICS[3], ***N. HAJOS**[5];
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Abstract: Neuronal circuits in the midbrain play a critical role in controlling defensive behavior. However, it is still elusive how different neuron types contribute to distinct behavioral outcomes during the presence of threat. In this study, we investigated a group of neurons in the ventral periaqueductal gray and dorsal raphe that express vasoactive intestinal polypeptide (VIP). Using viral tracing conducted in Vip-Cre mice, we observed that these VIP neurons innervated exclusively the bed nucleus of stria terminalis and central amygdala (CeA), the two main regions of the extended amygdala. Interestingly, neurons in these extended amygdala regions contributed to the innervation of midbrain VIP neurons to a large extent revealed by monosynaptic rabies tracing. In vitro electrophysiological recordings combined with optogenetics showed that light stimulation of VIP afferents activated ionotropic glutamate receptors in their postsynaptic partners in the extended amygdala. To clarify the role of the midbrain VIP neurons in fear acquisition, we inhibited their activity using chemogenetics during fear conditioning, and observed that this intervention impaired the contextual, but not cued fear memory recall on subsequent days. In line with the role of midbrain VIP neurons in fear learning, mild electrical shocks potently excited these neurons when tested by in vivo electrophysiological recordings.

Finally, using slice recordings combined with optogenetics, we found that the co-activation of ventral hippocampal and VIP-expressing PAG inputs forming synapses on CeA neurons underwent LTP, an observation that may provide a mechanistic explanation for the role of this subcortical pathway in the acquisition of contextual fear memory. These results collectively show that the excitatory midbrain-extended amygdala pathway expressing VIP contributes to contextual memory formation.

Disclosures: K. Müller: None. **B. Bruzsik:** None. **L. Rovira-Esteban:** None. **E. Paradiso:** None. **R. Karlócai:** None. **G.A. Nagy:** None. **O. Papp:** None. **Z. Fekete:** None. **F. Ferraguti:** None. **E. Mikics:** None. **N. Hajos:** None.

Poster

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Program #/Poster #: PSTR423.22/KK15

Topic: G.01. Fear and Aversive Learning and Memory

Support: CAPES
CNPq

Title: Characterization of defensive coping strategy induced by electrical footshock or chemical stimulation of the dorsolateral periaqueductal gray in female rats

Authors: *L. CORREA NAKATSUKASA TAKASUMI, L. A SOARES, A. P CAROBREZ; Pharmacol., Univ. Federal de Santa Catarina, Florianópolis, Brazil

Abstract: Rodents in life-threatening situations exhibit specific behaviors according to their defensive coping strategy. These behaviors can be classified as defensive (DB) (i.e., freezing, darting, and jumping) or risk assessment (RAB) (i.e., crouch sniffing, rearing, and stretch approach). The literature indicated that electrical footshock (EF) and the N-Methyl-D-Aspartate (NMDA) stimulation of the dorsolateral periaqueductal gray matter (dlPAG) elicited DB in male rats. Contrarily, female rats in EF protocols showed less DB than males, suggesting sex differences in their coping strategy in aversive situations, which is yet to be determined. This study was designed to characterize the defensive coping strategy expressed by female rats exposed to EF or dlPAG stimulation. In Experiment 1, female Wistar rats underwent an EF protocol of five 0 (control) or 0.8 mA EF (40 s interval; n = 20/group). For experiment 2, the rats implanted with guide cannulas directed at the dlPAG underwent a microinjection protocol in which vehicle (PBS; n = 10), NMDA 25 (n = 9), or 50 pmol (n = 7) was continuously infused in freely moving rats (0.2 µl final volume). All experiments were recorded, and data were expressed as percentage of time for DB and RAB. Kruskall-Wallis followed by multiple comparisons test evaluated the groups for each experiment. The results from Experiment 1 showed that female rats predominantly expressed RAB (53%) rather than DB (25%) after EF. Also, RAB and DB values were higher in EF than in the control group (33% and 0%,

respectively) ($p < 0.05$). The results obtained in Experiment 2 showed that female rats injected with NMDA 25 expressed more DB (DB = 51%; RAB = 28%) and NMDA 50 more RAB (DB = 23%; RAB = 32%) than PBS (DB = 10%; RAB = 10%) ($p < 0.05$). Freezing was the predominant DB in NMDA 25; crouch sniffing was the predominant RAB in NMDA 50. In summary, female rats showed a marked defensive response under different aversive stimuli, but also showed that the coping strategy is not homogeneously switching between DB and RAB. The results suggest that freezing alone, as widely applied in fear learning studies, may not provide a complete view on the defensive coping strategy found in female rats. More importantly, female rats exhibiting decreased DB could be erroneously described as expressing low fear, when in fact they are engaging in RAB.

Disclosures: L. Correa Nakatsukasa Takasumi: None. **L. A Soares:** None. **A. P Carobrez:** None.

Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

Location: WCC Halls A-C

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Program #/Poster #: PSTR423.23/KK16

Topic: G.01. Fear and Aversive Learning and Memory

Support: CIHR-PJT-153155
RGPIN-2016-04574
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Canadian Foundation for Innovation/Province of Ontario

Title: Molecular determinants of long-term memory formation ability in Lymnaea stagnalis

Authors: *J. BANDURA[1], C.-H. GUO[1], C. CHAN[2], H.-S. SUN[3], A. R. WHEELER[2,4,5], Z.-P. FENG[1];
[1]Physiol., [2]Chem., [3]Surgery, [4]Donnelly Ctr. for Cell. and Biomed. Res., [5]Inst. of Biomed. Engin., Univ. of Toronto, Toronto, ON, Canada

Abstract: Long-term memory (LTM) formation is essential for survival, and involves cellular and molecular mechanisms, such as CREB-dependent gene regulation and protein synthesis, that are conserved between vertebrates and invertebrates (Kandel 2001). The freshwater pond snail, mollusc *Lymnaea stagnalis*, forms LTM following aversive operant conditioning of aerial respiratory behaviour, as defined by a significant decrease in its respiratory activity (Lukowiak et al. 1996). Blocking CNS protein synthesis prevents LTM formation (Sangha et al. 2003), and our lab has previously reported that LTM formation is contingent on the expression of proteins such as CREB1 (Guo et al. 2010) and MEN1 (Dong, Senzel, Li et al. 2018). The ability of an animal to form aversive LTM in this model further correlates with activity in the pacemaker neuron of the respiratory central pattern generator (CPG), RPeD1, and can be predicted by behavioural responses during aversive operant conditioning, dividing animals into LTM and no-LTM groups

(Dong & Feng 2017). In this study, we hypothesized that the ability of animals to form LTM is associated with protein expression of defined molecular networks which are regulated in the CNS following aversive operant conditioning. To test this hypothesis, we first characterized spontaneous and evoked activity in RPeD1 from snails exhibiting LTM or no LTM. To identify proteins associated with differential LTM formation ability, we used bottom-up shotgun proteomics via liquid chromatography-tandem mass spectrometry (LC-MS/MS) for label-free quantification of protein abundance in the CNS of snails exhibiting LTM or no LTM (n=4 per group) and identified 293 differentially-expressed proteins. To determine the interaction network of critical proteins for LTM formation ability, we generated 6xHis-tagged LTM-regulated proteins and used affinity purification followed by LC-MS/MS and coimmunoprecipitation to identify interactions. Using this combined behavioural, electrophysiological, and large-scale proteomic and interactomic approach, we have for the first time characterized the protein interaction network differentially expressed in LTM animals relative to no-LTM animals and untrained controls. Together, we report the correlation between electrophysiological characteristics and the set of proteins associated with differential LTM formation ability following aversive operant conditioning, suggesting that essential molecular networks underlie inherent differences in LTM formation ability. The signaling pathways identified may thus constitute fundamental mechanisms of LTM formation cross-species.

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Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

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Program #/Poster #: PSTR423.24/KK17

Topic: G.01. Fear and Aversive Learning and Memory

Support: Actuated Medical Inc.
R01 MH122561

Title: Unit activity in the amygdalostriatal region and central nucleus of the amygdala during cue-evoked suppression of reward seeking

Authors: *K. J. ANDERSON[1], C. E. STELLY[2], A. M. HALL[1], P. STOLIN[1], J. P. FADOK[3]; [2]Cell & Mol. Biol., [3]Psychology and Tulane Brain Inst., [1]Tulane Univ., New Orleans, LA

Abstract: A common feature of many mental illnesses is dysregulated responding to different valences, yet the neural mechanisms that contribute to this are poorly understood. We have utilized a cue-evoked suppression of reward seeking paradigm designed to address both positive and negative valence using a combination of operant learning and Pavlovian conditioning. In addition, we have recorded single unit activity in awake, behaving mice during this conditioned suppression paradigm. With this work, we aim to track single unit activity in the

amygdalostriatal region (Astr) and central nucleus of the amygdala (CeA) in mice that have undergone conditioned suppression of reward seeking across three different stages of learning: naïve, conditioning, and extinction. We have found recorded single unit evidence to suggest neuronal activity in Astr is altered in response to reward, rather than the fear-associated cue presentation; further work is required in order to assess the extent of such reactivity. Additionally, we predict that single unit recordings in the CeA will demonstrate neuron reactivity in response to fear-associated cue presentation, which we have not identified as yet in such recordings from the Astr. The overall goal of this work is to identify the underlying mechanisms regarding behavior selection and valence responding.

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Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

Location: WCC Halls A-C

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Program #/Poster #: PSTR423.25/KK18

Topic: G.03. Motivation

Support: R00 DA045765
Bush Biomedical Research Grant FP00027458

Title: Orexin/hypocretin mediates sleep disturbances and drug seeking during initial cocaine abstinence

Authors: *U. GYAWALI, S. MERAI, A. BHAT, D. DE SA NOGUEIRA, M. JAMES; Rutgers Univ., Piscataway, NJ

Abstract: Sleep disturbances commonly occur in individuals with substance use disorders and worsens during withdrawal. Cocaine-exposed rats display disrupted sleep patterns, characterized by fragmented and reduced non rapid eye movement (NREM) and rapid eye movement (REM) sleep. Normalizing sleep during abstinence reduces drug cravings, indicating a potential relationship between these processes. The orexin neuropeptide system holds promise as a therapeutic target for medications designed to abrogate cocaine cravings and associated sleep disturbances, as orexins regulate both reward and wakefulness brain circuits. However, direct evidence linking orexin-sleep circuits with drug seeking behaviors is lacking. Here, we used conditioned place preference (CPP) to establish a contextual association with acute bolus injections of cocaine (10mg/kg). Rats then underwent 5d of drug abstinence followed by a place preference test. During abstinence, rats were treated daily with the dual orexin receptor antagonist suvorexant (0, 30 mg/kg, p.o.) immediately prior to the onset of the inactive period. In a subset of rats, electroencephalogram (EEG) and electromyogram (EMG) activity was monitored throughout the experiment. We observed sleep disturbances following cocaine

conditioning, characterized by increase in wake time, decrease in NREM and REM sleep along with sleep fragmentation (more frequent stage transitions). These changes persisted into abstinence but were abrogated by suvorexant treatment, which also facilitated extinction of cocaine seeking behavior. In a separate cohort, following the CPP expression test, we collected ventral tegmental area (VTA) and lateral hypothalamus (LH) and quantified orexin and orexin receptor 1 and 2 mRNA levels using qRT-PCR. We saw an increase in VTA orexin receptor 1 and LH orexin mRNA levels in cocaine vs. saline conditioned rats; these changes were positively correlated with cocaine preference. These data indicate that blocking orexin signaling during initial cocaine abstinence normalizes sleep and reduces drug seeking, indicating a potential causal link between these processes. Ongoing work is exploring VTA as a potential site through which orexin signaling might mediate both sleep and reward outcomes following cocaine.

Disclosures: U. Gyawali: None. **S. Merai:** None. **A. Bhat:** None. **D. De Sa Nogueira:** None. **M. James:** None.

Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR423.26/KK19

Topic: G.03. Motivation

Support: R00 DA045765
Busch Biomedical Research Grant FP00027458

Title: Administration of the dual orexin receptor antagonist suvorexant during the active vs. inactive period: Implications for cocaine behaviors

Authors: *S. L. O'CONNOR[1], U. GYAWALI[2], M. S. PALADINO[2], N. KRISHNAKUMAR[2], M. M. BILOTTI[3], D. J. BARKER[4], M. H. JAMES[2];
[1]Psychology, [2]Psychiatry, [3]Joint Grad. Program in Toxicology, Rutgers Univ., Piscataway, NJ; [4]Psychology, Rutgers Univ., Pisctaway, NJ

Abstract: Introduction: Cocaine self-administration in rats is associated with an increase in the number and activity of orexin (hypocretin) neurons. Orexin neurons are involved in reward and sleep, both of which are perturbed in cocaine use disorder. Thus, therapies that block orexin signaling might offer multifaceted therapeutic benefit. Here, we tested if the FDA-approved dual orexin receptor antagonist suvorexant, which is currently indicated to promote sleep in insomnia, can be used i) at low, non-sedating doses during the active period to reduce drug seeking, and ii) at higher doses to normalize sleep and subsequent drug seeking during cocaine abstinence. Methods: Male and female Long Evans rats were assessed for baseline economic cocaine demand using a within-session threshold procedure. Rats were then given daily intermittent access (IntA) to cocaine (5min access every 30min for 6h) for 2w. One group of rats (n=24) was treated with suvorexant (0, 3, 10, 30mg/kg; p.o., within-subjects) prior to being reassessed for

demand during the active period. To test for soporific effects of suvorexant, these rats were also tested on the rodent psychomotor vigilance task (rPVT), which requires rats to maintain attention for 30min to earn sucrose rewards; rats received suvorexant prior to testing, as above. A second group of rats (n=14) underwent extinction training for 7d; during this time, rats received suvorexant (0, 30mg/kg, p.o.) 30min prior to the onset of the inactive period. Results: Males and females both exhibited increased cocaine demand following IntA; this was reversed by suvorexant (10, 30mg/kg). Suvorexant did not impair performance on the rPVT. Repeated dosing with suvorexant during the inactive period facilitated extinction. Suvorexant efficacy in females was not affected by estrus stage. Discussion: Suvorexant can be used acutely during the active period to reduce drug motivation and during the inactive period to accelerate extinction of drug seeking. Ongoing studies will determine if the effects of suvorexant on extinction are linked with improved sleep outcomes.

Disclosures: S.L. O'Connor: None. **U. Gyawali:** None. **M.S. Paladino:** None. **N. Krishnakumar:** None. **M.M. Bilotti:** None. **D.J. Barker:** None. **M.H. James:** None.

Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

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Program #/Poster #: PSTR423.27/KK20

Topic: G.03. Motivation

Support: CEED Grant P30ES005022
NIDA R00 award DA045765

Title: Sex differences in the functioning of orexin (hypocretin) neurons and behavioral outcomes following peripubertal BPA exposure

Authors: *M. M. BILOTTI[1,2], V. ISSKANDAR[3], S. ANTHONY[1], A. YASREBI[4], N. T. BELLO[4], T. A. ROEPKE[4,1], M. H. JAMES[3,2];
[1]Joint Grad. Program in Toxicology, [2]Brain Hlth. Inst., [3]Psychiatry, Rutgers Univ., Piscataway, NJ; [4]Animal Sciences, SEBS, Rutgers Univ., New Brunswick, NJ

Abstract: Introduction: Human and rodent studies indicate that early life exposure to bisphenol-A (BPA), a weak estrogen receptor agonist, alters feeding and mood outcomes in adulthood. Separate literatures indicate that orexin-producing neurons in hypothalamus are 1) sensitive to estrogen, and 2) regulate feeding and motivational processes, making them a strong candidate system mediating the effects of early-life BPA exposure. Despite this, the effect of BPA on orexin system functioning, and any implications for behavioral outcomes in later life, have not been tested. Methods: Male (n=8-10/group) and female (n=8-12/group) Long-Evans rats were exposed to BPA (0, 25, 250µg/kg/day) in their drinking water from post-natal days (PND) 28-56. After BPA exposure, rats were tested for food motivation using three different assays: 1) binge-like eating of sweetened fat, 2) economic demand for sucrose, and 3) saccharine preference.

Following behavioral testing, brains were collected to determine orexin mRNA and protein levels. Results: In females, BPA rats exhibited reduced binge-like eating, demand for sucrose, and saccharine preference, indicating decreased food motivation. These behavioral changes were associated with lower orexin mRNA and protein levels in lateral hypothalamus. In males, BPA exposure was associated with increased food motivation and higher orexin mRNA and protein levels. Conclusions: Peripubertal BPA has opposing effects on food motivated behavior in males vs females. These behavioral changes were mirrored by alterations in orexin availability, indicating differential estrogen-mediated regulation of orexin neuron function in males vs. females

Disclosures: M.M. Bilotti: None. **V. Isskandar:** None. **S. Anthony:** None. **A. Yasrebi:** None. **N.T. Bello:** None. **T.A. Roepke:** None. **M.H. James:** None.

Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

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Program #/Poster #: PSTR423.28/LL1

Topic: G.03. Motivation

Support: NIH Grant DA 045765
New Jersey Health Foundation PC144-23
New Jersey Health Foundation PC98-22

Title: Binge-like eating restores hedonic tone in female rats maintained on a high fat diet via an orexin- (hypocretin) ventral tegmental area

Authors: U. GYAWALI, A. J. ARMANIOUS, J. B. MEHR, S. MERAI, ***M. H. JAMES**; Rutgers Univ., Piscataway, NJ

Abstract: *Introduction:* Binge eating has been argued to be 'self-medicating' against depressive states in persons of higher weight. Despite this, the neural systems that drive excessive food intake in the absence of caloric deficit remain to be fully characterized. Here, we tested if a diet-induced obesity model predisposes to binge-like eating in rats, and whether these binge-like episodes restore hedonic tone. We also examined whether an orexin-ventral tegmental area circuit, known to regulate other hedonic processes, governs binge-like eating in a diet-induced obesity model. *Methods:* Female Long Evans rats were maintained on a regular chow or high fat diet (HFD; 45% fat) for 8w; binge-like eating was then promoted via intermittent, restricted access to sweetened fat for 4w. Hedonic tone was measured using intracranial self-stimulation (n=6-7/group) and social preference (n=8/group) assays. Orexin release in VTA was measured using fiber photometry recordings of the OxLight1 sensor (n=5-6/group). In a separate group of rats, we also tested if binge-like eating differentially promotes 'addiction-like' food behaviors in lean (n=8) vs. HFD (n=9) rats by measuring sucrose demand, sucrose seeking during periods of signaled food non-availability, and reinstatement of extinguished sucrose seeking. *Results:* HFD

rats had higher ICSS thresholds and lower social preference compared to chow-fed controls. HFD rats exhibited greater escalation of binge-like eating, which partially normalized ICSS thresholds and social preference. In chow rats, food-associated stimuli elicited orexin release in VTA; this was blunted in HFD rats but partially restored by binge-like eating. Binge-like eating promoted higher 'food addiction' behaviors in HFD but not control rats. *Conclusions:* These data indicate that binge-like eating in obesity is i) governed by negative (rather than positive) reinforcement processes, ii) promotes 'addictive-like' eating behaviors, and iii) is mediated, at least in part, by the orexin-VTA circuit.

Disclosures: U. Gyawali: None. **A.J. Armanious:** None. **J.B. Mehr:** None. **S. Merai:** None. **M.H. James:** A. Employment/Salary (full or part-time):; Rutgers University.

Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

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Program #/Poster #: PSTR423.29/LL2

Topic: G.03. Motivation

Support: The Templeton World Charity Foundation
Narsad Young Investigator

Title: For the sake of safety: Orexinergic modulation of VTA for avoiding threats

Authors: *C. SILLER-PEREZ[1,2], E. C. ANDRADE[1], C. K. CAIN[1,2], J. E. LEDOUX[3], R. M. SEARS[1,2];
[1]Nathan Kline Inst., Orangeburg, NY; [2]Child and Adolescent Psychiatry, NYU Langone, New York, NY; [3]Ctr. For Neural Sci., New York Univ., New York, NY

Abstract: Identifying the neuromodulatory mechanisms that orchestrate survival behaviors is of critical importance to understanding anxiety- and stress-coping in health and disease. Perifornical (PFH) and lateral hypothalamus (LH) neurons expressing orexin (hypocretin) peptides project throughout the brain and mediate functions critical for survival behaviors including vigilance, attention, and action selection. Here we assessed the role of a key hypothalamic orexin system target using a model of proactive threat-coping behavior—signaled active avoidance (SigAA). Based on appetitive studies and physiology findings, we hypothesized that orexin would invigorate safety seeking via projections to a central hub in the reward pathway, the ventral tegmental area (VTA). Sprague Dawley rats received infusions of an orexin-specific viral vector containing an inhibitory opsin (AAV1-Ple112-Arch3.0-eYFP) into the PFH/LH, and optic fibers were implanted in the VTA. Following a 6-8-week incubation, rats were trained in the SigAA task. Animals received one Pavlovian trial (60 s white noise warning signal (WS) paired with an inescapable foot-shock (1.0/0.7 mA males/females; 0.5 s)). For all remaining trials, if animals shuttled during the WS (an avoidance response), a feedback (FB) tone was delivered (5 s, 80 dB) and indicated the animals were safe from harm (a safety signal). Failures to shuttle during the

WS resulted in shock, identical to trial 1. Rats received 15 trials per day until reaching criterion (80% successful avoidance) after which they were subjected to daily shock-free avoidance tests. Orexin◊ VTA axon terminals were inhibited (green laser 532 nm, 10 mW) during FB stimulus presentations only. On the first day, latencies and avoidance responses were unimpaired. However, inhibition on subsequent days increased latencies and impaired avoidance. These results suggest that 1) the FB cue is a reinforcer, perhaps through its association with safety, and 2) orexin communication with VTA is essential for safety-reinforced avoidance. Future studies will uncover the orexin system's role in adaptive coping behaviors and provide support for novel treatments of maladaptive coping, including active coping therapy combined with drugs to target the orexin system.

Disclosures: C. Siller-Perez: None. **E.C. Andrade:** None. **C.K. Cain:** None. **J.E. LeDoux:** None. **R.M. Sears:** None.

Poster

PSTR423. Neural Mechanisms of Motivation and Emotion

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR423.30/LL3

Topic: G.03. Motivation

Support: R00 045765

Title: Blockade of orexin 1 receptor signaling impairs sustained attention in rats

Authors: *N. KRISHNAKUMAR[1], M. PALADINO[1], J. WISKERKE[2], B. GRUSZKA[1], S. L. O'CONNOR[1], U. GYAWALI[1], M. H. JAMES[1];
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Abstract: Introduction: Preclinical studies support the utility of orexin-1 receptor (Ox1R) antagonists for the treatment of substance use disorder (SUD). However, the orexin system is also important for maintaining arousal, raising the possibility that such treatments may also interfere with cognitive functioning. Here, we tested whether the Ox1R antagonist SB334867 (SB) affects performance on the rodent psychomotor vigilance task (rPVT), an adapted version of a task used to measure cognitive fatigue and sustained attention in humans. **Methods:** Male (n=21) and female (n=11) Long Evans rats were trained to make rapid responses on a lever following presentation of a light cue that varied in onset time (3-10s after trial initiation); correct responses were rewarded with sucrose pellets and sessions ran for 30min. We first validated the task by testing the effect of two drugs with known attention-modulating properties (d-amphetamine, 0, 0.03, 0.1, 0.3 mg/kg; guanfacine, 0, 0.03, 0.1, 0.3 mg/kg i.p.). Next, all rats were tested following injections of SB (0, 3, 10, 30mg/kg; i.p.). All drugs were tested in a within-subjects design (order counterbalanced). **Results:** Across all measures, there was no effect of sex and thus data from males and females were analyzed together. At baseline,

performance (accuracy) worsened across the duration of the rPVT test session; this time-on-task effect was abrogated (accuracy was improved) by d-amphetamine and guanfacine, specifically in rats with low baseline performance. Accuracy was dose-dependently reduced by SB, however significant deficits were observed only at the highest dose tested (30mg/kg). **Conclusions:** Blockade of Ox1R tended to impair measures of sustained attention on rPVT, but meaningful changes were observed only at a relatively high dose of SB (30mg/kg). Given that SB reduces drug seeking at lower doses (5-20mg/kg), these data indicate that Ox1R antagonists might be useful for treating SUDs while having limited effect on general cognitive functioning.

Disclosures: N. Krishnakumar: None. **M. Paladino:** None. **J. Wiskerke:** None. **B. Gruszka:** None. **S.L. O'Connor:** None. **U. Gyawali:** None. **M.H. James:** A. Employment/Salary (full or part-time):; Rutgers University-Full Time.

Poster

PSTR424. Reward and Appetitive Learning and Memory: Acquisition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR424.01/LL4

Topic: G.02. Reward and Appetitive Learning and Memory

Title: The effects of naloxone on learned sexual behaviors in male Japanese quail on a partial schedule of reinforcement

Authors: E. CANTY, B. GLEESON, S. LEVY, S. NEWMAN, ***K. S. HOLLOWAY**; Vassar Col., Poughkeepsie, NY

Abstract: Determining the role that opioids have in learned sexual behavior has been complicated by inconsistent methods and divergent interpretations of data. In some cases, opioid blockade, typically with the antagonist naloxone, results in sharp attenuation of sexual conditioned behaviors. In others, naloxone has no effect. A review of this literature suggests that opioids may be particularly important in the maintenance of learned sexual behaviors during periods of non-reinforcement. Previous data from this laboratory supports this conclusion. Naloxone administration facilitates the extinction of Pavlovian sexual conditioned behavior but does not affect acquisition when reinforcement is present. This does not appear to be due to state-dependent learning effects. In a recent experiment, we extended the finding that naloxone facilitates extinction to a partial reinforcement Pavlovian sexual conditioning procedure. An important related question is whether opioid blockade with naloxone would affect the acquisition of a learned sexual response under a Pavlovian partial reinforcement schedule. To explore this, male Japanese quail were presented with an arbitrary visual stimulus paired 50% of the time with copulatory access to a quail hen. One group of subjects received naloxone (30 mg/kg) injections and another vehicle injections prior to each arbitrary stimulus presentation. Unlike the case for continuous reinforcement schedules, naloxone disrupted the acquisition of sexual behavior conditioned on a partial schedule. This finding is consistent with the view that opioids play a key role in the persistence of behavior when sexual reinforcement is not available. Further, it is

functionally important because in natural settings not every encounter with a conspecific, even during the breeding season, will result in copulation.

Disclosures: E. Canty: None. **B. Gleeson:** None. **S. Levy:** None. **S. Newman:** None. **K.S. Holloway:** None.

Poster

PSTR424. Reward and Appetitive Learning and Memory: Acquisition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR424.02/LL5

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIDA Grant (P30) DA048742
NIDA Grant (R01) DA034696
MINDs program (UMN)

Title: Dual Viral Approach allows Selective Expression in VTA Dopamine Neurons and Supports Optogenetic Intracranial Self Stimulation

Authors: J. C. BRENT, IV[1,2], **A. WELTER**[1], E. MITTEN[2], M. FREDERICK[1], A. SOUDERS[1], A. WICKMAN[1], K. WICKMAN[1], *E. MARRON[1];
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Abstract: The use of optogenetic tools has greatly enhanced our ability to probe key neuronal populations and brain regions implicated in reward and addiction. Selective expression of these tools relies on the use of transgenic mice, viral tools, or both. Although transgenic mice have proven to be invaluable to current research, using genetically modified mice can limit experimental design and it increases costs. Th-Cre or Dat-Cre mouse lines have been used extensively in combination with optogenetic tools to study the role of VTA dopamine neurons in reward, learning, mood, motivation, etc. In this study, we propose the use of a dual viral approach that allows selective expression in VTA dopamine neurons in C57BL/6J mice (wild-type, WT mice). Specificity is provided by the expression of Cre under the mTH promoter (AAV8-mTH-Cre) and a cre dependent opsin (AAV8-EF1-DIO-ChR2(H134R)-eYFP). WT mice (n=20, 7-10 wks; 10 males /10 females) underwent stereotaxic surgery for viral injection and fiber optic implantation. Subjects then completed our optical intracranial self stimulation (oICSS) paradigm, which included an 11 days acquisition period, a single stimulation frequency preference test session, and a 5 days extinction. DATCre heterozygotes (n=21, 7-12 wks; 11 males/10 females), served as a control group. These mice underwent the same surgical and behavioral manipulations as the WT mice, however they only received the ChR2-containing virus. Negative controls in the study included WT mice (n=5, 7wks; 3 males/2 females) injected with only the ChR2-containing virus and or WT mice (n=6, 7wks; 3 males/3 females) injected with the mTH-Cre, and a Cre-dependent eYFP control virus. These groups control for the possible cre-independent expression of the opsin and the effects of UV light stimulation,

respectively. In our preliminary results, no sex differences were found in the WT group (2-way ANOVA, p = 0.696); however, they were present in the Dat-Cre group (2-way ANOVA, p = 0.006). Both WT and Dat-Cre mice acquire optical self stimulation at a similar rate, however, WT mice showed a significantly higher number of nose-pokes when compared to DATCre mice (2-way ANOVA, p = 0.001). There were no differences in stimulation frequency preference, discrimination index during the acquisition or stimulation frequency preference test sessions, or the extinction profile. These results demonstrate that this dual viral approach is sufficient to support an oICSS behavioral paradigm. In conclusion, this dual viral approach is a valid alternative to the use of Dat-Cre or TH-Cre mouse lines, and could be extended to be used in other species like rats, hamsters or non-human primates.

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Poster

PSTR424. Reward and Appetitive Learning and Memory: Acquisition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR424.03/LL6

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH/NIMH Grant MH060379
NIH/NIMH Grant MH119467

Title: Dopamine responses in the dorsal striatum do not support the role of reward prediction error

Authors: M. J. KIM[1], ***D. J. GIBSON**[2], D. HU[2], A. MAHAR[2], C. SCHOFIELD[3], P. SOMPOLPONG[4], T. YOSHIDA[2], K. TRAN[2], A. M. GRAYBIEL[2];
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Abstract: Dopamine (DA) transmission is widely interpreted as representing a teaching signal, reward prediction error (RPE), posited by reward learning theory. We employed fiber photometry with G protein-coupled receptor-based dopamine sensors expressed in the central dorsal striatum to measure real-time dopamine release in mice as they learned a series of cue and reward associative learning tasks. Contrary to expectations based on the known RPE-like spiking patterns of DA-containing cells in the substantia nigra, we found several substantial departures from the RPE pattern in the DA release patterns in dorsal striatum. (1) Medially, there was no increased DA release in response to unexpected reward; (2) sustained DA responses developed with learning; (3) laterally, where outcome responses were strong, there was no progressive transfer of the phasic DA response from reward delivery to cue presentation. 63 head-fixed mice were successively trained in single cue conditioning (SC), then visual cue discrimination (CD)

and reversal cue discrimination (RD) based on the laterality of visual stimuli, and finally several different levels of probabilistic reward delivery. During initial SC, a brief burst of DA release was triggered by the visual stimulus at all sites probed. The strongest response occurred during the initial trial of cue presentation, gradually diminishing with training. The peak amplitude at cue onset was higher during SC than in CD and RD. A sustained plateau-like DA release response emerged in tandem with the development of cue discrimination behavior during CD and remained present through the remainder of training. The plateaus lasted throughout all or most of the cue presentation period, bridging the period between cue onset and outcome laterally, and were highest in the most successful learners, almost nil in non-learners. They were most frequently found in the more ventral regions explored. Responses to the outcome (cue off/reward) were sharply different between medial and lateral regions. DA responses to reward were absent or even slightly negative medially, but strong, phasic outcome responses occurred laterally and ventrally. The DA response at outcome remained similar across all stages of training, except for a gradual positive shift over the entire learning period, and - unlike an RPE signal - did not gradually transfer from reward presentation to cue presentation. These findings join a growing literature on heterogeneity in DA signaling during learning and performance.

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Poster

PSTR424. Reward and Appetitive Learning and Memory: Acquisition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR424.04/LL7

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant DA030444
NSF Grant IOS1901546

Title: Assessment of food preference behaviors and orexin neuronal activation in an intermittent feeding model of chronic binge eating behavior

Authors: *H. WARREN, S. JACKSON, K. RICHARDSON;
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Abstract: Binge eating disorder (BED) is characterized by the overconsumption of food over a short period of time. Various models in the literature have been noted as suitable paradigms for binge eating behavior. However, many of these models have not been substantiated over a chronic period (several months) of time as defined by the DSM-V for BED. We seek to validate the longevity and maintenance of palatable food preference across several months using the Boggiano intermittent test for identifying binge eating behavior and assess orexin neuronal activation. Orexin (or hypocretins) hypothalamic peptides are involved in the regulation of food

intake. Female Sprague Dawley rats (n=12/group, 250-300g) were given 30g of high fat/sugar pellets (PF) and the PF intake was measured at 0-hour, 1-hour, and 4-hour time increments. The median 4-hour PF intake was utilized to establish the upper, middle, and lower tertile where animals were characterized as high preference (HP), neutral preference (NP), or low preference (LP) across nine intermittent feeding tests. The HP and LP rats were also tested at 2 and 6 months after the feeding phenotypes were established. Data show that PF intake was significantly higher in HP versus LP rats and this pattern was maintained 2 and 6 months after phenotypes were established (p&lt 0.05). This finding validates the consistency of the feeding phenotypes across several months. This model provides a reliable and consistent paradigm to test preferences for high fat, high sugar diets over a chronic period and its use can help us gain a better understanding of the etiology of binge eating. Studies are underway to determine if there are differences in orexin neuronal activation within the lateral hypothalamus of the rats in each group.

Disclosures: H. Warren: None. **S. Jackson:** None. **K. Richardson:** None.

Poster

PSTR424. Reward and Appetitive Learning and Memory: Acquisition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR424.05/LL8

Topic: G.02. Reward and Appetitive Learning and Memory

Title: Neuropeptide Y neurons in nucleus accumbens promote palatable food memory

Authors: *Y.-B. KIM, D.-H. CHEON, S.-H. JUNG, L. HA, S. BAEK, H. CHOI;
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Abstract: The nucleus accumbens (NAc) has been recognized as a prime center for the reward. However, the mechanism by which neuron in NAc controls food-specific memory, especially for palatable food, remains unknown. Neuropeptide Y (NPY) has long been considered to have a profound orexigenic influence on the entire brain. Among the diverse molecular cell types in NAc, the role of NPY-expressing neurons is not yet fully understood. Here, we demonstrated that NPY neurons in NAc promote palatable food memory. Using calcium imaging, we showed that the NAc^{NPY} neurons respond to eating behavior. This response depended on the palatability of the food. Using optogenetics, we discovered that the NAc^{NPY} neuronal activation significantly increased palatable food (high-fat food) intake. Optogenetic activation of NAc^{NPY} neurons showed food-specific positive valence in real-time place preference test with palatable food context. Furthermore, optogenetic activation of NAc^{NPY} neurons served as an unconditioned stimulus for the formation of palatable food memory. In conclusion, these experiments provide strong evidence that NAc^{NPY} encodes positive memory for palatable food. Our findings could lead to the development of novel therapeutic strategies to prevent and treat obesity and food addiction.

Disclosures: **Y. Kim:** None. **D. Cheon:** None. **S. Jung:** None. **L. Ha:** None. **S. Baek:** None. **H. Choi:** None.

Poster

PSTR424. Reward and Appetitive Learning and Memory: Acquisition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR424.06/Web Only

Topic: G.02. Reward and Appetitive Learning and Memory

Support: KAKENHI 22K03200

Title: Pavlovian trace conditioning produces devaluation-insensitive, habitual form of conditioned response in mice

Authors: *R. SUZUKI, M. KOMURA, Y. KOSAKI;
Waseda Univ., Tokyo, Japan

Abstract: Unlike instrumental behavior which can become goal-insensitive under certain conditions (i.e., habit), the conditioned responses (CRs) engendered in Pavlovian conditioning procedures are usually sensitive to outcome (US) devaluation, regardless of the amount of training (e.g., Holland, 1998; Holland et al., 2008). This is especially true when the conditioned food-cup response (magazine entry) is measured as the target CR. However, the lack of evidence for habitual CRs may reflect the relative paucity of effort to find the phenomenon, rather than its indicating a general principle. Functionally, the expressions of CRs in the absence of a clear representation of sensory-specific features of the US would still be advantageous in some situations. In the current study, we investigated whether placing a temporal gap between the CS and the US would produce devaluation-insensitive habitual CRs using an appetitive trace conditioning procedure in mice. We trained one group of mice (Group Trace; n = 16) with a 4-s auditory CS followed by a 6-s trace interval that ended with the delivery of sucrose solution US. The other group of mice (Group Delay; n = 16) was trained with a 10-s CS that co-terminated with the US. After the mice achieved the acquisition criterion, the US was devalued for half the mice in each group by means of LiCl-induced taste aversion, while the value of the US was maintained for the other half with the unpaired control procedure. The results from the extinction test showed that the magazine CRs in Group Delay were substantially reduced by the US devaluation, whereas those in Group Trace were unaffected. The result indicates that the mice in Group Trace expressed the CRs which did not involve the specific representation of the US. The development of habitual CRs in the trace conditioning parallels the facilitation of instrumental habit formation under the delayed reinforcement paradigm (Urcelay & Jonkman, 2019). The placement of temporal gap between events might generally make it difficult for animals to use the specific sensory representation of the consequent event.

Disclosures: **R. Suzuki:** None. **M. Komura:** None. **Y. Kosaki:** None.

Poster

PSTR424. Reward and Appetitive Learning and Memory: Acquisition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR424.07/LL9

Topic: G.02. Reward and Appetitive Learning and Memory

Support: KAKENHI 19F19810
KAKENHI 22K15199
KAKENHI 22H05160
KAKENHI 22H00432
AMED Brain/MINDS JP19dm0207079
AMED-SICORP JP 22jm0210098

Title: Deciphering Arc-dependent control of circuit dynamics underlying the consolidation of associative memories

Authors: *L. S. BREBNER[1], E. KOYAMA[1], M. OKAMURA[1], Y. KONDO[1], T. YOKOYAMA[2], R. SHIMODA[1], K. OTA[1], R. KIM[1], H. OKUNO[3], H. BITO[1];
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Abstract: Cortical neurons expressing immediate early genes such as Arc following learning experiences encode associative memories. However, how these neurons come to encode associative memories is poorly understood, particularly when memories require repeated learning experiences. Crucially, the activity dynamics that Arc-expressing neurons display during these learning experiences remain unclear. Furthermore, while dorsal medial prefrontal cortex (dmPFC) neurons show altered signal processing in response to associative learning, the contributions of transient and targeted Arc expression in mediating these alterations are unknown.
Aim: Thus, our goal is to characterize the activity dynamics of dmPFC Arc-expressing neurons during associative learning.
Methods: To this aim, we performed longitudinal in vivo 2-photon imaging of the dmPFC in mice as they performed a cue-reward associative learning task. Imaged mice were injected with viral constructs coding for both the calcium indicator XCaMP-G and a red fluorophore driven by the Arc ensemble-marking E-SARE promoter. This method allowed us to track dmPFC Arc-expressing neurons across associative learning sessions and observe their activity patterns at different stages of learning.
Results: We observe that Arc-expressing neurons are progressively mapped to neurons displaying strong task-related activity over learning, resulting in the emergence of an Arc-expressing population with enhanced encoding capacity in late learning. Furthermore, past high Arc expression was not associated with subsequent modulations of activity per se but instead predicted long-term stabilization of activity dynamics.
Conclusion: Together, this suggests a dynamic two-way relationship between neuronal activity and Arc expression during associative learning. Over the course of associative learning, neurons

showing task-related activity are increasingly selected as Arc-expressing ensembles which in turn predicts persistence of their activity dynamics.

Disclosures: L.S. Brebner: None. **E. Koyama:** None. **M. Okamura:** None. **Y. Kondo:** None. **T. Yokoyama:** None. **R. Shimoda:** None. **K. Ota:** None. **R. Kim:** None. **H. Okuno:** None. **H. Bito:** None.

Poster

PSTR424. Reward and Appetitive Learning and Memory: Acquisition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR424.08/LL10

Topic: G.02. Reward and Appetitive Learning and Memory

Support: University of Vermont Department of Psychological Science

Title: Acute stress before instrumental conditioning promotes habit expression in female rats

Authors: *R. J. DOUGHERTY, Z. MOHAMMED, S. VONDOEPP, E. HILTON-VANOSDALL, D. J. TOUFEXIS;
Psychological Sci., Univ. of Vermont, Burlington, VT

Abstract: Much remains unknown about how stress affects the learning and memory processes involved in instrumental conditioning. Stress has been demonstrated to impair goal-directed action, but whether it does so by altering the acquisition of the behavior, or by affecting the expression of the goal-directed or habitual components of the response, has yet to be determined. In this study our objective was to explore the influence of acute stress on action control when administered prior to a minimal amount of acquisition training (Experiment 1), or prior to the test of behavioral expression (Experiment 2). In Experiment 1, 36 young female Long Evans rats received acute restraint stress (n=18) or a control behavioral treatment (n=18) before acquiring a nose-poking response to get a sucrose reward. Following training, the reward was devalued for half of the animals in each group by pairing it with lithium chloride to induce illness. In the critical test, behavioral sensitivity to reward devaluation was assessed in extinction conditions by comparing response rates between devaluation groups in a two-way analysis of variance (ANOVA). Results indicate that exposure to acute stress prior to instrumental conditioning is sufficient to support habitual responding, while the Non-Stressed group remained goal-directed. Experiment 2 was conducted identically to Experiment 1, apart from the installment of acute stress before the extinction test rather than before training. Results from this experiment indicate that rats receiving acute stress before the test responded in a goal-directed manner, as did those who received the control treatment. These data suggest that acute stress may promote habitual control over a minimally trained behavior through an effect on the learning and memory processes engaged during acquisition, but not during retrieval of the response memory. This study extends the contemporary understanding of the interaction between stress and instrumental learning by characterizing the effect of acute stress in female rats – which has not been done

before – when administered before conditioning or before the test. Progress in understanding how and why habits form has implications for understanding how they may go awry in compulsive pathologies such as addiction, obsessive compulsive disorder, and post-traumatic stress disorder. Further research is needed to determine the mechanism by which stress disrupts this conditioning process, and whether a sex difference exists in this regard.

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Poster

PSTR424. Reward and Appetitive Learning and Memory: Acquisition

Location: WCC Halls A-C

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Program #/Poster #: PSTR424.09/LL11

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Fondo de Investigación de la Universidad Anáhuac México 2023. (PI0000154)
Facultad de Psicología, Universidad Anáhuac

Title: Consumption of palatable food persists despite induced aversion during adolescence in rats

Authors: *L. RODRIGUEZ-SERRANO[1,2], M. CHAVEZ HERNANDEZ[3], A. LOPEZ-CASTILLO[4], F. LEYVA-GARCIA[2];
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Abstract: Palatable food (PF) intake can lead to chronic overconsumption and, subsequently, obesity. Furthermore, the classical view distinguishes that homeostatic feeding is necessary for basic metabolic processes and survival, while hedonic feeding (consumption PF) is driven by sensory perception or pleasure. In this regard, food properties have a main role in intake, in particular the palatability of food can lead to the development of obesity in susceptible individuals, produce metabolic syndrome and cognitive impairment, and enhance food intake by hedonic mechanisms that prevail over caloric necessities. The present study aims to evaluate binge-like intake of palatable food after inducing aversion during adolescence in rats. We used thirty male Sprague-Dawley rats (30 days postnatal); all animals were housed individually and had ad libitum access to a standard diet (SD) and water; animal weight and SD food intake were manually recorded every 24h. Rats were distributed on the following treatment: group 1 “CON-PF”, with PF access and no treatment; group 2 “PF-A”, with PF access and administration of LiCl to induce aversion; group 3 “PF-Veh” PF access and administration of vehicle. All groups had 1h access to PF (Oreo® Cookies Nabisco®) according to diet protocol intermittent with one-day access, one-day no-access. PF and SD caloric intake, and binge eating criterion (defined as

consuming ≥20% of total caloric intake per day during the 1h access to PF) were analyzed. Our results showed that PF-A reduced consumption of PF after inducing aversion; nevertheless, consumption increased rapidly and normalized with the other two groups. Overall, these results show that persistence in intake of PF during adolescence despite inducing aversion and that it can lead to chronic overconsumption in conditions of non-homeostatic feeding.

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Poster

PSTR424. Reward and Appetitive Learning and Memory: Acquisition

Location: WCC Halls A-C

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Program #/Poster #: PSTR424.10/LL12

Topic: G.02. Reward and Appetitive Learning and Memory

Support: University of Richmond
Stony Brook University
University of Massachusetts, Boston
University of Massachusetts Chan Medical School

Title: Preliminary Assessments of Flexible Cognition and Stress Hormone Profiles in Wild Brown Mouse Lemurs (Microcebus rufus)

Authors: *K. LAMBERT[1], R. G. HUNTER[2], P. WRIGHT[3], A. RAZAFINDRAKOTO[4], H. RAVELONJANAHARY[4], H. HESSELN[5], E. TRONICK[6], M. KENT[7], B. CROCKETT[1], J. DORION[1], S. HARTVIGSEN[1];
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Abstract: Mouse lemurs represent a valuable nonhuman primate model for Alzheimer's Disease (AD) due to shared age-related cognitive and neuropathology responses with humans (Piferri et al., 2019; Gary et al., 2018; Ho et al., 2021). Consequently, this small primate offers enhanced translational potential compared to shorter-lived rodents. In contrast to captive mouse lemurs, wild brown mouse lemurs (i.e., up to 8 years of age) failed to exhibit physical signs of senescence (Zohdy et al., 2014) suggesting that neurocognitive differences may exist between captive and wild aging mouse lemurs. In the current study, we assessed cognitive performance in wild brown mouse lemurs trapped near Madagascar's Ranomafana National Park. All animals were assumed to be young adults based on external evaluations; however, specific aging assessments were not conducted. Fecal samples were collected in male (n=9) and female mouse lemurs (n=5) to assess stress hormone profiles (cortisol and DHEA) at pre- (males and females) and post-trap time points (only males). For the flexible cognition assessment, male lemurs were

habituated to a dynamic foraging task consisting of three food wells that changed positions, each with a distinct visual pattern associated with a specific food value (i.e., specific pieces of banana). At appx 2100 hrs each evening, lemurs were assessed in habituation and test trials for three days for Group 1 (n=4 males) and one less test day for Group 2 (n=5). Preliminary results indicated significant sex differences in pre-trap DHEA/CORT ratios [x=0.18 and 0.94 for females and males, respectively; p=.04; higher ratios are correlated with resilience (Kent et al., 2018)]. Overall, 66% of the lemurs interacted with the dynamic foraging panel with minimal habituation; further, lemurs that interacted with the foraging task also solved a different grasping problem-solving task. Interestingly, the second cohort of animals failed to interact with the foraging panel during the habituation phase. This effect was likely due to relatively sparse foliage in the habitat compared to Cohort I, an effect that was reversed when sufficient foliage was added to the habitat. Hair samples are currently being assessed for integrative endocrine profiles. Although considerable variability in endocrine and behavioral responses was observed, no significant correlations were detected. These initial findings indicate that cognitive assessments can be conducted in wild mouse lemurs temporarily housed in ecologically appropriate habitats-- informing the developmental trajectory of cognitive performance in these small primates as they are evaluated for translational research opportunities.

Disclosures: **K. Lambert:** None. **R.G. Hunter:** None. **P. Wright:** None. **A. Razafindrakoto:** None. **H. Ravelonjanahary:** None. **H. Hesseln:** None. **E. Tronick:** None. **M. Kent:** None. **B. Crockett:** None. **J. Dorion:** None. **S. Hartvigsen:** None.

Poster

PSTR424. Reward and Appetitive Learning and Memory: Acquisition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR424.11/LL13

Topic: G.02. Reward and Appetitive Learning and Memory

Support: University of Richmond

Title: Behavioral, hormonal and neuroanatomical effects of appetitive anticipation in male and female rats.

Authors: *S. C. HARTVIGSEN[1], M. HOOPER[1], O. HARDING[1], E. WIENER[1], Y. SHATALOV[1], I. TOME[1], A. SAGE[1], L. BASTIAN[1], E. BARRINGER[1], C. OWENS[1], P. LUBY[1], B. CROCKETT[1], M. KENT[2], K. G. LAMBERT[1];
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Abstract: Developing more efficacious interventions for mental health disorders requires a better understanding of the long-term neural and behavioral impacts of emotionally salient events. While the effects of negative events have been widely studied, less is known regarding the effects of appetitive events on longstanding neural and behavioral changes. Our lab is interested in understanding how anticipation of positive events (“appetitive anticipation”)

influences stress resiliency, learning and memory. Prior experiments in our lab examined this by comparing stress-resilient behaviors, hormones and neuroanatomical markers in female Long Evans rats exposed to either five weeks of positive events preceded by anticipation or to five weeks of positive events that were not anticipated. Results from these experiments suggest appetitive anticipation may affect motivational and exploratory behaviors as well as receptor expression in the brain, though it remains unknown if similar effects are seen in males. Thus, the current study examines how appetitive anticipation in both male and female rats influences stress-related behaviors, hormones and receptor expression. Because optimism is associated with positive health outcomes in humans and animals, we also examined if anticipation of positive events affects optimistic cognitive bias, as determined by preference for a high-value or low-value reward in response to an ambiguous cue. We found a trend towards significance in which males exposed to appetitive anticipation chose a high-value reward more than males receiving unanticipated positive events (X^2 (1, N=11) = 3.592, p=0.0581), though future experiments are needed to examine if the high-value reward accurately reflects an optimistic cognitive bias. Comparing concentrations of corticosterone (CORT) before and after five weeks of anticipated or unanticipated positive events revealed a main effect of time (F(1,16) = 4.801 , p = 0.0436, N=24 rats total), where the direction of the effect was an increase in CORT in all groups except males exposed to appetitive anticipation, suggesting anticipatory training may blunt CORT increases in a sex-dependent manner. Because receptors can mediate effects of fluctuating stress hormones, ongoing analyses are examining glucocorticoid and mineralocorticoid receptor expression in brains of male and female rats exposed to anticipated versus unanticipated positive events. Overall, results from this study suggest increasing anticipation of positive experiences may offer a novel therapeutic avenue for improving stress resiliency and mental health.

Disclosures: S.C. Hartvigsen: None. **M. Hooper:** None. **O. Harding:** None. **E. Wiener:** None. **Y. Shatalov:** None. **I. Tome:** None. **A. Sage:** None. **L. Bastian:** None. **E. Barringer:** None. **C. Owens:** None. **P. Luby:** None. **B. Crockett:** None. **M. Kent:** None. **K.G. Lambert:** None.

Poster

PSTR424. Reward and Appetitive Learning and Memory: Acquisition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR424.12/LL14

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH grant DA054274

Title: Regulation of Pavlovian conditioning by stress and amphetamine: Learning of sensory cues with no predictive value

Authors: S. KARIMIHAGHIGHI[1], B. PAHLAVAN[2], H. R. WESSEL[1], M. R. DREW[1], G. A. RUEBEN[3], *H. MORIKAWA[1];
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Abstract: The ability to associate predictive environmental events with rewarding outcomes is essential for adaptive behaviors, while excessive learning of the incentive value of environmental cues can drive maladaptive behaviors, such as addiction to drug and non-drug rewards. It is well established that sensory cues need to precede reward for effective learning of cue-driven responses. However, it is not clear how daily experience regulates the cue-reward timing-dependence of learning. In this study, we investigated the impact of stress and amphetamine on timing dependence of reward learning in male rats. We employed a Pavlovian conditioned approach paradigm, where light cue at the food cup was paired with food pellet delivery, and the temporal relationships between the cue and food delivery were varied. To examine the effect of acute stress, male rats underwent 30-min social defeat stress. After a 10-min interval, stressed rats (n=11) and handled controls (n=12) underwent a single conditioning session (30 trials). While both handled and defeated rats developed comparable cue responses (i.e., food cup entry in response to the cue) with 5 sec cue-reward delay, only defeated rats developed cue responses when the cue onset and food delivery were simultaneous during conditioning. Neither group developed cue responses if food preceded the cue onset. Overall, defeat stress caused a leftward shift in cue-reward timing-dependence of conditioning and relieved the requirement of cue-reward delay. Acute defeat stress had no effect on food consumption during conditioning. Next, we tested different doses of amphetamine (0.1, 0.3, 1, 5 mg/kg, i.p.) injected ~15 min before each simultaneous conditioning session. Although saline-injected control rats failed to develop conditioned cue responses, all doses of amphetamine enabled simultaneous conditioning after three conditioning sessions (n=7-15 in each group). Interestingly, 5 mg/kg amphetamine virtually eliminated food consumption during conditioning even though conditioned cue responses were observed, suggesting that rats can learn using the pellet delivery sound learned previously. Finally, 1 mg/kg amphetamine caused robust rise in norepinephrine levels in the ventral tegmental area (VTA) measured with microdialysis (n=4), suggesting the potential role of norepinephrine, which has been shown to regulate the timing dependence of glutamatergic synaptic plasticity in the VTA. In conclusion, our results demonstrate that acute exposure to stress or amphetamine enables learning of the valence of sensory cues presented at the same time as reward, i.e., learning of cues that do not predict reward availability.

Disclosures: S. Karimihaghighi: None. **B. Pahlavan:** None. **H.R. Wessel:** None. **M.R. Drew:** None. **G.A. Rueben:** None. **H. Morikawa:** None.

Poster

PSTR424. Reward and Appetitive Learning and Memory: Acquisition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR424.13/LL15

Topic: H.10. Human Learning and Cognition

Support: Mori Manufacturing Research and Technology Foundation, Scholarship for Doctoral Program

Title: Psychophysiological Examination of Cognitive Models Interpreting User Thought Processes Leading to Product Acceptance

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Abstract: At a time when material wealth is saturated and a transition to a sustainable society is required, innovation is needed to create products with new functions that have never been seen before. Although there have been industrial developments that should be called innovations in the past, the process of discovery and acceptance often took a circuitous route; people did not easily accept and evaluate new functions that they had not yet encountered. In order for the value of a new feature of a product to be properly understood, its design must take into account the thought process leading to user acceptance. Models for evaluating user acceptance proposed by previous studies, such as the Technology Acceptance Model (Davis, 1989), are all static models that use subjective evaluation, and it is difficult to apply them to design in the early stages of development. When designing a product, it is necessary to include appropriate information in the product itself and its documentation that provides clues for understanding the functionality. Therefore, it is necessary to clarify the modality, presentation method, and the process of information required in the thought process leading to the user's comprehension. We aim to construct a theory that can be applied to product design based on the insights of cognitive neuroscience, and to model the thought processes that lead to a deeper comprehension and acceptance of product functions by users who are first given the product. In this study, we prepared highly professional tools or gadgets used for only a small part of daily life, and let users who used them for the first time observe the products until they understood their functions as experimental participants. We measured the EEG, eye gaze, and pupil diameter of the participants during the products observation. Whenever the participants became aware of a product feature during the observation, they were asked to write it down graphically and report it to us. These results showed that in addition to the state of "having accepted the product", which has been expressed by conventional models, we were able to capture the changes that lead to the acceptance of the product. In this presentation, we report on the characteristics of the biometric signals at each stage of the thought process, referring to the product characteristics that triggered acceptance, and show the possibility of constructing a model.

Disclosures: **N. Yamada:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Mori Manufacturing Research and Technology Foundation. **K. Ueda:** A. Employment/Salary (full or part-time):; CENTAN Inc.. **K. Nagato:** None. **M. Nakao:** None.

Poster

PSTR425. Reward and Appetitive Learning: Dopaminergic Circuit

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR425.01/LL16

Topic: G.02. Reward and Appetitive Learning and Memory

Title: Top down circuitry from anterior insular cortex to vta dopamine neurons modulates reward related memory

Authors: *E. ORTIZ[1], J. LUIS-ISLAS[3], R. GUTIERREZ[4], F. TECUAPETLA[5], F. BERMÚDEZ-RATTONI[2];
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Abstract: The insular cortex (IC) has been linked to the processing of interoceptive and exteroceptive signals associated with maintaining addictive behavior. However, whether the IC modulates the acquisition of affective reward states related to drugs by direct top-down connectivity with the ventral tegmental area (VTA) dopamine neurons is unknown. Our results demonstrated that photostimulation of the VTA terminals from the anterior insular cortex (aIC) pathway induced rewarding contextual memory, modulated VTA activity, and led to dopamine release within the VTA. Using neuronal recordings and neurochemical analysis, we confirmed the functional top-down organization of the circuit by transsynaptically and anterogradely tagging aIC presynaptic neuronal bodies with AAV1-Cre-dependent expression and identifying VTA recipient neurons. Furthermore, systemic administration of amphetamine altered the excitability of VTA neurons modulated by the aIC projection, enhancing contextual rewarding behavior. Our study reveals a circuit that plays a pivotal role in developing and retaining reward-related contextual memory, providing insight into the neurobiological basis of addictive behavior, and opening possibilities for developing therapeutic strategies for addiction.

Disclosures: E. Ortiz: None. **J. Luis-Islas:** None. **R. Gutierrez:** None. **F. Tecuapetla:** None. **F. Bermúdez-Rattoni:** None.

Poster

PSTR425. Reward and Appetitive Learning: Dopaminergic Circuit

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR425.02/LL17

Topic: G.02. Reward and Appetitive Learning and Memory

Support: R01 DC017311
U19 NS113201

Title: Temporal difference learning models explained dopamine activities during contingency degradation

Authors: *L. QIAN[1,2], M. BURRELL[1,2], J. HENNIG[2,3], V. N. MURTHY[1,2], S. GERSHMAN[2,3], N. UCHIDA[1,2];

[1]Dept. of Mol. and Cell. Biol., [2]Ctr. for Brain Sci., [3]Dept. of Psychology, Harvard Univ., Cambridge, MA

Abstract: Studies on animal learning have shown that the efficacy of both associative learning and responding depends on contingency, the extent to which the likelihood of an outcome changes by a presentation of a stimulus. The mesolimbic dopamine (DA) system, vital in associative learning, is believed to signal reward prediction errors which resemble temporal difference (TD) errors in reinforcement learning. Yet, how DA signals are modulated by contingency remains poorly understood. We examined how contingency changes affect conditioned responding and DA responses in mice (n=15). Mice were conditioned on two odors: one probabilistically rewarded (Odor A; 75%); the other, unrewarded (Odor B). Subsequently, the cue-reward contingency was degraded by delivering uncued reward. To control for possible satiety effects, a separate group of mice received additional rewards cued by a distinct odor (Odor C; cued-US group). Anticipatory licking decreased in Odor A trials in the degradation cohort ($p < 0.001$), while no significant change was observed in the cued-US group ($p=0.25$). DA axonal signals, recorded in the ventral striatum using photometry, tracked the contingency. The response to Odor A developed quickly during conditioning sessions ($p < 0.001$), and attenuated during degradation ($p < 0.01$). In contrast, the cued-US group did not exhibit a decrease ($p=0.14$). We next examined if DA responses in contingency degradation could be explained as TD errors. We found nearly all aspects of DA responses were explained by a TD learning model that harnesses a state representation reflecting the task structure, transitioning between inter-stimulus and inter-trial intervals (ISIs and ITIs, respectively). A recently proposed retrospective learning model, which claimed to replicate DA responses during contingency degradation (ANCCR; Jeong et al., 2022; Garr et al., 2023), failed to explain these responses, incorrectly predicting DA cue responses change identically in both degradation and cue-US groups as the retrospective contingency (P(Odor A | reward)) was the same in both groups. Additionally, we found that recurrent neural networks (RNNs), trained using TD learning, recapitulated nearly all aspects of DA responses. Trained RNNs developed state transition dynamics and the value function that matched those in the TD learning model with ISI and ITI states. Both models show DA cue response drops in degradation due to heightened ITI value predictions, reducing TD error. Our findings highlight TD learning can fully account for DA responses, if task states are carefully considered, and provide insights into the neural mechanisms underlying contingency learning.

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Poster

PSTR425. Reward and Appetitive Learning: Dopaminergic Circuit

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR425.03/LL18

Topic: G.02. Reward and Appetitive Learning and Memory

Title: Cued reward memories bias cognitive strategies: role of orbitofrontal circuits

Authors: *S. PETERSON[1], J. CHAVIRA[2], A. MAHERAS[3], R. KEIFLIN[2];
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Abstract: Reward-predictive cues can influence choice behavior and promote the pursuit of specific reward outcomes. This influence of cued reward memories on choice processes is often studied using outcome-specific Pavlovian to Instrumental Transfer (PIT). In this paradigm, stimuli established as Pavlovian predictors of specific outcomes can bias/invigorate specific instrumental actions. However, traditional PIT experiments fail to account for the fact that pursuit of specific outcomes often requires specific cognitive strategies (e.g. rule selection, selective attention, flexible working memory). The influence of cued reward memories on cognitive strategy selection has received less attention, and the neural circuits mediating this effect remains largely unknown. To address this gap in knowledge, we adapted the traditional PIT paradigm in rodents. In a first phase, rats acquired different cognitive strategies for decision-making in an instrumental sequence. Specifically, in separate blocks, they learned that a delayed nonmatching to sample (dNMTS) strategy was rewarded by outcome 1 (sample lever press - nosepoke - nonsampled lever press : O1) and that a visual cue tracking strategy was rewarded by outcome 2 (sample lever press - nosepoke - cued lever press : O2). In a second phase, two distinct auditory cues were established as Pavlovian predictors of the two outcomes (S1-O1; S2-O2). Finally, in nonrewarded probe tests we measured how the presentation of these Pavlovian cues biased the selection of a cognitive strategy. We found that Pavlovian predictors promoted the adoption of the strategy that corresponded to the cued outcome. Specifically, S1 promoted dNMTS strategy while S2 promoted a visual cue tracking strategy. This new behavioral paradigm will be used in combination with chemogenetic silencing to investigate the role of the orbitofrontal cortex and its projections to the prelimbic cortex in mediating cued outcome memories and their influence on cognitive strategy selection.

Disclosures: **S. Peterson:** None. **J. Chavira:** None. **A. Maheras:** None. **R. Keiflin:** None.

Poster

PSTR425. Reward and Appetitive Learning: Dopaminergic Circuit

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR425.04/LL19

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIDA Drug Supply Program
TCU/CSE Dean's Opportunity Fund
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Title: Frustrative nonreward: Role of the accumbens-globus pallidus connections in reward downshift

Authors: *M. R. PAPINI, C. HAGEN, P. OGALLAR;
Psychology, Texas Christian Univ., Fort Worth, TX

Abstract: Reward downshift is used as a model of frustrative nonreward, a negative valence trait related to the response to loss, psychological pain, and frustration. Most neurobiological research on reward downshift has centered on the amygdala, but recent results implicate the basal ganglia. Activation of the nucleus accumbens (NAc) and globus pallidus externus (GPe) with excitatory DREADDs (designer receptors exclusively activated by designer drugs) enhanced and reduced consummatory suppression, respectively, after a 32-to-2% sucrose downshift (Guarino et al., Neurobiol Learn Mem, 2023). We hypothesized that these effects were mediated by the direct NAc-GPe connection and tested this hypothesis using a double-infection technique to selectively excite the pathways connecting the NAcàGPe and NAc-GP internus (GPi). Male Wistar rats (90 days old at the start of surgery) were used. Viral vector constructs carrying the excitatory Cre-dependent DREADD [AAV8-hSyn-DIO-hM3Di-mCherry] containing a red fluorescent reporter (mCherry) and a DNA fragment for the engineered muscarinic receptor M3 were infused bilaterally into the NAc (departure area). Retrogradely transported viral vector constructs (AAV9-hSyn-eGFP-Cre) carrying the Cre protein and a green reporter (eGFP) were infused bilaterally into the GPe or GPi (destination areas). Thus, only projection neurons containing the Cre protein expressed the excitatory DREADDs in the NAc, as assessed by fluorescent microscopy. Groups infused with a control virus were also included. All animals received systemic injections of clozapine N-oxide or vehicle during four 32-to-2% sucrose downshift sessions. In contrast to our hypothesis, activating the NAc-GPe pathway did not affect consummatory behavior after reward downshift relative to vehicle and virus controls. However, activating the NAc-GPi pathway enhanced consummatory suppression after the downshift. Thus, the previously described effect of NAc activation was at least in part related to its direct connection with the GPi. There was no evidence that activation of these pathways had motor effects in the open field. Unlike previous studies that focused on single brain regions, our approach uncovered for the first time the importance of specific brain pathways in frustrative nonreward.

Disclosures: **M.R. Papini:** None. **C. Hagen:** None. **P. Ogallar:** None.

Poster

PSTR425. Reward and Appetitive Learning: Dopaminergic Circuit

Location: WCC Halls A-C

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Program #/Poster #: PSTR425.05/LL20

Topic: G.02. Reward and Appetitive Learning and Memory

Support: This work was supported by the DICBR of the NIAAA [ZIA AA000455 to AJK].

Title: Medial septum glutamate neurons play a role in strategy switching potentially via modulating nucleus accumbens dopamine responses to reward-related stimuli

Authors: *A. KESNER, S. RAMOS-MACIEL, N. WESTCOTT;
Unit on Motivation and Arousal, NIAAA, Rockville, MD

Abstract: The septum was first region discovered by Olds and colleagues to support electrical intracranial self-stimulation in the rat. Further studies by Heath in the 1970s showed humans will similarly press a button to earn intra-septal electrical stimulation. Despite these landmark studies, further interest in the septum, in particular the medial septum (MS), as a locus for reward related behaviors remained limited. We previously found that mice will lever press to earn optogenetic stimulation of the MS, and in particular MS glutamate neurons (MS-GLUn), and MS-GLUn in turn project to the VTA to influence dopamine (DA) release in the nucleus accumbens (NAc) (Kesner et al., 2021). Little else is known about the role of MS-GLU neurons during natural reward-seeking behaviors. To address this knowledge gap, we recorded MS-GLUn population activity using GCaMP7F and fiber photometry techniques while mice performed various operant and Pavlovian reward-seeking behaviors. We found that MS-GLUn indeed respond differentially to reward-related stimuli (e.g., active vs inactive lever presses, reward consumption, and reward predictive cues). We next modulated MS-GLUn activity using a chemogenetic approach (Gi and Gq DREADDs, or mCherry control), and found that enhancing MS-GLUn excitability increased the rate that mice incorporated new information to obtain goals, i.e., strategy switching. Since we know optogenetic stimulation of MS-GLUn can increase NAc-DA, we next hypothesized that the effect on strategy switching behavior from chemogenetic modulation of MS-GLUn may be driven by resultant changes in NAc-DA during these tasks. We recorded NAc-DA via fiber photometry of dLight1.3b during the same operant and Pavlovian strategy switching behaviors while MS-GLUn were chemogenetically manipulated. We observed differences in NAc-DA during these tasks dependent on chemogenetic modulation of MS-GLUn that appears to correspond to NAc-DA responses to new reward related cues/stimuli. These findings are an important step in understanding the role of this understudied population of neurons in an understudied brain region related to reward and motivational processes, and could lead to novel therapeutic interventions for treating psychiatric disorders related to maladaptation in motivated behaviors.

Disclosures: **A. Kesner:** None. **S. Ramos-Maciel:** None. **N. Westcott:** None.

Poster

PSTR425. Reward and Appetitive Learning: Dopaminergic Circuit

Location: WCC Halls A-C

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Program #/Poster #: PSTR425.06/LL21

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Stanford NeuroChoice Initiative Pilot Award to NE
Simons Foundation Bridge to Independence Award to NE

Title: Striatal integration of inverse dopamine and serotonin signals gates learning

Authors: *D. F. CARDOZO PINTO[1], M. B. POMRENZE[1], M. Y. GUO[1], B. S. BENTZLEY[2], N. ESHEL[1], R. C. MALENKA[1];
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Abstract: The neuromodulators dopamine (DA) and serotonin (5-hydroxytryptamine; 5HT) are powerful regulators of associative learning. Similarities in the activity and connectivity of these neuromodulatory systems have inspired competing models of how DA and 5HT interact to drive the formation of new associations. However, these hypotheses have yet to be tested directly because it has not been possible to precisely interrogate and manipulate multiple neuromodulatory systems in a single subject. Here, we establish a double transgenic mouse model enabling simultaneous genetic access to the brain's DA and 5HT systems. Anterograde axon tracing revealed the nucleus accumbens (NAc) to be a putative hotspot for the integration of convergent DA and 5HT signals. Simultaneous recordings of DA and 5HT input activity in the NAc posterior medial shell revealed that DA axons are excited by rewards while 5HT axons are inhibited. Optogenetically blunting DA and 5HT reward responses simultaneously blocked learning about a reward-predictive cue. Optogenetically reproducing both DA and 5HT responses to reward, but not either one alone, was sufficient to drive the acquisition of new associations. Altogether, these results demonstrate that striatal integration of inverse DA and 5HT signals is a crucial mechanism gating associative learning.

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Poster

PSTR425. Reward and Appetitive Learning: Dopaminergic Circuit

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR425.07/LL22

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant R01037327

Title: Parallel dopamine circuits from the VTA to Nucleus Accumbens and their response to natural rewards and opioids

Authors: *O. CULVER, IV;
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Abstract: Dopamine (DA) neurons in the VTA play key roles in motivation and addiction, including addiction to opioids. However, these roles are increasingly recognized to be heterogeneous, as prior studies showed that VTA DA neurons projecting to medial vs. lateral nucleus accumbens respond differently to motivational stimuli. Prior studies also showed that medial accumbens-projecting dopamine neurons exhibit greater c-Fos activation by heroin as compared to lateral accumbens-projecting DA neurons. However, these prior studies did not examine other opioids such as fentanyl nor did they examine withdrawal effects, and also did not examine whether DA release mirrors these c-Fos patterns. Therefore, we examined fentanyl-induced (single 0.1 mg/kg sc dose) c-Fos in VTA neurons that were retrogradely labeled from the medial and lateral nucleus accumbens. We also examined fentanyl-induced DA release in medial and lateral accumbens using fluorescent DA sensors. Our c-Fos experiments extended previous findings, as medial accumbens-projecting DA neurons had significantly higher c-Fos activation as compared to lateral accumbens-projecting DA neurons after a single fentanyl dose. Fentanyl also increased accumbens DA differentially between animals, but further analysis is required to correlate these differences with anatomical location.

Disclosures: O. Culver: None.

Poster

PSTR425. Reward and Appetitive Learning: Dopaminergic Circuit

Location: WCC Halls A-C

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Topic: G.02. Reward and Appetitive Learning and Memory

Support: VA CDA IK2BX006125
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VA CDA IK2BC003308

Title: Altered gamma oscillations in a prefrontal striatal network associated with social preference impairments after traumatic brain injury

Authors: *M. SALIMI[1,2], M. NAZARI[3], H. AFSHAR[1,2], N. LEE[1,2], K. MOKHA[1,2], T. WALKER[1,2], M. FRANCOEUR[1,2], D. RAMANATHAN[1,2];
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Abstract: Background and objectives: Traumatic Brain Injury (TBI) leads to cognitive and behavioral, impairments, including social preference deficits. Physiological studies have identified the mediation of gamma frequency oscillations for social behaviors. This study aims to investigate how frontal TBI affects cortical gamma frequency activity and coordination across distributed networks resulting in chronic social preference impairments. We simultaneously recorded local field potentials (LFP) from 32 brain areas: an approach study advantageous to studying distributed brain networks and offers translational potential to human studies.
Materials and Methods: We compared social preference behavior and concurrent data across two groups of Male Long-Evans rats. One group received a severe frontal TBI (n = 12 TBI rats) and control (n=10 rats). One week after we implanted 32 electrodes in regions focal, and distal to the site of injury. 3 weeks post-injury using three-chamber social interaction maze locomotor activity and social preference were measured. LFP analysis performed offline with Matlab software included Power spectral density (PSD) and coherence connectivity calculations to assess frequency bands and brain areas supporting social preference. Histological analysis was performed to measure tissue damage and verify electrode placement.**Results:** Frontal TBI decreased social preference and increased overall locomotor activity ($F_{1,38} = 29.8$, $p < 0.0001$). TBI animals spent more time and made more visits to the non-social chamber compared to controls ($t_{(38)} = 3.23$, $p = 0.002$). TBI increased locomotor activity in the ($F_{1,38} = 5.73$, $p = 0.02$). Power Spectral Density showed a deficit in brain regions focal (prelimbic cortex), local (anterior lateral motor cortex, anterior insula), and distal (nucleus accumbens shell) to the injury site. Sham animals exhibited elevated prefrontal-ventral striatum coherence of gamma frequency oscillations (25-60 Hz) in the social chamber compared to the non-social chamber. TBI animals showed less pronounced differences in gamma coherence between the social and non-social chambers compared to sham animals.**Conclusion:** This study demonstrates that damage to the frontal cortex reduces social preference driven by alterations in gamma oscillations within frontostriatal networks. Even areas beyond the focal injury site showed gamma frequency deficits that persisted weeks after the injury. Understanding the impact of gamma oscillation disruptions on social preference deficits in TBI can provide valuable insights for developing targeted interventions to improve social outcomes in individuals with TBI.

Disclosures: M. Salimi: None. **M. Nazari:** None. **H. Afshar:** None. **N. Lee:** None. **K. Mokha:** None. **T. Walker:** None. **M. Francoeur:** None. **D. Ramanathan:** None.

Poster

PSTR425. Reward and Appetitive Learning: Dopaminergic Circuit

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR425.09/LL24

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NSERC Grant RGPIN-2016-06703

Title: Divergence between the rewarding effect of MFB stimulation and photometric measurement of dopamine release

Authors: *J. VOISARD, Z. MOHAMMADREZAEE, A. ARVANITOGIANNIS, P. SHIZGAL;
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Abstract: Rodents work vigorously to earn electrical stimulation of the Medial Forebrain Bundle (MFB). The prevalent account attributes the highly rewarding effect of MFB stimulation predominantly, if not entirely, to activation of midbrain dopamine neurons. We challenged this widely held view by coupling fiber-photometric recording of dopamine release to behavioral measurement of reward effectiveness. At multiple pulse frequencies, stimulation current was varied to drive responding from asymptotic to negligible levels. Dopamine release in the Nucleus Accumbens (NAc) was measured using the molecular sensor, GRAB-DA2m, both during self-administered and passively received MFB stimulation. We determined the current required to hold either behavior or dopamine release constant at each pulse frequency. The resulting behavioral and chemometric trade-off functions were similar at low pulse frequencies but diverged sharply at higher ones. These results parallel those obtained previously by means of fast-scan cyclic voltammetry. Together with recent optogenetic findings, the photometric and voltammetric results support a model in which midbrain dopamine neurons and the directly activated, non-dopaminergic, MFB neurons subserving self-stimulation project in parallel to the behavioral final common path. If so, identifying the non-dopaminergic MFB neurons and their efferents is crucial to achieving a comprehensive account of brain reward circuitry.

Disclosures: J. Voisard: None. **Z. Mohammadrezaee:** None. **A. Arvanitogiannis:** None. **P. Shizgal:** None.

Poster

PSTR425. Reward and Appetitive Learning: Dopaminergic Circuit

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Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR425.10/LL25

Topic: G.02. Reward and Appetitive Learning and Memory

Support: PSC-Cuny

Title: Phosphorylation of ribosomal protein S6 as a marker of reward identity and time prediction errors throughout the rat brain

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Abstract: The concept of a “reward prediction error (RPE)” is central to our understanding of how learning and neuroplasticity takes place. While RPEs are key to many influential models of associative learning, most neuroscientists define this in terms of a mismatch between obtained and anticipated values of reward. However, distinct sensory and temporal features of reward can also regulate learning at the behavioral level of analysis, although less neuroscience research has

focused on RPEs defined in these terms. To what extent does learning about different reward features rely on the same or different neural processes? Some data suggest that different neural systems may be required for learning about reward identity and time (e.g., Delamater et al, 2018). Here, we used immunofluorescence techniques to begin exploring the neural systems invoked by reward identity and time RPEs during a Pavlovian magazine approach task. Across two experiments, rats were trained to associate two stimuli with reward, followed by an unexpected reversal of the stimulus-outcome contingencies. During the critical test session, control groups were given stimuli and rewards without reversal of those contingencies. Brains were processed for phosphorylation of ribosomal protein S6 (p-rPS6; a cytoplasmic marker of cell activity) and tyrosine hydroxylase (dopamine precursor) 20 min after this test session, and sections were analyzed in several cortico-striato-limbic and mid-brain regions. In our first experiment, one 10 s stimulus was paired with grain pellets and another with liquid sucrose rewards at stimulus offset. In the second study, two 60 s stimuli were paired with grain pellets but for one those pellets were delivered at 15 s and for the other at 60 s after stimulus onset. The number of dopaminergic and non-dopaminergic cells activated, as well as their activation intensities, in each brain region was measured following the test session involving a switch in the stimulus-outcome contingencies or not to assess which brain regions processed reward identity and/or time RPEs. Our findings suggest that reward identity and time RPEs recruit distinct neurobiological circuits, with the nucleus accumbens playing a significant role in identity RPEs and nigrostriatal, VTA, and hypothalamic-BLA pathways playing a role in reward time RPEs.

Disclosures: D. Siegel: None. **N. Hussein:** None. **A.R. Delamater:** None.

Poster

PSTR425. Reward and Appetitive Learning: Dopaminergic Circuit

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Program #/Poster #: PSTR425.11/LL26

Topic: G.02. Reward and Appetitive Learning and Memory

Support: The Brain and Behavior Research Foundation NARSAD Young Investigator Award
Mary E. Groff Foundation
Trinity University Murchison and Department of Biology funds

Title: Characterizing the Interhemispheric Projections from the Pedunculopontine Tegmental Nucleus to the Substantia Nigra Pars Compacta Dopaminergic Neurons

Authors: *A. BALDWIN, H. ARIAS, N. FERNANDEZ, M. NEELALA, M. RODRIGUEZ, A. ELFEZOUATY, I. KHETARPAL, D. PENNY, C. HOFFMAN, L. MUZYKA, G. M. BEAUDOIN, III;
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Abstract: The neurotransmitter dopamine has been shown to be involved in motivation and reward, notably in the nigrostriatal dopaminergic pathway. Within this pathway, our research focuses on the synapse between the midbrain regions pedunculopontine tegmental nucleus (PPN) and substantia nigra pars compacta (SNc). The PPN is unusual as it sends both ipsilateral and contralateral projections, unlike other brain structures that generally confine their innervations to either ipsilateral or contralateral. To understand the nature of these inputs, the current study focuses specifically on the glutamatergic excitatory projections and the GABAergic inhibitory projections within the PPN-SNc synapse using electrophysiological and confocal microscopic methods. These projections have not been previously studied due to a lack of tools. The goal of this study is to investigate how hemisphere-specific inputs differ in their synaptic strength. Optogenetics allows us to characterize the ipsilateral and contralateral PPN inputs to SNc both functionally and structurally. We use unilateral stereotaxic surgery to deliver virus expressing a depolarizing light-gated ion channel, Channelrhodopsin (ChR2), which is tagged with either a yellow or red fluorescent protein. The virus labels glutamatergic and GABAergic neurons in the PPN. Thus, we use electrophysiology to characterize the light-activated, PPN-innervated synapses to SNc dopaminergic neurons either ipsilateral or contralateral to the injection site. Finally, the PPN innervation of the SNc is further confirmed using imaging by immunostaining with markers for dopamine neurons, GABAergic synapses, and glutamatergic synapses. Confocal images were collected to quantify the macroscopic distribution of any ipsilateral and contralateral inhibitory projections in the SNc. The results of these anterograde tracing technique are being corroborated with retrograde tracing by using retrograde bead injections into the SNc to label projecting neurons in the PPN.
Current findings suggest that only the SNc ipsilateral to PPN receives GABAergic inputs, while both ipsilateral and contralateral SNc receive glutamatergic inputs from PPN. The PPN exhibits stronger glutamatergic innervations ipsilaterally to SNc DA neurons than contralaterally. Our research will permit greater clarity for future studies which seek to understand the underlying neural network and synaptic-level processes of the nigrostriatal dopaminergic pathway.

Disclosures: **A. Baldwin:** None. **H. Arias:** None. **N. Fernandez:** None. **M. Neelala:** None. **M. Rodriguez:** None. **A. Elfezouaty:** None. **I. Khetarpal:** None. **D. Penny:** None. **C. Hoffman:** None. **L. Muzyka:** None. **G.M. Beaudoin:** None.

Poster

PSTR425. Reward and Appetitive Learning: Dopaminergic Circuit

Location: WCC Halls A-C

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Program #/Poster #: PSTR425.12/LL27

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIDA IRP/NIH
The Center on Compulsive Behaviors/NIH

Title: Paradoxical findings concerning behavioral function of supramammillary neurons projecting to the lateral preoptic area

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Abstract: The supramammillary region (SuM) projects to both the septal area and the hippocampal formation. This connectivity led to investigations of the SuM with respect to hippocampal functions such as theta oscillations, spatial and episodic memories, and spatial navigation. By contrast, our group found that excitation of SuM glutamatergic neurons (GluN) projecting to the medial septum (SuM-MS GluN) reinforces approach behavior and triggers dopamine release in the nucleus accumbens (NAc). Because SuM neurons are known to respond to salient stimuli, we hypothesized that the SuM is important in coordinating approach behavior toward not only rewards but also other salient stimuli. To further understand such coordination role of the SuM and its mechanisms, first, we examined if SuM-MS GluN send collateral projections to multiple brain regions. We confirmed that about 95% of SuM-MS neurons were GluN using retrograde tracing and mRNA in situ hybridization. Then, we examined collateral projections of SuM-MS GluN by viral tracing. We found that SuM-MS neurons densely project to the lateral preoptic area (LPO). Because the LPO is known to modulate reward-seeking behavior, we then examined if optogenetic stimulation of SuM-LPO GluN instigates approach behavior using an optogenetic intracranial self-stimulation test. Mice quickly learned to press the lever that activated SuM-LPO GluN, suggesting that the stimulation of SuM-LPO GluN instigates and reinforces approach behavior. Finally, we examined if SuM-LPO neurons respond to positive and negative stimuli, we used a fiber photometry with a calcium indicator GCaMP to detect their activity in response to water in thirsty mice and aversive footshock. Intriguingly, water reward and the tone cue paired with water decreased the activity of SuM-LPO neurons, while footshock and the tone cue paired with shock increased their activity. Moreover, we found that salient sensory stimuli bright light and loud noise increased their activity. In summary, our observations together with previous findings led us to hypothesize that SuM-LPO and SuM-MS GluN respond to salient stimuli that are uncertain and require immediate attention, but not salient stimuli that are familiar, particularly consumables and that these neurons facilitate investigatory behavioral processes in stressful or uncertain conditions. In addition, we suggest that water and its conditioned cue decreased their activity because the activation of SuM-LPO neurons induces investigatory behavioral processes, which are not compatible with consuming ingestible stimuli such as water, thereby being inactivated during consumption. Future studies will test the hypotheses.

Disclosures: Y. Arima: None. **S. Ikemoto:** None.

Poster

PSTR425. Reward and Appetitive Learning: Dopaminergic Circuit

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR425.13/LL28

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIDA IRP
DFG Grant MA 8509/1-1

Title: Anticipatory dopamine ramps are decorrelated from acetylcholine dips

Authors: *K. M. COSTA[1], Z. ZHANG[1], Y. ZHUO[2], G. LI[2], Y. LI[2], G. SCHOENBAUM[1]; [1]Behavioral Neurophysiol. Neurosci. Section, NIH, NIDA IRP, Baltimore, MD; [2]PKU-THU Ctr. for Life Sci., Peking Univ., Beijing, China

Abstract: Striatal dopamine release, heavily implicated in learning, movement, and motivation, is regulated by cholinergic interneurons. Previous work has demonstrated that peaks in dopamine release are typically correlated with a characteristic pause in firing in cholinergic interneurons. The coordination of phasic dopamine peaks with dips in cholinergic tone is thought to create a critical plasticity window for dopamine to act as a teaching signal. Acetylcholine can also rapidly trigger dopamine release by acting directly on dopamine axons. However, dopamine dynamics are not always phasic. For example, dopamine release in the nucleus accumbens slowly ramps up as animals approach a desired goal, and there is still controversy on how these slower signals contribute to behavior. Here, we tested whether dopamine ramps in the nucleus accumbens are coincidental with dips in acetylcholine release. We used multi-channel fiber photometry and two newly developed genetically encoded optical sensors, rDA3m and gAch4h, to simultaneously record dopamine and acetylcholine release, respectively, in the nucleus accumbens of freely moving rats. These methods avoid the known confounds of measuring bulk calcium signals from neurons. Rats (male, N=4) were water restricted and trained on task that required them to attend to a light cue that indicated trial start, then perform a nose poke, hold for 0.5 seconds, and then perform a second entry into a fluid well to receive a droplet of water. We found that dopamine signals slowly ramped up prior to the initial nose poke, as expected. However, to our surprise, this dopamine ramp was not coincidental with changes in acetylcholine release. On the other hand, the initiation of the fluid well response and the receipt of rewards led to phasic dopamine peaks that were correlated with clear dips in cholinergic signals. We also found that the light cues that indicated trial start and end led to measurable phasic increases in acetylcholine release, which precludes the possibility of a ceiling effect in the fluorescence signal. Our results reveal that anticipatory dopamine ramps in the nucleus accumbens are not coincidental with major changes in acetylcholine release, and that seemingly only phasic dopamine peaks related to reward and directly rewarded actions are correlated with cholinergic dips. This finding has important implications for the understanding of the behavioral relevance of dopamine signals at different time scales, and how these are coordinated with acetylcholine signals during learning and decision making.

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Poster

PSTR425. Reward and Appetitive Learning: Dopaminergic Circuit

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Program #/Poster #: PSTR425.14/MM1

Topic: G.02. Reward and Appetitive Learning and Memory

Support: National research foundation

Title: Investigation of neural circuits underlying hedonic consumption

Authors: *S. CHOI, S. LEE, J. PYO, J.-H. KIM;
POSTECH, Pohang-si, Korea, Republic of

Abstract: Consumption is generally triggered by homeostasis due to nutritional imbalance. However, not in nutritional imbalance, people can consume palatable food to fulfill their preferences. Consumption of palatable food could be an addictive behavior. Like substance addiction, food addiction involves a compulsive and uncontrollable relationship with food, leading to negative consequences for physical health, emotional well-being, and overall quality of life. To investigate the neural circuits that mediate hedonic consumption, especially compulsive consumption, we established a mouse model behavior paradigm. We found differential activation in an epithalamus brain region in a compulsive consumption group. We tried functional approaches using optogenetics and observed neural populations.

Disclosures: S. Choi: None. **S. Lee:** None. **J. Pyo:** None. **J. Kim:** None.

Poster

PSTR425. Reward and Appetitive Learning: Dopaminergic Circuit

Location: WCC Halls A-C

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Program #/Poster #: PSTR425.15/MM2

Topic: G.02. Reward and Appetitive Learning and Memory

Support: 2020M3E5D9080734

Title: Effects of the chemogenetic lateral habenula activation on the association of CS with the nonoccurrence of US in Pavlovian appetitive conditioning

Authors: *I.-B. JIN, S.-P. PARK, J.-S. HAN;
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Abstract: The presentations of an unconditioned stimulus (US) contingent upon the occurrence of a conditioned stimulus (CS) establishes excitatory conditioning or conditioned excitation, such as approaching a food-cup area. Conversely, associating a CS with the nonoccurrence of a US generates inhibitory conditioning or conditioned inhibition, such as not approaching a food-cup area. While previous research has predominantly focused on conditioned excitation, less attention has been given to the neural mechanisms underlying conditioned inhibition. In a previous study, our objective was to demonstrate the effects of chemogenetic inactivation of the lateral habenula (LHb) on the establishment of conditioned inhibition. Inhibiting LHb activity

during unpaired training eliminated the delayed acquisition of subsequent excitatory learning following inhibitory learning. However, LHb inhibition during paired training had no effect. In the present study, we examined whether the chemogenetic LHb activation facilitates the association of CS with the nonoccurrence of US. We adapted the same experimental design described above and employed the chemogenetic method to test it. In this study, rats went 3 days of unpaired training, which did not retard the acquisition of the subsequent excitatory conditioning, along with chemogenetic LHb activation. And then paired training was followed. Rats with chemogenetic LHb activation and the 3-day unpaired training exhibited delayed acquisition of subsequent excitatory conditioning. These results suggest that LHb activation promotes inhibitory learning. These findings contribute to our understanding of the neural mechanisms underlying conditioned inhibition and shed light on the role of the LHb in this process.

Disclosures: **I. Jin:** None. **S. Park:** None. **J. Han:** None.

Poster

PSTR425. Reward and Appetitive Learning: Dopaminergic Circuit

Location: WCC Halls A-C

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Program #/Poster #: PSTR425.16/MM3

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Korea Research Foundation Grant 2020M3E5D9080734

Title: Chemogenetic inactivation of the lateral habenula impairs the association of a conditioned stimulus with the absence of an unconditioned stimulus, as assessed by the superconditioning task

Authors: *N.-H. KIM, S.-Y. SIM, D.-H. KIM, J.-S. HAN;
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Abstract: The present study aimed to explore the neural substrates involved in conditioned inhibition, which occurs when a conditioned stimulus (CS) is associated with the absence of an unconditioned stimulus (US). Recent research suggests that the lateral habenula (LHb) plays a role in the association of a CS with the nonoccurrence of a US. To investigate this further, we conducted experiments using chemogenetic inhibition of the LHb to examine its effect on the inhibitory property of a CS generated by explicitly unpaired CS and US procedures. We employed the superconditioning task to assess the inhibitory property of CS, which consisted of three phases. During the initial phase, animals underwent six days of explicitly unpaired training of a light (CS1) with food in the behavioral chamber. In the subsequent phase, the animals received six days of paired training involving a light (CS1)-tone (CS2) compound paired with food. In the final phase, food-cup responses to each CS were measured over two days, with four trials of each CS per day. A comparison group of rats only underwent the second phase of the paired training, followed by the third phase. The results revealed a significantly higher food-cup

response to the tone (CS2) was significantly higher in animals that underwent explicitly unpaired training during the first phase, compared to the comparison group. These findings demonstrate the successful generation of conditioned inhibition through explicitly unpaired training. Additionally, we investigated the effects of experimental manipulations throughout the explicitly unpaired training on the conditioned inhibitory properties. Animals with chemogenetic LHb inhibition throughout the unpaired training showed diminished conditioned inhibitory properties. These results indicate that chemogenetic LHb inhibition impairs the association of a CS with the absence of a US, thereby supporting the notion that LHb mediates a conditioned inhibition.

Disclosures: **N. Kim:** None. **S. Sim:** None. **D. Kim:** None. **J. Han:** None.

Poster

PSTR425. Reward and Appetitive Learning: Dopaminergic Circuit

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Program #/Poster #: PSTR425.17/MM4

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant 3R01MH120131

Title: Flow of reward prediction error signaling during appetitive Pavlovian conditioning in mice

Authors: *S. BISWAS[1], S. SHABEL[2];
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Abstract: Reward prediction error (RPE) - the difference between the value of received reward and the expected reward value - is thought to be a computation that animals use to predict reward and change behavior accordingly. Neurons in certain brain regions, such as the globus pallidus (GPi), lateral habenula (LHb), and ventral tegmental area (VTA) encode RPE bidirectionally, i.e., these regions encode positive RPE and negative RPE in opposite directions. These regions are connected through direct and indirect projections and are key nodes in the reward system of the brain. Previous studies have examined the nature of RPE encoding in each of these regions. However, it is unclear whether and how this information changes as it flows from one region to another. To investigate this, we are analyzing data from multi-site fiber photometry during appetitive Pavlovian conditioning in mice. Data were collected from two groups of 4 mice - in one group, we recorded from the LHb and GPi terminals in the LHb, and in the other group, we recorded from the LHb, dopamine neurons in the VTA, and dopamine terminals in the nucleus accumbens. Preliminary data indicate that changes in neural activity during conditioned and/or unconditioned stimuli are sometimes distinct in different regions within the same mice. This suggests that information may not be simply passed on from one region to another but that there is additional processing in some regions of the circuit.

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Poster

PSTR425. Reward and Appetitive Learning: Dopaminergic Circuit

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Topic: G.02. Reward and Appetitive Learning and Memory

Support: UDRF
GUR
DE-CTR
DE-INBRE
INCRE-CCAA
NIGMS-MIRA

Title: Investigating the role of a cluster of dopaminergic neurons in the regulation of motivational state in Drosophila

Authors: *Y. PARK[1,2], D. B. BUSHEY[3], M. ITO[3], M. ENDRES[2], L. SHAO[2];
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Abstract: The motivation to consume rewards, including food and sex, depends on the internal state and the value of the rewarding stimuli. Understanding how motivation is represented and regulated in the nervous system is essential for understanding reward-driven behaviors as well as pathophysiology of mental disorders, such as depression and addiction. Dopaminergic neurons (DANs) have been shown to signal for reward value and motivation in various species. Yet the functions of DANs in reward processing remains to be fully explored due to the heterogeneity in their location, projection, and transcriptional profiles. Many clusters of DANs have been identified in the *Drosophila* brain that mediate distinctive aspects of reward processing. Recently, we identified a cluster of DANs that may involve in the animal's motivation to engage in reward-driven behaviors. Specifically, we identified two cell types in the paired posterior medial 3 (PPM3) cluster of DANs that projects to the fan-shaped body. We show that optogenetic activation of these PPM3 neurons is rewarding to the flies. Interestingly, these neurons also involve in the regulation of feeding and mating behaviors. Further research is in need to determine the role of PPM3 DANs in the regulation of reward-driven behaviors in *Drosophila*.

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Poster

PSTR425. Reward and Appetitive Learning: Dopaminergic Circuit

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Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH DA042895
MH129370
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NIH P30 DA048742

Title: Basolateral amygdala dopamine and D1 neurons track dynamic drug seeking behaviors

Authors: *M. A. BRICKNER, J. M. PROHOFSKY, A. R. WOLFF, M. J. THOMAS, B. T. SAUNDERS;
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Abstract: Environmental cues can reinforce actions and evoke strong, dynamic motivational states during drug seeking and craving to prompt relapse. The basolateral amygdala (BLA) is critical for the associative learning that underlies this process, via the linking of affective valence to sensory stimuli that predict biologically relevant outcomes. Dopamine signaling is also critical for learning and cue-driven motivational states. The BLA receives dense dopaminergic projections and is enriched with D1 dopamine receptors (D1DRs). Microdialysis and electrophysiology studies indicate that cocaine-predictive stimuli increase extracellular dopamine levels and activate neurons within the amygdala, potentially via D1DRs. In recent work, we and others demonstrated that intermittent access to cocaine promotes a sensory gated, state-level control of motivation that results in binge-like seeking profiles thought to approximate human drug use. How BLA dopamine and D1DR activity functions in addiction remain largely unexplored. Here, we used fiber photometry to measure *in vivo* dopamine signaling and D1-neuron activity in the BLA during an intermittent access to cocaine self-administration (IntAc) paradigm using dLight and GCaMP, respectively, with high spatiotemporal precision. The IntAc paradigm includes periods of drug available and unavailable periods, and the shifts between the two are indicated by changes in sensory stimuli to probe whether the BLA is involved in state-level encoding associated with drug seeking. Both BLA dopamine and D1-neuron signals emerged in response to cocaine infusions and came to track the presence of dynamic, sensory-guided drug seeking motivational states during the transitions between drug availability periods. Dopamine and D1-neuron signals exhibited different temporal profiles during state transitions and cues, suggesting that they may underlie unique facets of drug-related behaviors. In ongoing studies, I am exploring the functional role of D1-containing BLA neurons in state-level control of drug seeking using inhibitory optogenetics.

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Poster

PSTR425. Reward and Appetitive Learning: Dopaminergic Circuit

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NIH Grant T32 DA007234
NIH Grant F31 DA055482
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Title: Superior colliculus influences Pavlovian motor learning via excitation of dopamine and GABA neurons in the VTA and SNc

Authors: *C. L. POISSON[1], A. R. WOLFF[2], B. T. SAUNDERS[2], C. R. HERUBIN[2];
[1]Neurosci., Univ. of Minnesota Twin Cities, Minneapolis, MN; [2]Dept. of Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Animals associate rewarding or threatening events with specific sensory stimuli (i.e. cues) through Pavlovian conditioning. It is well known that the ventral midbrain (ventral tegmental area- VTA, and substantia nigra pars compacta- SNc) are key to the formation and expression of cue-guided behaviors. However, it is unclear how these dopamine (DA) rich regions rapidly integrate sensory information about cues in order to create conditioned behavior. In addition, the ventral midbrain is noted to have a significant population of GABA neurons that influence appetitive conditioning. A candidate structure that processes sensory information and sends excitatory projections to both DA and GABA neurons is the superior colliculus (SC). Here, we use a combination of optogenetics and fiber photometry to manipulate SC projections while simultaneously recording DA and GABA neurons in the ventral midbrain. Fiber photometry recordings demonstrate that optogenetic stimulation of SC terminals in the VTA/SNc increases DA neuron activity. Interestingly, SC terminal excitation also increased activity of local inhibitory GABAergic neurons in the VTA. These results suggest that SC inputs can influence the ventral midbrain not only via excitation of dopamine neurons, but also more subtly through the recruitment of local inhibitory neurons. In order to determine how SC inputs influenced Pavlovian conditioning, we optogenetically excited SC terminals to the VTA and SNc during a cue conditioning task. Unlike direct DA neuron stimulation, optogenetic activation of SC-DA projections does not drive learning to create conditioned behavior to an otherwise neutral cue. Instead, excitation of these projections produces unique motor output - changes in head, neck, and body orientation. Stimulation of SC-DA projections does not create real time place preference or support intracranial self stimulation, suggesting that the information signaled by this projection does not have a strong positive valence. These studies suggest that excitatory input to DA and GABA neurons from the SC has a subtle role in learning, including movement invigoration and postural control during reward-seeking behaviors. In ongoing studies we are recording deep layer SC neuron activity during a visual cue-sucrose Pavlovian conditioning task with fiber photometry. These results reveal SC dynamics that are time-locked to reward consumption and are modulated by the presence of predictive sensory cues. Overall, our work implicates that deep layer SC neurons are involved in Pavlovian conditioning, and that projections to dopamine regions influence movements associated with cue-reward learning.

Disclosures: **C.L. Poisson:** None. **A.R. Wolff:** None. **B.T. Saunders:** None. **C.R. Herubin:** None.

Poster

PSTR425. Reward and Appetitive Learning: Dopaminergic Circuit

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR425.21/MM8

Topic: G.02. Reward and Appetitive Learning and Memory

Title: The Role of Striosomal Inhibition on Midbrain Dopamine Neurons in the context of Reward-Seeking Behavior

Authors: *J. MANKEL[1], J. READ[2], S. LAMMEL[2], J. ROEPER[1];
[1]Goethe-University Frankfurt, Frankfurt, Germany; [2]Mol. and Cell Biol., Univ. of California Berkeley, Berkeley, CA

Abstract: Midbrain dopamine neurons (mDAN) in the substantia nigra pars compacta (SNc) receive direct synaptic input from various brain regions, including the dorso-medial (DMS) and dorso-lateral striatum (DLS) (Lerner et al., 2015). Recent research showed direct inhibitory input from the striosomal compartment to induce enhanced rebound firing in a subset of mDANs (Evans et al., 2020). However, the presence and functional role of DA rebound in vivo remains unclear. We aim to investigate the connectivity between striosomes and projection-defined mDANs to elucidate the role of dopamine rebound in the basal ganglia network. We hypothesize relevant differences between DMS and DLS-projecting DANs, since the later are intrinsically rebound ready and might thus be more susceptible to inhibition by striosomes. A deeper understanding of this circuitry might have implications for the modulatory role of dopamine during value-based decision making. To test our hypothesis, we selectively expressed channelrhodopsin in striosomes of *pdyn-cre* mice and utilized in-vitro patch-clamp recording paired with retrograde tracing to assess their connectivity to projection-defined mDANs. While optogenetic patch-terminal activation evokes $GABA_B$-mediated GIRK- currents in both mDAN-populations (DLS-proj.: 37.41 +/- 6.58 pA; DMS-proj.: 31.41 +/- 4.27 pA), striosomes in the DMS showed similar connectivity to DMS- and DLS-projecting DANs (DLS-proj.: 86%; DMS-proj.: 75%). However, DLS-striosomes preferentially innervated DLS-projecting neurons (DLS-proj.: 76%; DMS-proj.: 40%), which is in accordance with the ascending-spiral model proposed by Haber et al. (2000). Next, we tested the effect of optogenetic patch-terminal stimulation on dopamine release in vivo. By using rGrab-DA 3.0 to monitor striatal dopamine transients (Sun et al., 2020), we observed a strong inhibition of dopamine release following optogenetic patch stimulation compared to baseline (mean Z-score: -3.2). In contrast, optogenetic activation of dMSNs using *Drd1-cre* mice enhances striatal dopamine levels relative to baseline (mean Z-score: +2.3). This finding indicates that the striosomal compartment exerts a strong inhibitory control on SNc DANs, while the matrix compartment disinhibits them.To gain a better understanding of the effect of striosomal activation on reward-seeking behavior, we are chemogenetically manipulating DMS-striosomes in a probabilistic reversal-learning task.

Additionally, we intend to examine the contribution of mDANs to value-based decision making by directly recording from projection-defined mDANs in a head-fixed reversal-learning task using Neuropixel-probes.

Disclosures: **J. Mankel:** None. **J. Read:** None. **S. Lammel:** None. **J. Roeper:** None.

Poster

PSTR425. Reward and Appetitive Learning: Dopaminergic Circuit

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR425.22/MM9

Topic: G.02. Reward and Appetitive Learning and Memory

Support: BBRF NARSAD grant
IBRO

Title: Neurotensin in the nucleus accumbens regulateshedonic feeding and obesity.

Authors: ***N. GAZIT**[1], A. TOSE[1], C. SENG[2], Y. JIN[3], H. YANG[4], J. P. VERHAREN[1], C. LIU[1], E. HU[1], L. W. TANG[1], J. READ[1], B. LIM[5], L. TIAN[3], C. FOLDY[2], S. LAMMEL[1]; [1]MCB, UC Berkeley, Albany, CA; [2]Brain Res. Inst., Univ. of Zurich, Zurich, Switzerland; [3]Univ. of California, Davis, Davis, CA; [4]Neurobio., Zhejiang Univ., Hangzhou, China; [5]Biol. Sci., UCSD, La Jolla, CA

Abstract: The neuropeptide neurotensin (NTS) and its receptors are widely expressed in the mammalian brain. NTS regulates many physiological functions including sleep, feeding, blood pressure, body temperature and locomotion, but its role in the nucleus accumbens is unclear. Here, we combined patch-seq, *in vivo* and *ex vivo* electrophysiology, optogenetics and imaging to study the anatomy and function of NTS in neurons in the lateral nucleus accumbens projecting to the ventral tegmental area (NAcLat→VTA). We found that NTS is highly expressed in this pathway and optogenetic stimulation promotes NTS release. In addition, we found that neural activity of NAcLat→VTA cells is directly correlated with consumption of palatable food but not regular chow or other behaviors. Accordingly, optogenetic activation of NAcLat→VTA induced hedonic feeding in a NTS dependent manner. Furthermore, in the high fat diet (HFD) obesity mouse model, we found a diet dependent uncoupling of NAcLat→VTA activity and hedonic feeding behavior. This was accompanied by reduced NTS expression and release. Lastly, when we selectively overexpressed NTS in the NAcLat→VTA pathway of mice exposed to HFD, we observed a significant reduction in weight gain compared to control mice. Together, these data reveal an unexpected role for NTS in the NAcLat→VTA pathway for regulating hedonic feeding behavior and obesity. Our findings contrast the well-known anorexic effects of NTS in the lateral hypothalamus suggesting that circuit-specific manipulations of NTS neurons may be necessary in order to harness the translational potential of NTS in the treatment of obesity.

Disclosures: **N. Gazit:** None. **A. Tose:** None. **C. Seng:** None. **Y. Jin:** None. **H. Yang:** None. **J.P. Verharen:** None. **C. Liu:** None. **E. Hu:** None. **L.W. Tang:** None. **J. Read:** None. **B. Lim:** None. **L. Tian:** None. **C. Foldy:** None. **S. Lammel:** None.

Poster

PSTR425. Reward and Appetitive Learning: Dopaminergic Circuit

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR425.23/MM10

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant R03DA045913
NIH Grant K01DA051662

Title: Optogenetic stimulation of dopamine neurons disrupts the acquisition of sign-tracking behavior and cue encoding in the nucleus accumbens

Authors: E. MCLAUGHLIN, K. LEAR, S. ZENG, K. DUFFER, ***S. MORRISON**; Neurosci., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: If a Pavlovian cue predicts a reward in a different location, some rats will preferentially approach and interact with the cue (sign tracking) and others will approach the site of reward delivery (goal-tracking). Sign tracking, but not goal tracking, has been shown to involve the modulation of dopamine release in the nucleus accumbens (NAc) over the course of cue-reward learning: in sign trackers, dopamine release in response to the cue strengthens over time, while dopamine release in response to reward delivery diminishes (Flagel et al., 2011). We have previously demonstrated that reward-evoked activity in the NAc reflects the different patterns of dopamine release in sign trackers vs. goal trackers (Gillis and Morrison, 2019). However, the causal relationships among dopamine release, NAc neural activity, and sign tracking have not been tested. In particular, it remains unknown whether cue- or reward-specific stimulation of dopamine neurons can alter sign-tracking behavior or NAc responses.
To address this question, we used male and female TH-Cre (tyrosine hydroxylase-Cre) rats to specifically express channelrhodopsin in dopamine neurons of the ventral tegmental area (VTA). We found that reward-concurrent stimulation of VTA dopamine neurons during early stages of cue-reward learning had little effect on sign-tracking behavior at first, but, surprisingly, disrupted the continued acquisition of sign tracking in sessions after stimulation was discontinued. Meanwhile, in another group of TH-Cre rats performing sign tracking and/or goal tracking, we recorded from individual neurons in the NAc while stimulating VTA dopamine neurons concurrent with either cue or reward. We observed complex effects of stimulation on neural activity, including enhancement of excitatory responses, but also induction of later inhibitory responses, often within the same neuron. These findings largely support the idea that NAc dopamine release enhances cue- and reward-evoked excitations, which in turn drive Pavlovian conditioned approach towards a reward-associated cue. However, they reveal additional complexity in the relationships among dopamine release, neural signaling, and behavior; this

includes a key role of the specific timing of dopamine neuron activation, on both short time scales (i.e. within a trial) and long (i.e. across sessions).

Disclosures: E. McLaughlin: None. **K. Lear:** None. **S. Zeng:** None. **K. Duffer:** None. **S. Morrison:** None.

Poster

PSTR425. Reward and Appetitive Learning: Dopaminergic Circuit

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR425.24/MM11

Topic: G.02. Reward and Appetitive Learning and Memory

Support: ICRG Seed Grant
NIH Grant R03DA045913
NIH Grant K01DA051662

Title: Propensity for risky choices despite lower cue-reactivity in adolescent rats

Authors: *S. ZENG, E. MCLAUGHLIN, K. LEAR, S. E. MORRISON;
Neurosci., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Reward-associated cues play an important role in the development of addiction and relapse, including the development of gambling disorder. Notably, the ability of reward-related cues to elicit interaction varies widely among individuals; this may be modeled in animals using a Pavlovian conditioned approach procedure. If a cue is repeatedly predictive of reward in another location (e.g., lever extension followed by sugar pellet delivery to a food cup), some rats will present a preference for lever approach and interaction—a behavior known as sign tracking (ST)—while others will approach the site of reward delivery, a behavior known as goal tracking (GT). We recently showed (Swintosky et al., 2021) that a propensity towards sign tracking is associated with suboptimal behavior—more risky choices and fewer optimal ones—in a rodent gambling task (rGT) with win cues (modified from Barrus and Winstanley, 2016). However, we and others have shown that adolescent males exhibit less sign tracking and more goal tracking than adults (Rode et al., 2020), implying lower cue reactivity than adults. Therefore, we assessed the sign-tracking tendency of adolescent male rats (n = 48), as well as their performance on the rGT, and compared their behavior to that of adults (n = 64).
Confirming our previous report, we found that adolescents displayed markedly less sign tracking and more goal tracking than adults. At the same time, adolescents were much more likely to make risky choices, and less likely to behave optimally, on the rGT with win cues. Unlike adults, in adolescents, increased sign tracking behavior was not associated with riskier choices on the rGT; in fact, there was a tendency towards the opposite (i.e., a propensity towards goal tracking being associated with risk-taking). This implies that suboptimal behavior on the rGT may be driven by a different factor - e.g., increased reward sensitivity - in adolescents, rather than a motivational pull towards reward-associated cues. Finally, we found that adolescents who were

trained on the rGT in adolescence retained their risk-taking preferences into adulthood, resulting in a markedly different behavioral profile compared to subjects who began "gambling" as adults. This may have important implications for adolescent exposure to gambling as well as other potentially addictive behaviors.

Disclosures: S. Zeng: None. **E. McLaughlin:** None. **K. Lear:** None. **S.E. Morrison:** None.

Poster

PSTR425. Reward and Appetitive Learning: Dopaminergic Circuit

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR425.25/MM12

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant #5R25HL103180-12
SFARI & NIH Grant R01MH104450
NIMH F32

Title: Nucleus accumbens neurons encode social reward

Authors: *J. RIMORIN[1], C. W. TSCHUMI[2], L. S. ZWEIFEL[3];
[1]Univ. of Washington Undergraduate Neurobio. Program, Seattle, WA; [3]Pharmacol., [2]Univ. of Washington, Seattle, WA

Abstract: Prosocial behavior is important to many species and its disruption is a hallmark symptom of many neurodevelopmental and neuropsychiatric disorders including autism, schizophrenia, and depression. The neurotransmitter dopamine modulates neuronal activity in the nucleus accumbens (NAc) and plays a critical role in the regulation of prosocial behavior. While the role of dopamine (DA) is well studied in the context of social behavior, little is known about neuronal activity in the NAc during social behavior. Here we used a viral vector to drive the expression of a genetically encoded fluorescent calcium sensor combined with mini-scope imaging to monitor calcium dynamics in the NAc. Calcium dynamics were recorded during an operant task in which an experimental mouse presses a lever to gain access to a conspecific social partner. We identified subpopulations of neurons within the striatum that encode for social reward, but not for other discrete cues or actions associated with the task. Findings from this study will improve our understanding of how prosocial behavior is encoded in the ventral striatum and how perturbations in these signals contribute to social behavioral disruption that will likely be relevant to disorders in which prosocial behavior is impaired.

Disclosures: J. Rimorin: None. **C.W. Tschumi:** None. **L.S. Zweifel:** None.

Poster

PSTR426. Treatment and Drugs for Mood Disorders

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR426.01/MM13

Topic: G.05. Mood Disorders

Support: RO1MH1182939
RO1MH132806

Title: Analysis of miRNA and mRNA gene networks in Major Depression in a large postmortem brain sample

Authors: *Z. N. TAYLOR[1], J. DRAKE[1], A. DENHAM[1], S.-A. BACANU[3], J. SHIN[4], J. KLEINMAN[5], T. HYDE[6], V. I. VLADIMIROV[2];
[2]Psychiatry, [1]Univ. of Arizona, Phoenix, AZ; [3]VCU, Richmond, VA; [4]Lieber Inst. for Brain Develop., College Station, TX; [5]Lieber Inst. for Brain Develop., Baltimore, MD; [6]Lieber Inst. For Brain Develop., Baltimore, MD

Abstract: Major Depression (MDD) is a disorder characterized by low mood and anhedonia that affects roughly one out of every six adults worldwide. Genome-wide significant variants associated with MD have been identified in large GWAS studies but GWAS alone provides little insight into the functional impact of such variants. Analysis of MDD associated transcriptome changes in postmortem brain tissues offers a complimentary approach to uncovering the underlying neuropathology of MDD.
MicroRNA (miRNA), a class of small non-coding RNA with gene regulatory functions and high expression in the brain, has been investigated in different multiple neuropsychiatric phenotypes. Here, we used miRNA-Seq to compare the expression of over 1000 miRNAs between 150 MDD patients and 150 matched controls in the subgenual anterior cingulate cortex (sACC) and Amygdala. Our analysis includes differential expression analysis (DEA) of individual miRNAs, weighted gene co-expression analysis (WGCNA) of miRNA and mRNA expression, and miRNA/mRNA correlation-based analyses to identify miRNAs with a converging role in the etiology of MDD. We identified multiple differentially expressed miRNAs (at FDR of 5%) including several previously associated with MDD and/or associated with neurodevelopment or other psychiatric illness. Our network analyses detected both significant miRNA and mRNA modules associated with MDD at the Bonferroni corrected $p \leq 0.05$. Using correlation analysis, miRNA target site prediction, and gene enrichment analysis a number of mRNAs and their attendant gene ontologies were identified potential miRNA regulatory targets.
Ours is the largest to date postmortem brain miRNA expression study of major depression, and our ongoing analyses provides solid evidence of the importance of miRNA as a contributing factor to the development of MDD.

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Poster

PSTR426. Treatment and Drugs for Mood Disorders

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR426.02/MM14

Topic: G.05. Mood Disorders

Support: FRQS Doctoral Award

Title: Extracellular vesicle miRNAs from depressed brain are dysregulated in a sex-specific manner

Authors: *P. IBRAHIM[1,2], R. DENNISTON[1], H. MITSUHASHI[1,2], J. YANG[1], C. NAGY[1,2], G. TURECKI[1,2];
[1]Douglas Hosp. Res. Ctr., Verdun, QC, Canada; [2]McGill Univ., Montreal, QC, Canada

Abstract: Major Depressive Disorder (MDD) is a leading cause of disability worldwide. MicroRNA's (miRNA) are disrupted in MDD, and they can be packaged into extracellular vesicles (EVs), along with other bioactive molecules. We hypothesize that EV cargo from the anterior cingulate cortex, a brain region highly implicated in MDD, will have a disease specific profile that could mediate disease development. EVs were isolated from post-mortem human brain tissue of 40 (20M/20F) individuals who had depression and died by suicide, as well as 40 (20M/20F) individuals with no psychiatric illness, via size exclusion chromatography. The quality was assessed by western blots, transmission electron microscopy (TEM), and microfluidic resistive pulse sensing. MiRNA profiling and differential analysis were then performed. Western blots showed no contamination with cellular debris and enrichment of EV marker CD9. TEM images showed cup-shaped vesicles with sizes mostly between 30 and 200 nm and labelled with CD81, another EV marker. Differential analyses revealed sex-specific dysregulation in miRNAs, where miR-92a-3p was downregulated in depressed females, while miR-129-5p was downregulated in depressed males. MiRNA target genes were predicted *in silico*, and functional analysis suggested that these miRNAs are involved in neurotransmission and synaptic plasticity. This is the first study to profile brain-derived EV miRNA in the context of depression. This study will provide novel mechanistic insights into the pathophysiology of MDD, which could serve as a starting point for the development of targeted therapeutic strategies as well as prevention measures for depression.

Disclosures: P. Ibrahim: None. **R. Denniston:** None. **H. Mitsuhashi:** None. **J. Yang:** None. **C. Nagy:** None. **G. Turecki:** None.

Poster

PSTR426. Treatment and Drugs for Mood Disorders

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR426.03/MM15

Topic: G.05. Mood Disorders

Support: GT holds a Canada Research Chair (Tier 1)
Canadian Institute of Health Research Grant FDN148374
Canadian Institute of Health Research Grant ENP161427
ERA-NET ERA PerMed

Title: Mir-151a-5p cargo in neuron-derived extracellular vesicles mediates antidepressant response.

Authors: *D. ZURAWEK[1,2], A. MORGUNOVA[1,3], L. FIORI[2], J. YANG[2], C. BELLIVEAU[1,3], P. IBRAHIM[2,3], S. H. KENNEDY[4,5], R. W. LAM[6], R. MILEV[7], S. ROTZINGER[4], C. N. SOARES[8,9], V. H. TAYLOR[10], R. UHER[11,12], J. A. FOSTER[13,4], B. N. FREY[13], C. FLORES[1,14,15], C. NAGY[2], G. TURECKI[2];
[1]Douglas Mental Hlth. Univ. Institute, McGill Univ., Montreal, QC, Canada; [2]McGill Group for Suicide Studies, Douglas Mental Hlth. Univ. Institute, Dept. of Psychiatry, McGill Univ., Montreal, QC, Canada; [3]Integrated Program in Neuroscience, McGill Univ., Montreal, QC, Canada; [4]Dept. of Psychiatry, Univ. Hlth. Network, Krembil Res. Institute, Univ. of Toronto, Toronto, ON, Canada; [5]St Michael's Hospital, Li Ka Shing Knowledge Institute, Ctr. for Depression and Suicide Studies, Toronto, ON, Canada; [6]Dept. of Psychiatry, Univ. of British Columbia, Vancouver, BC, Canada; [7]Departments of Psychiatry and Psychology, Queens University, Providence Care Hosp., Kingston, ON, Canada; [8]Dept. of Psychiatry, St Michael's Hospital, Univ. of Toronto, Toronto, ON, Canada; [9]Dept. of Psychiatry, Queen's Univ. and Providence Care Hosp., Kingston, ON, Canada; [10]Dept. of Psychiatry, Univ. of Calgary, Calgary, AB, Canada; [11]Dept. of Psychiatry, Dalhousie Univ., Halifax, NS, Canada; [12]Nova Scotia Hlth. Authority, Halifax, NS, Canada; [13]Dept. of Psychiatry and Behavioural Neurosciences, McMaster University, and St Joseph's Healthcare Hamilton, Hamilton, ON, Canada; [14]Dept. of Neurol. and Neurosurgery, Fac. of Medicine, McGill Univ., Montreal, QC, Canada; [15]Dept. of Psychiatry, McGill Univ., Montreal, QC, Canada

Abstract: Clinical care for major depressive disorder (MDD) relies on a trial-and-error approaches to select the most effective therapy. The difficulty of studying the molecular processes of the living brain has been a limitation in advancing our understanding of the molecular mechanisms that mediate the antidepressant response. However, recent technical advances have enabled us to overcome this limitation. Brain can communicate with peripheral tissues in a quasi-endocrine manner by secreting nano-sized extracellular vesicles that can cross blood-brain barrier and carry cell-type specific molecular cargo. Thus, the levels of brain-derived extracellular vesicles (BDEVs) and their cargo composition may reflect the actual molecular state of the brain and help understand mediators of antidepressant response. In this study, we used 430 human plasma samples from CAN-BIND-1 clinical trial (clinicaltrials.gov, NCT01655706) that were collected at two time points — before and after an 8-week escitalopram treatment—from healthy controls and depressed patients who either responded or did not respond to the treatment. We isolated a population of BDEVs from plasma samples by using a combination of size exclusion chromatography and immunoprecipitation against SNAP25, a protein exclusively expressed in brain neurons and present on the surface of BDEVs. Small RNA-seq profiling followed by RT-qPCR validation revealed that the levels of miR-151a-5p in BDEVs differentiated depressed patients from healthy controls and predicted a positive response to escitalopram (interaction time x treatment response $F(2,403) = 3,623$, $p<0.05$, n=71-73/group). Before treatment, depressed patients had lower levels of miR-151a-5p in BDEVs than

controls while this levels significantly increased over time only in group of depressed patients who responded to antidepressant treatment ($q<0.05$). Further analysis showed good miR-151a-5p predictive value in discriminating groups of responders and non-responders (AUC = 0.65, $p<0.01$). *In vitro* and *in sillico* experiments showed that miR-151a-5p negatively regulates a set of genes enriched in the prefrontal cortex and being responsible for the regulation of synaptic glutamate release. Engineered BDEVs enriched with miR-151a-5p displayed antidepressant properties *in vivo* and effectively ameliorated the behavioral deficits induced by chronic social defeat stress paradigm in stress-susceptible mice when delivered to the prefrontal cortex (interaction time vs treatment: $F(3, 29) = 6{,}194$, $p<0.01$, n=6-11/group). Our observations suggest that miR-151a-5p in BDEVs may be a molecular mediator of effective antidepressant treatment response.

Disclosures: **D. Zurawek:** None. **A. Morgunova:** None. **L. Fiori:** None. **J. Yang:** None. **C. Belliveau:** None. **P. Ibrahim:** None. **S.H. Kennedy:** None. **R.W. Lam:** None. **R. Milev:** None. **S. Rotzinger:** None. **C.N. Soares:** None. **V.H. Taylor:** None. **R. Uher:** None. **J.A. Foster:** None. **B.N. Frey:** None. **C. Flores:** None. **C. Nagy:** None. **G. Turecki:** None.

Poster

PSTR426. Treatment and Drugs for Mood Disorders

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR426.04/MM16

Topic: G.05. Mood Disorders

Title: Behavioral and neurochemical effects of increased MAO-A activity in the prefrontal cortex in mice

Authors: *R. LEBEAU[1], R. ZHOU[2], T. TOMODA[3], J. MEYER[4], E. SIBILLE[5], J.-P. GUILLOUX[1];
[1]Univ. Paris Saclay - INSERM 1018 CESP, team Moods, Orsay, France; [2]Univ. of Toronoto, Toronto, ON, Canada; [4]Dept. of Psychiatry, [3]Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada; [5]CAMH - Univ. of Toronto, Toronto, ON, Canada

Abstract: The Monoamine Oxidase A (MAOA) is a mitochondrial enzyme involved in the catabolism of monoaminergic neurotransmitters, more precisely serotonin (5HT) and norepinephrine (NE). A pathological feature of major depressive disorder (MDD) that may underlay decreased monoamines, depressive symptoms, and variable response to antidepressant (AD) treatments is elevated levels of MAOA. PET imaging studies revealed a ~34% increase in MAOA brain levels in unmedicated MDD patients compared to healthy controls, with greater levels in the prefrontal cortex (PFC). MAOA levels are also correlated with the severity of MDD symptoms. This higher expression and activity of MAOA is suspected to be involved in the MDD etiology but also in the resistance to AD, an hypothesis never been formally tested.We propose to develop a murine model of human MAOA overexpression to determine whether increased MAOA activity could result in decreased 5-HT extracellular levels, change in

expression in monoamine-related genes, and impact emotional behavior.An adeno-associated viral (AAV) vector containing the human MAOA (hMAOA) gene and the fluorescent marker GFP (or GFP only for control mice) was injected in the PFC of adult CamKII-Cre mice to increase selectively hMAOA expression. The effects of increased hMAOA were tested 2-3 weeks after the AAV injection. A microdialysis probe was implanted in the same region and in vivo microdialysis in awake mice was performed. Depression and anxiety-like phenotype were tested in a second cohort, using Elevated Plus Maze, Open Field, Novelty Suppressed Feeding and Splash tests. Microdialysis experiments revealed that hMAOA overexpression decreases basal [5-HT]ext levels by 40% in the PFC. Emotional behavior analyses showed that hMAOA overexpression did not affect anxiety-related behavior, but decreased grooming behavior in the Splash test, suggesting an anhedonic effect of hMAOA overexpression. Molecular biology experiments confirmed an increase in the level of expression and activity of hMAOA but neither endogenous MAOA or SERT expressions were affected.Overall, our results confirmed that increased hMAOA activity results in lower 5-HT neurotransmission associated with changes in emotional behavior. Whether these regio-selective neurochemical changes can lead to greater susceptibility to stress and lead to AD treatment resistance remains to be tested. Moreover, overexpression of hMAOA will be tested in other brain regions related to emotional behavior.

Disclosures: **R. Lebeau:** None. **R. Zhou:** None. **T. Tomoda:** None. **J. Meyer:** None. **E. Sibille:** None. **J. Guilloux:** None.

Poster

PSTR426. Treatment and Drugs for Mood Disorders

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR426.05/MM17

Topic: G.05. Mood Disorders

Support: GRF 15100018

Title: Involvement of medial prefrontal cortex in the rapid onset of antidepressant effects of acute physical exercise

Authors: *C. TONG, Y. SUK YU;
Hong Kong Polytechnic Univ., Kowloon, Hong Kong

Abstract: Major depressive disorder (MDD) is a widespread mental health disorder. Emerging clinical studies have demonstrated the rapid onset of antidepressant effects of single-bout of physical exercise. This study investigated the underlying neural mechanism of antidepressant effects of acute exercise in an animal model. We subjected the adult mice with C57BL/J to moderate-intensity acute exercise intervention and investigated the molecular mechanism of rapid antidepressant response involving the medial prefrontal cortex (mPFC). Our pilot data have shown that acute exercise elicited a rapid (30 min post-exercise) and sustained antidepressant effect (last to 24 hours post-exercise). Acute exercise also induces significant activation of

neurons in the mPFC, mainly glutamatergic neurons, and its activation lasts at least 2 hours. This study provides preliminary mechanistic insights into the novel roles of acute exercise in counteracting depression.

Disclosures: C. tong: None. **Y. Suk Yu:** None.

Poster

PSTR426. Treatment and Drugs for Mood Disorders

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR426.06/Web Only

Topic: G.05. Mood Disorders

Title: Response to cannabidiol administration in a murine model of depression

Authors: *E. ROBLES[1], A. GONZALEZ HORTA[1], D. MONTIEL CONDADO[1], M. BERMUDEZ DE LEON[3], B. GONZALEZ HERNANDEZ[2];
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Abstract: RESPONSE TO CANNABIDIOL ADMINISTRATION IN A MURINE MODEL OF DEPRESSION
Major depressive disorder (MDD), also known as unipolar depression, is one of the most serious and common psychiatric disorders worldwide, MDD is a condition characterized by pathological sadness accompanied by psychophysiological signs. About 30% of patients taking antidepressants are considered drug resistant, so it is necessary to study alternatives, one of which is cannabidiol (CBD), a non-psychotropic phytocannabinoid derived from the Cannabis plant. Methods: Wistar rats (200-250g) were used, the model of depression used was the forced swimming test combined with a "time-sampling" analysis: 1) pre-session of 15min. and 24hrs. later, 2) experimental session of 5min, in which behaviors are recorded. Treatments: Saline, cannabidiol (10mg/kg, 20mg/kg and 30mg/kg), imipramine (30mg/kg) and ACEA (3mg/kg). The following behaviors were analyzed: immobility (depression) as passive behavior, climbing and swimming as active behavior. Results: 1) Males, immobility decreased significantly with the five treatments, showing better effect CBD 10mg and CBD 20mg, swimming increased in all five conditions showing better effect CBD 10mg and CBD 20mg. Climbing decreased with imipramine, CBD 20mg, CBD 30mg and ACEA. 2) Females: immobility decreased significantly with the five treatments, swimming increased in all conditions showing better effect in imipramine, CBD 20mg and ACEA, but no difference was found in climbing. Conclusion: CBD shows a dose-dependent antidepressant profile, with better effects in males than in females.
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Poster

PSTR426. Treatment and Drugs for Mood Disorders

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR426.07/MM18

Topic: G.05. Mood Disorders

Title: Signaling snapshot of cholecystokinin receptor-2 unravels G-protein coupling selectivity

Authors: *Y. LIAO, Y. DING, X. LI;
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Abstract: Signaling snapshot of cholecystokinin receptor-2 unravels G-protein coupling selectivity Yu-Ying Liao, Yu Ding, Xiao-Ming Li
AbstractCCK2R is primarily expressed in the brain, particularly in the cortex and the limbic structures including the amygdala, the hippocampus, and the nucleus accumbens and selected regions in the gastrointestinal tract, including gastric epithelial parietal cells, pancreatic acinar cells, myenteric neurons, and human peripheral blood mononuclear cells. In this paper, we determined two high-resolution cryo-EM structures of CCK2R signaling complexes. The analysis of structures, together with mutagenesis studies, revealed distinct features of the CCK2R selectivity for gastrin-17, and thus highlighted the basis for G-protein coupling promiscuity and ligand recognition by CCK2R.
Key Words: GPCR; Cholecystokinin; G-protein

Disclosures: Y. Liao: None. **Y. Ding:** None. **X. Li:** None.

Poster

PSTR426. Treatment and Drugs for Mood Disorders

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Program #/Poster #: PSTR426.08/MM19

Topic: G.05. Mood Disorders

Support: NIHR01AG029493
NIHR01DC020528
East Tennessee State University Research Development Committee-Major Grants Program

Title: Female-specific role of progesterone-activated astrocyte focal adhesion kinase in inhibiting stress responses

Authors: *C. JIA, W. D. GILL, C. LOVINS, R. W. BROWN, T. HAGG;
East Tennessee State Univ., Johnson City, TN

Abstract: Astrocytes have been implicated in stress responses. We found that ciliary neurotrophic factor (CNTF), which is only produced and released by astrocytes in the brain, plays a sex-specific role in stress responses and that CNTF is upregulated by inhibition of focal adhesion kinase (FAK). Here, we found that inducible conditional gene deletion of FAK in astrocytes or systemic treatment with an FAK inhibitor promoted despair or passive coping behavior, i.e., immobility in an acute forced swim stress in female, but not male, mice. Strikingly, four weeks of chronic unpredictable stress did not further increase immobility in female astrocytic FAK knockout mice but exacerbated it in female wildtype mice and male mice of both genotypes. These data suggest that chronic stress might act through astrocyte FAK in females. CUS indeed reduced phospho-FAK in the female medial amygdala, an area involved in female stress responses, as we previously found. Stress reduces systemic progesterone levels, whereas progesterone attenuates ovariectomy-induced stress responses and inhibits CNTF expression in the amygdala. Here, progesterone treatment after ovariectomy activated FAK in the amygdala and alleviated ovariectomy-induced immobility in wildtype, but not astrocytic FAK knockout females. This suggests that progesterone-mediated activation of FAK in amygdala astrocytes underlies a beneficial mechanism in reducing female stress responses. Finally, astrocytic FAK knockout or FAK inhibitor treatment increased CNTF expression in the medial amygdala of both sexes, although not in the hippocampus. However, as we previously found, CNTF in the medial amygdala promotes stress responses only in females explaining the female-specific role of astrocytic FAK inhibition. Together, this study reveals a novel female-specific progesterone-astrocytic FAK pathway that inhibits stress responses and points to opportunities for developing treatments for stress-related disorders in women.

Disclosures: C. Jia: None. **W.D. Gill:** None. **C. Lovins:** None. **R.W. Brown:** None. **T. Hagg:** None.

Poster

PSTR426. Treatment and Drugs for Mood Disorders

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Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR426.09/MM20

Topic: G.05. Mood Disorders

Support: CIHR

Title: Intravenous Reelin treatment in a rodent model of chronic stress: Putative implications for treatment of depression

Authors: *B. S. K. REIVE[1], R. ROMAY-TALLON[2], L. E. KALYNCHUK[1], H. J. CARUNCHO[1];

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Abstract: Major depression has been linked to inflammation and is marked by multiple depressive episodes. Our lab has shown antidepressant-like actions of intravenous Reelin in a rodent model of chronic stress, where we evaluated whether peripheral Reelin treatment impacts stress-induced alterations to immune function in brain and/or periphery. Spleens and brains were collected from male and female rats exposed to 21 days of corticosterone or vehicle injections and 2 injections of either Reelin or vehicle (delivered on days 11 and 21 of chronic stress) for analysis of spleen white pulp and hippocampal microglia morphology. To evaluate if Reelin maintains antidepressant-like effects after multiple episodes or "bouts" of depression, we incorporated Reelin treatment in a cyclic chronic stress model involving multiple episodes or bouts of depression. In this model, 1 cycle involves 21 daily corticosterone injections followed by a 21-day injection-free recovery period. Antidepressant-like effects were evaluated following both 1.3 and 2.6 cycles of chronic stress and subsequent Reelin treatment. Our results indicate chronic stress atrophied spleen white pulp, reduced lengths and complexity of microglia processes and increased microglia cell body sizes, consistent with disrupted immune regulation in brain and periphery following chronic stress. Reelin treatment was associated with recovery of white pulp atrophy and normalization of morphological alterations to hippocampal microglia induced by chronic stress. In evaluating antidepressant-like effects of Reelin in the cyclic corticosterone model, we found rats treated with Reelin after chronic stress were more mobile in the forced swim test, indicating a single peripheral injection of Reelin continues to show antidepressant-like properties following both 1.3 cycles and 2.6 cycles of chronic corticosterone injections. As Reelin treatment reversed both peripheral and central inflammatory alterations induced by chronic stress and a single peripheral injection continues to have antidepressant-like properties after multiple bouts of chronic stress induced depression-like behaviour, our results support the continued assessment of Reelin-based therapeutics in the context of major depression.

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Poster

PSTR426. Treatment and Drugs for Mood Disorders

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR426.10/MM21

Topic: G.05. Mood Disorders

Title: Phenotypic profiling of psychedelics in mice using SmartCube to find the next generation of drug candidates to treat psychiatric disorders.

Authors: *A. PEHRSON, G. ZHOU, A. AMBESI, D. BRUNNER, D. BLEAKMAN, M. A. VARNEY;
PsychoGenics, Inc., Paramus, NJ

Abstract: SmartCube® is an artificial intelligence-enabled phenotypic screening platform that rapidly evaluates and categorizes the behavioral effects of drugs in mice. In partnership with Sunovion, this platform resulted in the discovery of ulotaront, a novel antipsychotic candidate currently in late-phase clinical trials. We have used SmartCube to screen a library of more than 7,000 behaviorally active compounds for similarity to psychedelic, dissociative, and entactogen reference compounds. Molecules similar to psilocin, ketamine, or MDMA served as starting points for discovery programs aimed at identifying novel treatments for major depressive disorder or post-traumatic stress disorder (PTSD) without producing psychedelic-like side effects. Mice were treated intraperitoneally with test or reference compounds and placed in SmartCube, which presents challenges and monitors behavior for 45 min. Behavioral features were identified using deep learning, and machine learning derived similarity scores from those features. Promising compounds were screened for functional activity at 5-HT_{2A} and NMDA receptors. Those with behavioral similarity to psilocin or ketamine (and without 5-HT_{2A} agonist or NMDA antagonist activity) were evaluated in the mouse tail suspension test (10/group) as a screen for antidepressant-like potential. MDMA-similar compounds were evaluated in the mouse extinction of conditioned fear test (15/group) as a predictive screen for PTSD. Finally, compounds were tested for the mouse head twitch response (10/group), which may predict hallucinogenic activity, and the rat prepulse inhibition model (15/group), which is disrupted by serotonergic hallucinogens, NMDA receptor antagonists, and stimulants like MDMA. Here we present results for PGI-33 and PGI-81, which had antidepressant-like and psychedelic similar behavioral signatures but no 5-HT_{2A} agonist or NMDA antagonist activities. Both molecules had favorable pharmacokinetic profiles, and reduced tail suspension immobility with a minimally effective dose of 3 mg/kg for PGI-33 and ≤ 10 mg/kg for PGI-81. PGI-33 also reduced freezing in the cued fear test. Neither compound induced head twitch up to 100 mg/kg nor did they impair prepulse inhibition at up to 30 mg/kg. Thus, these molecules may represent novel antidepressant-like compounds with limited potential for hallucinogenic or dissociative side effects.

Disclosures: **A. Pehrson:** A. Employment/Salary (full or part-time):; Psychogenics, Inc. **G. Zhou:** A. Employment/Salary (full or part-time):; Psychogenics, Inc. **A. Ambesi:** A. Employment/Salary (full or part-time):; Psychogenics, Inc. **D. Brunner:** A. Employment/Salary (full or part-time):; Psychogenics, Inc. **D. Bleakman:** A. Employment/Salary (full or part-time):; Psychogenics, Inc. **M.A. Varney:** A. Employment/Salary (full or part-time):; Psychogenics, Inc..

Poster

PSTR426. Treatment and Drugs for Mood Disorders

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR426.11/MM22

Topic: G.05. Mood Disorders

Title: Functional in vitro Screening of 5-HT2A receptor modulators in neuronal network cultures

Authors: O. H. SCHROEDER, L. SCHULTZ, A.-M. KNOSPE, M. WINKLER, *K. JÜGELT; NeuroProof Systems GmbH, Rostock, Germany

Abstract: The 5-HT2A receptor plays a significant role in the mode of mechanisms of psychedelic drugs. Psychedelic drugs can change the treatment paradigm for deep depression and traumatic brain injury (Khan et al. 2021).
Psychedelics cause hallucinations and are therefore called hallucinogenic drugs (Lowe et al. 2021). Hallucinogenic drugs are classified into dissociative drugs, such as dextromethorphan (DXM), ketamine and phencyclidine (PCP), and classic serotonergic and dopaminergic hallucinogens.
Psilocybin and its metabolic psilocin are studied in many psychiatric diseases, especially depression.
5-HT2A agonists demonstrate fast anti-depressive actions with unknown mechanisms responsible for their anti-depressive effects.
The 5-HT2A is a GPCR of the type Gαq with the effector PLCβ; this catalyzes phosphoinositide PI into IP3, which triggers Ca2+ release (Pottie and Stove 2022). 5-HT2A agonists exhibit a rapid effect in the CNS with a strong afterward desensitization, which is challenging for in vitro modeling.
We have tested 5-HT2A modulators in our microelectrode array approach on primary frontal cortex cultures from mice cultivated 28 days in vitro, such as risperidone, DOI, DMT, MDMA, and psilocin. We tested all 5 compounds at 5 concentrations and analyzed their response in time intervals of 15 minutes for one hour. We found a significantly different behavior in these time intervals.
Our experiments showed that psychedelics show a strong variation of activity in the first hours of application. The time course of the dose responses could be a clear descriptive biomarker for assessing psychedelics and potentially anti-depressive compounds.
References
Khan, S. M., G. T. Carter, S. K. Aggarwal, and J. Holland. 2021. 'Psychedelics for Brain Injury: A Mini-Review', Front Neurol, 12: 685085.
Lowe, H., N. Toyang, B. Steele, H. Valentine, J. Grant, A. Ali, W. Ngwa, and L. Gordon. 2021. 'The Therapeutic Potential of Psilocybin', Molecules, 26.
Pottie, E., and C. P. Stove. 2022. 'In vitro assays for the functional characterization of (psychedelic) substances at the serotonin receptor 5-HT2A R', J Neurochem, 162: 39-59.

Disclosures: O.H. Schroeder: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroProof Systems GmbH. **L. Schultz:** A. Employment/Salary (full or part-time):; NeuroProof Systems GmbH. **A. Knospe:** A. Employment/Salary (full or part-time):; NeuroProof Systems GmbH. **M. Winkler:** A. Employment/Salary (full or part-time):; NeuroProof Systems GmbH. **K. Jügelt:** A. Employment/Salary (full or part-time):; NeuroProof Systems GmbH.

Poster

PSTR426. Treatment and Drugs for Mood Disorders

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR426.12/MM23

Topic: G.05. Mood Disorders

Title: First ^{18}F radiolabeling and PET-imaging of NLX-204 in rat brain: a 5-HT$_{1A}$ receptor biased agonist with antidepressant-like activity

Authors: *V. RICHIN[1,3], C. BOUILLOT[4], S. BOUVARD[2], P. COURAULT[2,5], F. HERBRECHT[3], A. NEWMAN-TANCREDI[3], L. ZIMMER[2,5,4], W. ZEINYEH[2];
[2]Ctr. de Recherche en Neurosciences de Lyon, [1]Univ. Claude Bernard Lyon 1, Villeurbanne, France; [3]Neurolixis SAS, Castres, France; [4]CERMEP - Imagerie du vivant, Lyon, France; [5]Hospices Civils de Lyon, Lyon, France

Abstract: The serotonin 1A receptor (5-HT$_{1A}$R) is involved in central nervous system functions such as mood, pain, and motor control and in psychiatric and neurodegenerative disorders. In particular, agonist activation of 5-HT$_{1A}$R mediates the antidepressant activity of diverse classes of antidepressant drugs. NLX-204 is a highly selective 5-HT$_{1A}$R biased agonist which preferentially activates ERK1/2 phosphorylation. This molecule presents an efficacious antidepressant-like activity in a variety of mouse and rat models (1,2,3). Positron emission tomography (PET) imaging with agonist radiotracers allows exploration of the targeted receptors in their activated coupling state. PET imaging with different radiolabeled biased agonists is therefore a promising strategy to investigate whether functional selectivity for a signaling pathway *in vitro* is associated with activation of a specific region in the brain. In this study, we radiolabeled NLX-204 and performed PET imaging to explore the brain regions involved in the antidepressant-like activity of NLX-204. To produce [^{18}F]NLX-204, we developed a nitro precursor allowing an isotopic radiolabeling with a good specific activity (>444 GBq/µmol). The radiochemical yield was 1% and the chemical and radiochemical purities were >98%. MicroPET imaging in healthy Sprague-Dawley rats showed a rapid brain penetration of [^{18}F]NLX-204 within 5 min after its IV administration, with pseudo-plateau kinetics. The reproducibility of brain labeling by [^{18}F]NLX-204 was evaluated by a Test-Retest exploratory study on 4 healthy rats. Standardized Uptake Values Ratio to cerebellum (SUVR) determinations were reproducible with a variability <8% over the different regions of interest (ROIs). Radiometabolites of [^{18}F]NLX-204 were measured in rat plasma (N=2) and revealed that 69% of [^{18}F]NLX-204 remained unchanged 1 hour after IV injection. A competition study with 8-OH-DPAT (a reference 5-HT$_{1A}$R agonist) showed that 80 to 85% of [^{18}F]NLX-204 binding was blocked (N=4). Over those two studies, SUVR values ranged from 1.1 to 1.6 depending on brain region. Notable ROI labeled by [^{18}F]NLX-204 were the dorsal raphe nucleus, brain stem, thalamus and hypothalamus, hippocampus and cortical areas. Brain regions labeled by [^{18}F]NLX-204 are associated with control of mood and cognition and are consistent with the potential antidepressant properties of the drug. Future studies will investigate the relationship between these data and the therapeutic-like effects of NLX-204.
(1)Sniecikowska J, et al. J Med Chem (2019); (2)Głuch-Lutwin M, et al. Behav Brain Res (2023); (3)Papp, M., et al. Psychopharmacology (2023)

Disclosures: **V. Richin:** A. Employment/Salary (full or part-time):; Neurolixis. **C. Bouillot:** None. **S. Bouvard:** None. **P. Courault:** None. **F. Herbrecht:** A. Employment/Salary (full or

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Poster

PSTR426. Treatment and Drugs for Mood Disorders

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR426.13/MM24

Topic: G.05. Mood Disorders

Support: Centre National de la Recherche Scientifique

Title: Tcb-2, a non-selective serotonin$_{2a}$ receptor agonist reduces correlative links for neurotransmitter systems across mouse brain regions

Authors: J. J. BUTLER, M. VIRGILI, *P. DE DEURWAERDERE;
Univ. of Bordeaux, Bordeaux, France

Abstract: The mechanism of action of classical psychedelics in the brain remains unknown, in particular the possible widespread influence of these compounds on the activity of neurotransmitter systems across the brain. In mice, we studied the effect of non-selective 5-HT2A receptor (5-HT2AR) agonist TCB-2 (0.3, 3, and 10mg/kg) and the combination TCB-2 (3 mg/kg) / MDL100,907 (5-HT2AR antagonist; 0.2 mg/kg) on the tissue content of neurotransmitters [GABA, glutamate, noradrenaline (NA), dopamine (DA), serotonin (5-HT) and their metabolites] 1-h after agonist administration. Post-mortem, tissue content was measured by HPLC in 30 brain regions belonging to various neurobiological networks. The neurochemical study was preceded by the behavioural measurement of head twitches in isolated cages to determine the efficacy of the treatments. Quantitatively, TCB-2 dose-dependently decreased 5-HT turnover (usually an increase in 5-HT) in all brain regions. It reduced the ratio 3-methoxytyramine/DA in the striatum, and enhanced markers of the DA system in a few cortices (cingulate, somatosensorial) and NA in the cingulate cortex and the ventral hippocampus. Despite the ability of MDL100,907 to prevent TCB-2-induced head twitches in these animals, the decrease in 5-HT turnover induced by TCB-2 was generally insensitive to MDL100,907. However, MDL100,907 blocked TCB-2-induced 5-HT increase in the cingulate and auditory cortices and the ventral hippocampus. It also blocked some DA and NA effects notably in the anterior cingulate cortex. TCB-2 alone or combined with MDL-100,907 did not modify amino acid tissue contents. Qualitatively, TCB-2 dramatically decreased the correlative links assessed by Pearson’s correlations for a neurotransmitter between brain regions, whatever the neurotransmitter system considered. Of note, MDL100,907 also reduced the correlative links. The disruptive effect of TCB-2 was partially counteracted by MDL100907 for 5-HT, glutamate,

and GABA. Irrespective of its questionable action beyond 5-HT2AR, the data indicate that TCB-2 dramatically alters the activity of 5-HT neurons in the brain and disrupts the correlative links between brain regions for all neurotransmitters.

Disclosures: **J.J. Butler:** None. **M. Virgili:** None. **P. De Deurwaerdere:** None.

Poster

PSTR426. Treatment and Drugs for Mood Disorders

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR426.14/MM25

Topic: G.05. Mood Disorders

Title: Discovery and preclinical characterization of 2,5-dimethoxy-4-thiofluoroalkyl phenethylamines as potent and long-acting serotonin 5-HT_2 receptor agonists

Authors: *G. B. VARTY[1], C. E. CANAL[3], J. A. HARTSEL[2], R. TYAGI[3], K. AVERY[2], M. E. MORGAN[2], T. A. MUELLER[2], A. C. REICHELT[2], P. PATHARE[2], E. STANG[2], M. G. PALFREYMAN[2], A. NIVOROZHKIN[2];
[1]Cybin, Annandale, NJ; [2]Cybin, Toronto, ON, Canada; [3]Mercer Univ., Atlanta, Georgia

Abstract: Novel chemical synthesis and structure-activity studies led to the discovery of 4-thiofluoroalkyl-substituted phenylalkylamine serotonin 5-HT_2 receptor ligands with varying efficacies and durations of action in the mouse head-twitch response model of 5-HT_{2A} receptor activation. Several potent 5-HT_2 receptor ligands exhibited varying degrees of 5-HT_{2A} receptor agonism in vitro and elicited robust head-twitch responses, while other compounds did not elicit an in vivo response despite binding to the 5-HT_{2A} receptor in vitro. The phenylalkylamine, 2,5-dimethoxy-4-(trifluoromethyl)thiophenethylamine (CYB210010) is an example molecule with high agonist potency at 5-HT_{2A} and 5-HT_{2C} receptors, modest agonist selectivity over 5-HT_{2B}, 5-HT_{1A}, 5-HT_6, and α_{2A} receptors, and >100-fold binding selectivity over 70+ other proteins including monoamine transporters. CYB210010 dosed either subcutaneously or orally elicited a robust, dose-dependent head-twitch response, and could be administered sub-chronically at threshold doses that occupy brain 5-HT_{2A} receptors without any behavioral tolerance. In studies measuring target engagement and gene expression, CYB210010 occupied frontal cortical 5-HT_{2A} receptors and increased the expression of genes implicated in neuroplasticity in the frontal cortex, but not the hippocampus. Pharmacokinetic studies demonstrated that CYB210010 was orally bioavailable in three species, exhibited high brain penetration, and similar pharmacokinetic profiles in three compartments; plasma, brain, and CSF. Based on these properties, CYB210010 represents an important molecule for investigating the downstream effects of serotonergic receptor activation and the therapeutic potential of serotonin 5-HT_2 receptor agonists administered via sub-psychedelic chronic dosing regimens.

Disclosures: **G.B. Varty:** A. Employment/Salary (full or part-time):; Cybin. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder,

excluding diversified mutual funds); Cybin. **C.E. Canal:** A. Employment/Salary (full or part-time):; Mercer 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.; Cybin, Mercer University. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cybin. **J.A. Hartsel:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cybin. **R. Tyagi:** 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.; Mercer University. **K. Avery:** A. Employment/Salary (full or part-time):; Cybin. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cybin. **M.E. Morgan:** A. Employment/Salary (full or part-time):; Cybin. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cybin. **T.A. Mueller:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cybin. **A.C. Reichelt:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cybin. **P. Pathare:** A. Employment/Salary (full or part-time):; Cybin. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cybin. **E. Stang:** A. Employment/Salary (full or part-time):; Cybin. **M.G. Palfreyman:** 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.; Cybin. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cybin. **A. Nivorozhkin:** A. Employment/Salary (full or part-time):; Cybin. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cybin.

Poster

PSTR426. Treatment and Drugs for Mood Disorders

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR426.15/NN1

Topic: G.05. Mood Disorders

Title: Novel 5-HT2A receptor agonists exhibit translational antidepressant and psychedelic drug-like profiles in a model of treatment-resistant depression

Authors: *C. A. BOWEN, I. PREMOLI, T. A. KHAN, R. PERNI, P. KLEINE, S. RAO, G. F. SHORT, III;
EntheogeniX Biosciences, Inc., Encinitas, CA

Abstract: Mood disorders, particularly treatment-resistant depression, remain a significant unmet medical need. Clinical and preclinical research suggest the potential for serotonergic psychedelic compounds, such as psilocybin, to produce rapid and lasting antidepressant activity after a single dose. Increased rapid eye movement (REM) sleep is a hallmark of depression in humans that is suppressed by antidepressant treatment. Wistar Kyoto (WKY) rats exhibit behavioral, neurobiological and endocrine phenotypes that are consistent with symptoms observed in clinical depression. In particular, this strain shows increased REM sleep that is resistant to suppression by antidepressant drugs, making it a potentially useful genetic model of treatment-resistant depression. Novel hallucinogenic 5-HT2A receptor agonists discovered using an artificial intelligence (AI)/machine learning (ML) approach were investigated for antidepressant drug-like effects using translational electroencephalography (EEG)-based measures in the WKY rat model of treatment-resistant depression. Adult male WKY rats (n=7) were implanted with epidural EEG electrodes, an electromyography (EMG) electrode and an intraperitoneal telemetry transmitter to record EEG, EMG, body temperature and locomotor activity. During weekly testing, at approximately 2 hours after light onset, rats received one of the test conditions (negative control, psilocybin positive control (10 mg/kg IP), or novel 5-HT2A agonist EGX-A (3-30 mg/kg IP) or EGX-B (1-30 mg/kg IP)) in a pseudo-randomized cross-over fashion. EEG recording continued for at least 22 hours after dosing. Automatic sleep-wake scoring was performed, and the hourly distribution of wakefulness, non-REM (NREM) and REM sleep was determined. In addition, sleep latency and spectral frequency power by vigilance state was analyzed. Compared to vehicle, significant increases in latency to REM sleep and decreases in the amount of REM sleep were found with psilocybin, EGX-A and EGX-B, indicative of antidepressant drug-like responses. Also similar to psilocybin, EGX-A and EGX-B significantly decreased NREM low-frequency delta and theta power. The profiles of EGX-A and EGX-B on high-frequency gamma power during wake, locomotor activity and body temperature were distinguished from each other and psilocybin. The data suggest that these novel 5-HT2A receptor agonists exhibit significant, dose-related antidepressant and hallucinogenic drug-like activity in a model of treatment-resistant depression and demonstrate the use of translational EEG-based measures in antidepressant drug discovery.

Disclosures: **C.A. Bowen:** A. Employment/Salary (full or part-time):; Employee of atai Life Sciences, EntheogeniX Biosciences, Inc., is an atai platform company. **I. Premoli:** A. Employment/Salary (full or part-time):; Employee of atai Life Sciences, EntheogeniX Biosciences, Inc., is an atai platform company. **T.A. Khan:** A. Employment/Salary (full or part-time):; Employee of atai Life Sciences, EntheogeniX Biosciences, Inc., is an atai platform company. **R. Perni:** F. Consulting Fees (e.g., advisory boards); Consultant for atai Life Sciences; EntheogeniX Biosciences, Inc., is an atai platform company. **P. Kleine:** Other; Paid intern of atai Life Sciences; EntheogeniX Biosciences, Inc., is an atai platform company. **S. Rao:** A. Employment/Salary (full or part-time):; Employee of atai Life Sciences, EntheogeniX Biosciences, Inc., is an atai platform company. **G.F. Short:** A. Employment/Salary (full or part-time):; Employee of atai Life Sciences, EntheogeniX Biosciences, Inc., is an atai platform company.

Poster

PSTR426. Treatment and Drugs for Mood Disorders

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR426.16/NN2

Topic: G.05. Mood Disorders

Title: Psilocybin: the relationship between head twitch response, locomotor activity, 5-HT2A ex vivo receptor occupancy and plasma and brain levels of psilocin

Authors: *S. CHEETHAM[1], M. ACKLEY[2], E. BILLINGHAM[1], M. BURNETT[1], A. CARTER[1], A. CHAND[1], M. CONWAY[1], I. DAVIES[1], G. LAI[2], T. PISER[2], A. PLUMB[1], D. RIAL[1], U. SHAH[2], C. TAYLOR[1], S. VICKERS[1], L. JAGGER[1];
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Abstract: The use of psychedelics, such as, psilocybin, as therapy for mental health disorders is receiving significant interest. The mode of action of psychedelics may involve 5-hydroxytryptamine$_{2A}$ (5-HT$_{2A}$) agonism. Psilocybin, a prodrug, is metabolised to psilocin which has moderate affinity for mouse frontal cortical 5-HT$_{2A}$ receptors (inhibition constant: K_i = 58 ± 7 nM; Hill slope 0.87 ± 0.03: mean ± sem, n=5). We have evaluated the effect of psilocybin on head twitches, locomotor activity, 5-HT$_{2A}$ ex vivo receptor occupancy and plasma and brain levels of psilocin in male C57BL6J mice (20 - 25g). Psilocybin (0.3 - 3 mg/kg sc) was dosed to mice and head twitches manually counted by blinded observers and locomotor activity measured using Ethovision. In a second study, mice given psilocybin (1 - 10 mg/kg ip) were terminated one hour later by a schedule 1 method and a post-mortem blood sample collected and plasma prepared by centrifugation. Frontal cortex and rest of brain were dissected and frozen on dry ice. Frontal cortex was homogenised in 50 mM Tris-HCl, pH 7.4 containing 4 mM $CaCl_2$ (3.75 mg wet weight of tissue/ml) and incubated with [^{3}H]cimbi-36 (0.075nM) for 30 minutes at room temperature. Plasma and brain levels of psilocin were measured in the rest of brain by mass spectrometry. Psilocybin (0.3 - 3 mg/kg sc) increased the number of head twitches (Vehicle 0.90 ± 0.52; psilocybin 0.3 mg/kg 13 ± 1; 1 mg/kg 18 ± 1; 3 mg/kg 19 ± 1; all $p<0.001$ vs vehicle; values are mean number of head twitches in 1 hour ± sem; n=7-8). This was associated with a significant decrease in locomotor activity at the highest dose (Vehicle 6583 ± 212; psilocybin 0.3 mg/kg 7043 ± 312; 1 mg/kg 7005 ± 436, 3 mg/kg 3503 ± 523, $p<0.001$ vs vehicle; values are distance travelled in 30 minutes in cm ± sem; n=7-8) with less time spent in the central zone of the arena (Vehicle 6583 ± 212; psilocybin 0.3 mg/kg 7043 ± 312; 1 mg/kg 7005 ± 436 $p<0.05$, 3 mg/kg 3503 ± 523 $p<0.001$; values are sum of time/pixel; n=7-8). Frontal cortical 5-HT$_{2A}$ receptor occupancy increased with increasing dose of psilocybin (Vehicle 1174 ± 24, 0%; psilocybin 1 mg/kg 1080 ± 24, 8%, $p<0.01$; 3 mg/kg 1009 ± 24, 14%; $p<0.001$; 10 mg/kg 811 ± 26, 31%, $p<0.001$ (values are mean specific binding (dpm) ± sem, mean receptor occupancy (%); n = 5-8). There was a good correlation between plasma and brain levels of psilocin and ex vivo receptor occupancy. Thus, psilocybin increases head twitch behaviour which is associated with an increase in 5-HT$_{2A}$ receptor occupancy which correlates with plasma and brain levels of psilocin. Rigorous in vitro, ex vivo and in vivo characterisation of psychedelics may prove useful in the discovery and screening of novel therapeutics acting at the 5-HT$_{2A}$ receptor.

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Poster

PSTR426. Treatment and Drugs for Mood Disorders

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR426.17/NN3

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant R01MH127737
NIH Grant R01AG071787
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Max Planck Fellowship

Title: Distinct behavioral effects of a serotonergic psychedelic and a non-hallucinogenic analog

Authors: K. LUKASIEWICZ[1], ***J. LU**[2], J. BAKER[2], Y. ZUO[2];
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Abstract: Psychedelics induce profound changes in conscious experiences and have shown promise as therapeutics for neuropsychiatric disorders. Human studies suggest long-lasting psychological impacts of psychedelics on both healthy individuals and psychiatric patients. However, it is challenging to disambiguate biological, experiential, and placebo effects due to psychedelic-induced perturbations to consciousness. Animal models may allow a clearer understanding of the biological effects of psychedelics on behaviors that are less reliant on conscious experience. Computational neuroethology approaches are uniquely advantageous for such studies as they provide a comprehensive, unbiased assessment of behavioral patterns in animals. In this study, we characterized the exploratory behavior of mice using an unsupervised machine learning algorithm and revealed distinct acute and long-term effects of a classical serotonergic psychedelic (2,5-dimethoxy-4-iodoamphetamine hydrochloride, DOI) and a recently synthesized non-hallucinogenic psychedelic analog (tabernanthalog, TBG). This approach can be applied to studies on psychedelics' effects on mouse spontaneous behaviors in the context of aging, neurodevelopmental, and neuropsychiatric disorders.

Disclosures: K. Lukasiewicz: Other; .PROT Ltd, Bialystok, Poland.. **J. Lu:** None. **J. Baker:** None. **Y. Zuo:** None.

Poster

PSTR426. Treatment and Drugs for Mood Disorders

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR426.18/Web Only

Topic: C.01. Brain Wellness and Aging

Title: Theoretical-computational study and molecular modelling of the interactions between DMT-derived psychedelic ligands as potential natural serotonergic agonist antidepressant candidates on 5-HT-type receptors in neuropharmacological systems.

Authors: *A. F. LATORRE PALOMINO;
Chem., Univ. Nacional Mayor San Marcos, Lima, Peru

Abstract: Depression, according to WHO, affects about 280 million people worldwide. This disease is characterized by altered levels of serotonin uptake in the body, the use of natural psychedelics such as serotonergic derivatives containing the Amazonian preparation ayahuasca (DMT, harmine and harmaline) has potential and promising effects against depression. However, to our knowledge, there are few theoretical-computational studies of affinity mechanisms of these derivatives for serotoninergic receptors of the 5HT type (5-HT1A, 5-HT2A, 5-HT1B, 5-HT2B, sigma 1 and the serotonin transporter SERT). Therefore, it is proposed to theoretically evaluate their chemical reactivity and to analyze, by molecular docking, DFT and molecular modeling, the natural ligands derived from the pharmacophore N,N-dimethyltryptamine (DMT): (serotonin, N-methylserotonin, psilocin, 5-MeO-DMT, bufotenin, psilocybin, baeocystin, norbaeocystin, LSD and mescaline) on the mentioned receptors to choose the ligand/receptor systems that present lower energies and interact with the residues of interest of the receptors. Subsequently, we will evaluate temporal stability, free and interaction energies for the selected systems using molecular mechanics and quantum mechanical calculations. With this we hope to estimate their efficiency as possible serotonergic agonists and propose them as antidepressant drug candidates. In addition to the molecular approach, we intend to contribute to the field of study of psychedelics by using the database of interaction of the neurotransmitter seronotonin with the receptors of interest, as well as taking advantage of the availability of data collected using psylocibin, DMT and LSD, thus giving a total-brain analysis approach to determine specific regions that respond to the use of psychedelics in depression

Disclosures: A.F. Latorre Palomino: None.

Poster

PSTR426. Treatment and Drugs for Mood Disorders

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR426.19/NN4

Topic: G.05. Mood Disorders

Title: Preclinical and translational profile of GM-1020, a novel, orally bioavailable NMDA antagonist

Authors: *Z. A. HUGHES[1], A. KLEIN[1], D. DVORAK[1], E. AUSTIN[1], M. PAPP[2], J. SPORN[1], S. GATTI[3], G. J. MAREK[1], L. KISS[1], A. KRUEGEL[1];
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Abstract: Despite ketamine providing rapid and sustained antidepressant efficacy, patient access is limited by poor bioavailability and sedative and dissociative side effects. Here we describe the novel NMDA receptor (NMDAR) antagonist, GM-1020 which was designed to be orally bioavailable and cause limited sedation and dissociation at therapeutic doses.
GM-1020 displaces [^{3}H]MK-801 binding in rat cortex (Ki = 3.25 μM) and is a voltage-dependent antagonist of human GRIN1/GRIN2A-containing NMDAR (IC50 = 1.19 μM at -70 mV and 265.21 μM at +60 mV). Oral bioavailability (%F) ranged from 27-43% in 4 non-clinical species which was predicted by microsomal clearance (%F=32-52%); human %F was predicted to be >60%.Antidepressant efficacy was demonstrated in Wistar Han rats exposed to the chronic mild stress paradigm (CMS). Exposure of rats to CMS produced a robust reduction in sucrose intake that was maintained throughout exposure to stress in vehicle treated rats. GM-1020 (1.5-9 mg/kg, i.p.), like ketamine (10 mg/kg, i.p.) produced a restoration of sucrose intake in stressed rats 24 h after a single administration. Efficacy was maintained through 5 weeks of once weekly dosing. After cessation of drug treatment efficacy of GM-1020 and ketamine was maintained for ≥2 weeks despite continued exposure to stressors. In a separate study GM-1020 and ketamine (both 3.2-32 mg/kg, s.c.) were evaluated for ataxia in the rotarod assay in SD rats. Both compounds produced dose-dependent ataxia (decreased latency to fall); for GM-1020 the ED50 = 17.4 mg/kg, while for ketamine ED50 = 3.4 mg/kg. A cohort of SD rats implanted with skull screw electrodes above the frontal cortex, was used to test the effects of GM-1020 (1-10 mg/kg, s.c.) on EEG spectral power. GM-1020 increased gamma and decreased theta power at exposures associated with antidepressant efficacy.
In summary, GM-1020 had rapid-onset, durable efficacy in CMS at plasma concentrations ~14-fold lower than ataxic doses. In contrast the dose of ketamine that was efficacious in CMS produced plasma concentrations ~35% lower than those that produced ataxia acutely after dosing. After dosing with GM-1020 EEG spectral power changed in a plasma concentration-dependent manner indicating that decreases in low frequency and increases in high frequency power provide translational biomarkers of NMDA receptor target engagement. Quantitative EEG can be used clinically to determine whether significant target engagement can be achieved without causing dissociation or sedation.

Disclosures: Z.A. Hughes: None. **A. Klein:** A. Employment/Salary (full or part-time):; Gilgamesh Pharmaceuticals. **D. Dvorak:** A. Employment/Salary (full or part-time):; Gilgamesh Pharmaceuticals. **E. Austin:** A. Employment/Salary (full or part-time):; Gilgamesh Pharmaceuticals.com. **M. Papp:** None. **J. Sporn:** A. Employment/Salary (full or part-time):; Gilgamesh Pharmaceuticals. **S. Gatti:** None. **G.J. Marek:** A. Employment/Salary (full or part-time):; Gilgamesh Pharmaceuticals. **L. Kiss:** A. Employment/Salary (full or part-time):; Gilgamesh Pharmaceuticals. **A. Kruegel:** A. Employment/Salary (full or part-time):; Gilgamesh Pharmaceuticals.

Poster

PSTR426. Treatment and Drugs for Mood Disorders

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Program #/Poster #: PSTR426.20/NN5

Topic: G.05. Mood Disorders

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JSPS JP22J13259 (R.Y.)
JSPS JP22KJ2161 (R.Y.)

Title: R-ketamine enhances anterior insular cortex activity associated with social cognition

Authors: *R. YOKOYAMA[1], Y. AGO[2], M. TANUMA[1], Y. SHIMAZAKI[1], K. SEIRIKI[1], T. NAKAZAWA[3], K. HASHIMOTO[4], A. KASAI[1], H. HASHIMOTO[1];
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Abstract: Recent studies have revealed that (R)-ketamine, the enantiomer of (S)-ketamine (esketamine) which is an FDA-approved antidepressant, may be effective for treating depression and the associated cognitive dysfunction. Although many studies provide insights into the neural mechanisms of racemic ketamine and (S)-ketamine, the mechanism of (R)-ketamine remains largely unclear. We have found that (R)-ketamine has more potent antidepressant-like effects than (S)-ketamine in rodent models of depression. Then, to identify brain regions that contribute to effects of (R)-ketamine, we have performed brain-wide mapping of immediate early gene Arc expression and investigated the effects of temporary suppression of neural activity by Gi-DREADD in social isolation-reared male C57BL6/J mice treated with the ketamine enantiomers. Our previous results suggest that activation of the anterior insular cortex (aIC) is necessary to exert the effects of (R)-ketamine on immobility in the forced swim test (SfN Annual Meeting: Neuroscience 2022). In this study, we aimed to clarify the roles of aIC activation in the effects of (R)-ketamine on social cognition. First, we analyzed aIC calcium activity by using fiber photometry before and after administration of the ketamine enantiomers in the three-chamber test. Isolation-reared mice treated with saline exhibited a similar aIC activity pattern after contact with an object and mouse. (R)-ketamine, but not (S)-ketamine, caused a significant increase in aIC activity after contact with a mouse compared with the activity after contact with an object, similar to group-reared mice treated with saline. We next conducted the 5-trial social memory test in social isolation-reared mice expressing Gi-DREADD in the aIC. Under a vehicle-treated normal condition, (R)-ketamine rapidly decreased the time investigating the same intruder, indicating promoting social memory formation. Under DREADD-ligand CNO-treated condition which inhibited aIC activity, there was no difference in investigation time between mice treated with saline and (R)-ketamine. Our results indicate that activation of the aIC plays an important role in mediating the effects of (R)-ketamine on social cognitive behaviors and might provide a promising mechanism for restoring cognitive dysfunction in depression.

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Poster

PSTR426. Treatment and Drugs for Mood Disorders

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Topic: G.05. Mood Disorders

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U.S. Department of Veterans Affairs Merit Awards 1I01BX004062
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Title: Metaplastic effects of ketamine metabolite (2R,6R)-hydroxynorketamine on hippocampal synaptic plasticity in mice

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Abstract: Ketamine metabolite (*2R,6R*)-hydroxynorketamine (HNK) maintains the rapid and prolonged preclinical antidepressant-like effects of ketamine without adverse effects and has completed human phase I clinical trials. Metaplasticity is an alteration in plasticity induced by previous synaptic activity that influences synaptic function and may mediate sustained antidepressant action. To study *in vitro* HNK metaplastic effects, we incubated slices collected from mice with HNK for 60 min followed by ACSF washout for 35 min. To study the sustained effects of HNK *in vivo,* mice were treated with HNK and sacrificed for electrophysiology experiments 24 h later. Primary outcomes assessed were input/output excitatory postsynaptic potential slope (fEPSP), paired-pulse facilitation (PPF), and long-term potentiation (LTP). Incubation with HNK enhanced presynaptic-mediated synaptic transmission at the Schaffer collateral-CA1 synapse in a concentration-dependent manner as indicated by enhanced basal fEPSP responses and reduced PPF while also impairing LTP magnitude. These effects were blocked by preincubation with AC or PKA inhibitors. Preincubation with the NMDAR antagonist D-APV neither blocked HNK's effects nor exerted metaplastic effects on its own, and

no effects were observed with ketamine itself. *Ex vivo* recordings revealed dose-dependent enhancement of LTP without significant alterations in basal synaptic transmission or PPF. Our *in vitro* findings suggest that rapid HNK effects are induced by a presynaptic mechanism that enhances glutamatergic transmission whereas our *ex vivo* results suggest sustained HNK effects are maintained by a postsynaptic metaplastic mechanism. These findings provide insight into HNK's rapid and sustained mechanism of action that may facilitate novel antidepressant discovery.

Disclosures: K.A. Brown: None. **T.D. Gould:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); TDG has received research funding from Allergan Pharmaceuticals during the preceding 3 years.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); TDG is listed as an inventor in patents and patent applications related to the pharmacology and use of a ketamine metabolite, (2R,6R)-hydroxynorketamine, in the treatment of depression, anxiety, anhed.

Poster

PSTR426. Treatment and Drugs for Mood Disorders

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

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Topic: G.05. Mood Disorders

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National Natural Science Foundation of China (81625022, 91853205, 81821005)
Shanghai Municipal Health Commission in China (18431907100 and 19XD1404700)

Title: Structural basis of ketamine action on human NMDA receptors

Authors: *Y. ZHANG[1,2], F. YE[3], T. ZHANG[1,2], S. LV[1,2], L. ZHOU[2,4], F. GUO[4], C. LUO[2,4], S. ZHU[1,2];
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Abstract: Ketamine is a non-competitive channel blocker of N-methyl-d-aspartate (NMDA) receptors. A single sub-anaesthetic dose of ketamine produces rapid (within hours) and long-

lasting antidepressant effects in patients who are resistant to other antidepressants. Nevertheless, the precise mechanism by which ketamine binds to NMDA receptors remains to be elucidated. In this study, we first resolved the three-dimensional structure of the ketamine-bound human NMDA receptor by single particle cryo-electron microscopy (cryo-EM), and uncovered the binding pocket of ketamine is located within the central vestibule between the channel gate and selectivity filter in the transmembrane domain. Furthermore, combining site-directed mutagenesis and electrophysiological recordings, we found that mutations of asparagine 616 on GluN1 (N1-N616) and leucine 642 on GluN2A (2A-L642) largely reduced the potency of ketamine inhibition on NMDA receptors. Molecular dynamics simulation further revealed that two hydrophobic groups of ketamine (phenyl ring and cyclohexane) formed hydrophobic interaction with 2A-L642, while the amino-group of ketamine selectively formed hydrogen bond with N1-N616. In addition, the ketamine derivatives with modification of ketone group and chlorine still effectively inhibit the activity of NMDA receptor, indicating that ketone group and chlorine were not necessary for ketamine to bind with NMDA receptor. Altogether, we propose the mechanism of ketamine action on NMDA receptor: ketamine forms hydrophobic interaction with its hydrophobic groups facing 2A-L642 on the sides of vestibule, and hydrogen bond with its amino-group pointing to N1-N616 at the bottom of vestibule to bind in the vestibule and block ion flow. Based on our structure, we conducted high-throughput virtual screening in the chemical databases, and identified 102 candidate compounds likely to bind in the vestibule of NMDA receptor. Electrophysiological recordings validated seven compounds with inhibition potency comparable to ketamine. Preliminary behavioral tests showed that one compound elicited rapid antidepressant effects in mice with chronic restraint stress (CRS) one hour post drug administration. These findings show structurally how ketamine binds to and acts on human NMDA receptors, and pave the way for the future development of ketamine-based antidepressants.

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Poster

PSTR426. Treatment and Drugs for Mood Disorders

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Program #/Poster #: PSTR426.23/NN8

Topic: G.05. Mood Disorders

Support: IRP-NIMH-NIH
ZIA MH002857

Title: Predictors from initial ketamine administration with neuropsychiatric patients for long-term ketamine treatment utilization

Authors: A. TILLMAN[1], K. HURST[1], D. GREENSTEIN[1], *J. GILBERT[2], C. ZARATE, Jr.[1], E. BALLARD[1];
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Abstract: Depression is the leading psychiatric cause of morbidity and disability, with few rapid-onset treatments. Ketamine is a glutamatergic modulator that can produce rapid improvements in depression symptoms within hours of administration. Mixed evidence has linked the dissociative effects of ketamine with better antidepressant response. Community utilization of ketamine and esketamine has increased, but less is understood about who seeks out ketamine treatment and why. Participants from the National Institute of Mental Health (NIMH) represent some of the first individuals to receive ketamine in a controlled trial, providing a unique opportunity for long-term follow-up. We explored the association between ketamine's antidepressant and dissociative effects observed during NIMH clinical trials and patients' decision to receive ketamine post-discharge. Follow-up assessments were completed by 41 adults (65% female) with unipolar depression (n=28) or bipolar disorder (n=13) who had previously participated in NIMH ketamine trials, including two-period placebo-controlled crossover trials (n=29). All trials measured antidepressant response using the Montgomery-Asberg Depression Rating Scale (MADRS) or the Hamilton Depression Rating Scale (HAM-D); dissociation using the Clinician-Administered Dissociative States Scale (CADSS); and anhedonia using the Snaith-Hamilton Pleasure Scale (SHAPS). Logistic regression was used to determine if depressive symptoms one day post-ketamine or dissociation immediately post-ketamine was associated with ketamine and/or esketamine utilization in the years post-discharge. A separate logistic regression analysis was performed to control for placebo response in the crossover studies. Each model included time post-discharge, study, and baseline measures as covariates. We did not detect relationships between either depressive symptoms or dissociation (all p values > 0.10) post-ketamine and ketamine/esketamine utilization post-discharge. Results were similar when limited to participants from two-period crossover trials, controlling for placebo response. Leveraging data collected over 20+ years, we did not find relationships between ketamine response in inpatient clinical trials and subsequent ketamine/esketamine use in the community. Due to the length of follow-up, data are limited by the relatively low recruitment rate. Future research is needed to examine other factors that may impact patients' ketamine treatment post-discharge, including logistical/financial barriers to access and long-term symptom profiles.

Disclosures: A. Tillman: None. **K. Hurst:** None. **D. Greenstein:** None. **J. Gilbert:** None. **C. Zarate:** None. **E. Ballard:** None.

Poster

PSTR426. Treatment and Drugs for Mood Disorders

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Topic: G.05. Mood Disorders

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Title: A simple protein biomarker may reveal a continuity of antidepressant properties, at the cellular level, for monoaminergic antidepressants, ketamine and psychedelics.

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Abstract: The heterotrimeric G protein, Gsalpha (Gsα), when ensconced in lipid rafts shows impaired stimulation of adenylyl cyclase (AC). Both decreased Gsα-AC coupling and an increase in lipid raft Gsα are observed in MDD subjects. Antidepressants (AD) accumulate in, and trigger the translocation of Gsα out of lipid rafts resulting in the sustained cAMP elevation observed during antidepressant treatment. "Rapid-acting" ADs (e.g) ketamine, show this effect on an accelerated timescale. Clinical data suggest that psychedelics and dissociative compounds have similar properties with a variable speed of onset and variable durability of effect. We hypothesize that increased accumulation of Gsα in lipid rafts is a biomarker for depression and that the translocation of Gsα from those rafts is a biomarker for clinical response to AD. Platelets were collected from studies with depressed subjects, treated with various ADs. Activation of adenylyl cyclase by Gsα was determined by comparing activity in the presence of prostaglandin E1 (PGE1) vs no activator. Greater lipid raft localization of Gsα is reflected by less PGE1 activation of the enzyme. Statistical measures were used to compare pre- and post-treatment MDD severity (measured by HAM-D, MADRS and QIDS) with PGE-1 activation of AC. AD effects on Gsα-mediated cAMP accumulation were also measured in cultured neurons and glia, exposed to ADs and psychedelic compounds. These preclinical studies allow assessment of speed of onset and durability of AD effect. Preclinical studies on cultured neural or glial cells, with escitalopram, desipramine, ketamine, psilocin, LSD and MDMA, all showed augmented Gsα activation of AC; mood stabilizers and antipsychotics had no effect. While the monoaminergic ADs required 3 days to achieve this effect, ketamine required 15 minutes and the psychedelics, one hour. Only the psychedelics had a persistent effect. In two clinical studies, platelets from the first study showed Gsα-activated AC (but not intrinsic enzyme activity) was elevated in healthy controls vs. depressed subjects. Subjects responding to AD therapy returned to the level of PGE1 activation seen in healthy controls where non-responders were unchanged in this biomarker value. Similar results were seen in the second trial.
Lipid-raft localization of Gsα, as determined by Gsα-activated AC, is a potential biomarker for depression and therapeutic success of ADs. While the onset and durability of action differ between traditional ADs, ketamine and psychedelics, the cellular model system may be a useful tool to predict antidepressant properties of a compound.

Disclosures: A. Gunay: None. **S.D. Targum:** None. **W. Dunn:** A. Employment/Salary (full or part-time):; UCLA. **O.A. Ajilore:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); KeyWise AI, Inc.

F. Consulting Fees (e.g., advisory boards); Boehringer Ingelheim, Embodied Labs, Blueprint, Sage Therapeutics, Otsuka USA. **M.M. Rasenick:** A. Employment/Salary (full or part-time):; Veterans Administration. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Lundbeck. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pax Neuroscience.

Poster

PSTR426. Treatment and Drugs for Mood Disorders

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR426.25/NN10

Topic: G.05. Mood Disorders

Title: Ropanicant (SUVN-911), an α4β2 nAChR antagonist: A phase-2a study evaluating the safety and efficacy in participants with moderate to severe major depressive disorder

Authors: *V. PALACHARLA, V. BENADE, J. RAVULA, S. JETTA, V. GOYAL, A. MOHAMMED, R. SUBRAMANIAN, S. PANDEY, P. JAYARAJAN, A. SHINDE, R. NIROGI;
Suven Life Sci. Ltd, Hyderabad, India

Abstract: Ropanicant (SUVN-911) is a potent and selective α4β2 nAChR antagonist and has demonstrated more than more than 100 fold selectivity for α4β2 nAChR receptors (NovaScreen selectivity panel and closely related α3β4 nAChR receptors). Ropanicant showed good oral bioavailability in tested preclinical species (rats, mice and dogs). Upon oral administration in rats, it showed good brain exposures that translated well into dose dependent receptor occupancy at α4β2 receptors. Good oral exposures translated into robust antidepressant like effects in animal models of depression and significantly increased cortical serotonin and norepinephrine levels. It showed faster onset of antidepressant activity, enhanced cognitive function and did not induce sexual dysfunction in animal models thus, addressed major limitations of the currently used antidepressants. Ropanicant showed good margin of safety in toxicity studies and is non-mutagenic and non-clastogenic in nature. Ropanicant was well tolerated and safe up to the highest tested dose of 60 mg single dose and 45 mg once daily for 14 days in humans. Ropanicant has shorter half-life in humans following once daily (QD) dosing, and twice a day (BID) dosing could provide sufficient target coverage and maximize the therapeutic potential. An open-label parallel group study to evaluate the safety and efficacy of ropanicant in participants with moderate to severe major depressive disorder (MDD) is being initiated. The primary objective of the study is to evaluate the safety of ropanicant upon BID dosing. The secondary objectives include assessment of ropanicant treatment in reducing depressive symptoms and to evaluate pharmacokinetics. Approximately 36 participants will be randomized to receive ropanicant either 45 mg QD, 30 mg BID, or 45 mg BID in a ratio of 1:1:1. Following a screening period of up to 4 weeks, participants will be treated for 2 weeks. Safety assessments will include adverse events, physical examination, vital signs, ECG, clinical laboratory tests, and suicidal ideation/behavior evaluation by Columbia Suicidal Severity Rating Scale (C-SSRS). The

efficacy assessments will include change from baseline in Montgomery-Asberg Depression Rating Scale (MADRS) and Clinical Global Impression of severity (CGI-S). Pharmacokinetics will be evaluated on day 1 and day 14 in subjects receiving BID dosing. This open-label study will be a preface to a large double-blind placebo controlled study of ropanicant in participants with MDD.

Disclosures: V. Palacharla: A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **V. Benade:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **J. Ravula:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **S. Jetta:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **V. Goyal:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **A. Mohammed:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **R. Subramanian:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **S. Pandey:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **P. Jayarajan:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **A. Shinde:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **R. Nirogi:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd..

Poster

PSTR426. Treatment and Drugs for Mood Disorders

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR426.26/NN12

Topic: G.03. Motivation

Support: NIH Grant R01MH108605

Title: Tnf antagonism enhances motivation and dmpfc encoding of subjective value in patients with major depression and high inflammation

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Abstract: Background: It has been shown that chronic inflammation may act on the cortical-striatal effort-based decision-making circuitry, namely the dorsal anterior cingulate cortex, to aggravate motivational deficits in individuals with Major Depression (MD). In our study, a single dose challenge of infliximab, a TNF antagonist that reduces inflammation, was given to patients with MD and high inflammation, to study the drug's effect on motivation deficits and dACC functioning.Methods: 37 medically stable, unmedicated, MD patients with high inflammation (C-Reactive Protein > 3.0 mg/l) participated in a double-blind, placebo-controlled randomized clinical trial (NCT03006393). A reward/effort-based decision-making task was

administered during an fMRI scan, both before infliximab/placebo infusion and 14 days post-infusion. Using a group-by-time comparison, we examined changes and correlations in (1) behavioral effort/reward choices and the effort discounting parameter from a subjective value model; (2) brain signals in the cortical-striatal network, especially the dACC; as well as (3) peripheral levels of TNF inflammation markers.Results: Compared to the placebo group, patients who received infliximab showed significant decreases, from baseline to 14-days post-infusion, in behavioral effort discounting ($p < .05$) and peripheral TNFRII ($p < .005$) levels. Increased willingness to expend effort was associated with greater reductions in TNFRII ($p = 0.017$) and anhedonia symptoms ($p = 0.083$). Decreases in effort discounting ($p < .001$) and TNF ($p = 0.017$) were additionally associated with decrease in the level of dACC encoding of the subjective value of monetary reward. Moreover, dACC subjective value encoding played a mediating role in the correlation between TNFRII and effort expenditure behavior (bootstrapped CI: [-1.7, -.01]). Finally, using gene expression maps from the Allen Brain Atlas, we found that the areas impacted by infliximab showed greater expression of TNF as compared to other inflammatory markers.Conclusion: Our results suggest that, for MD patients with high inflammation, a single dose of infliximab may lead to decreased effort discounting and increased motivation. These results may be mediated by the reduced engagement of dACC during reward/effort-based decision making.

Disclosures: **S. Liu:** None. **S. Betters:** None. **J. Kubert:** None. **S. Etuk:** None. **J.A. Cooper:** None. **E. Haroon:** None. **J. Felger:** None. **A.H. Miller:** F. Consulting Fees (e.g., advisory boards); Cerevel Therapeutics, Sirtsei Pharmaceutical. **M.T. Treadway:** F. Consulting Fees (e.g., advisory boards); Boehringer Ingelheim, Neumora.

Poster

PSTR426. Treatment and Drugs for Mood Disorders

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR426.27/Web Only

Topic: G.05. Mood Disorders

Support: JSPS KAKENHI 22K15764

Title: The mood-improving effects and changes in brain activity brought about by visual stimulation with images of the natural environment in patients with depressive and anxiety disorders: A near-infrared spectroscopy study

Authors: *T. MIZUMOTO[1], H. IKEI[2], K. HAGIWARA[1], T. MATSUBARA[1], F. HIGUCHI[1], M. KOBAYASHI[1], T. YAMASHINA[1], J. SASAKI[1], N. YAMADA[1], N. HIGUCHI[1], K. HARAGA[1], F. KIRIHARA[1], C. CHEN[1], Y. MIYAZAKI[2], S. NAKAGAWA[1];
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Abstract: Introduction: Whereas nature therapies have been attracting much attention, few studies have examined their effects in clinical patients. Here, we aimed to examine the mood-improving effects of viewing images of nature and the accompanying brain activation changes in patients with depressive and anxiety disorders. Methods: We conducted a randomized crossover trial with sixty adult outpatients diagnosed with a depressive or anxiety disorder based on DSM-5. Subjects were randomized to view images of nature and city in a counterbalanced order. We used greenish natural images for nature and building-centered urban images for city (as control images). Each intervention had 12 images presented for 3 minutes in total. Frontal brain activity, specifically orbitofrontal cortex (OFC), was measured using 2-channel near-infrared spectroscopy device (Pocket NIRS Duo, Hamamatsu, Japan). Immediately after each intervention, participants reported their mood in the moment with a visual analog scale in term of comfortableness, relaxation, and vigor. This study was approved by the IRB of Yamaguchi University Hospital and conducted in line with the Declaration of Helsinki. All participants provided written informed consent.Results: Viewing images of nature improved mood, as shown by increased comfortableness, relaxation, and vigor, in patients with only depressive (n=26) and anxiety (n=24) disorders. In contrast, in patients with comorbid depressive and anxiety disorders (n=10), viewing images of nature increased only comfortableness. During viewing images of nature, patients with depressive disorders showed increased concentration of oxygenated hemoglobin (Oxy_Hb) in the right OFC. In patients with anxiety disorders, in contrast, whereas the trend towards a decrease in the concentration of Oxy_Hb in the OFC during viewing images of nature was nonsignificant, there was a significant negative correlation between the changes in Oxy_Hb in the left OFC and changes in mood such that the lower the concentration of Oxy_Hb, the greater the mood-improving effect. In patients with comorbid depressive and anxiety disorders, however, there was no such difference nor correlation.Discussion: To our knowledge, this is the first study to examine the effects of visual stimulation with images of nature in patients with depressive and anxiety disorders. Whereas both disorders showed mood improvement, the effect was limited when they comorbid. Furthermore, the patterns of frontal brain activity were opposite in the two disorders. These findings may advance our knowledge of the unique neuropsychopathological changes in depressive and anxiety disorders and their comorbidity.

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Poster

PSTR427. Animal Models of Mood Disorders: Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR427.01/NN13

Topic: G.05. Mood Disorders

Support: Dr. Giovanni Colella's donation

Title: Blood biomarkers potentially exploitable to predict vulnerability and resilience to stress.

Authors: *M. RINAUDO, F. NATALE, M. SPINELLI, R. PIACENTINI, M. D'ASCENZO, S. FUSCO, C. GRASSI;
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Abstract: When exposed to similar stressors, individuals' responses may largely differ, with some people developing mood alterations, including depression, while others being virtually unaffected. However, the molecular and cellular mechanisms underlying susceptibility and resilience to stress are largely unknown. Aim of the present study was to identify a molecular fingerprint of stress vulnerability to unravel biomarkers potentially allowing to predict the risk to develop mood alterations in response to stress. Experiments were performed in male C57Bl6 mice exposed to the Unpredictable Chronic Mild Stress (UCMS) paradigm for two weeks. Blood samples were collected before starting the UCMS and behavioral assessment of the depressive-like phenotype was conducted at different time points during the stressing protocol. Employing a clustering algorithm, animals were categorized into two sub-populations: one exhibiting consistent depressive-like symptoms throughout the observation period and another group never developing such phenotype. Subsequently, two -omic analyses were performed on blood samples previously collected: i) untargeted metabolomic analysis and ii) transcriptome analysis of miRNAs extracted from circulating brain-derived extracellular vesicles. Supervised and unsupervised multivariate data analysis of the -omic datasets revealed distinct metabolic and miRNome profiles associated with the identified sub-populations of vulnerable and resilient mice. Specifically, enrichment analysis indicated significant alterations in energetic pathways such as the pentose phosphate pathway, Krebs' Cycle and amino sugar and nucleotide metabolism. Furthermore, single metabolic and miRNomic variables showed strong correlation with behavioral data. In conclusion, our findings suggest the existence of specific biomarkers, both in terms of circulating metabolites and brain-derived microRNAs, which are quantifiable before stress induction and may harbor information about stress vulnerability or resilience, thus potentially providing predictive insights into stress responses of the population at risk.

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Poster

PSTR427. Animal Models of Mood Disorders: Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR427.02/NN14

Topic: G.05. Mood Disorders

Title: Network structure and gene expression patterns of sex-specific brain-behavior correlates of chronic variable stress in mice

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Abstract: Intro: Stress is a major risk factor for depression. Female mice exposed to Chronic Variable Stress (CVS) display greater stress susceptibility. However, the sex-specific impacts of CVS on whole-brain network organization is unknown. Here, we used *in vivo* magnetic resonance imaging (MRI) and the Allen Institute's mouse connectivity and gene expression data to uncover putative mechanisms underlying sex-specific brain-behavior correlates of CVS in mice. **Methods**: We acquired T1w MRI (100 um isotropic voxels, Bruker 7T) and behavioral measures (social preference and open field; OFT) on 8 week old C57BL/6 male and female mice, pre- and post- sex-specific CVS protocols: 28 or 6 days of 1hr daily stressors (foot shocks, tail suspension, restraint), respectively (n=~10 group/sex). Brain (voxel-wise Jacobian determinants) and behavior relationships were examined with partial least squares correlation (PLSC), using 1) post- minus pre-CVS and 2) post-CVS measures. We used the spatial pattern of voxels significantly correlated with behavior (negative or positive) to examine their network structure (rich club; RC, community structure, and participation coefficient; PC), and correlation to spatial gene expression patterns.**Results:** In females, behavioral and neuroanatomical change covaried significantly (p=0.03, covariance explained=44.1%; Fig 1A). Decomposing the structural network of negative or positive voxels into 9 and 5 communities revealed the highest PC in the amygdala and cerebellum, respectively (Fig 1B). In males, CVS-induced neuroanatomical adaptations covaried with the OFT (p=0.04, cov. exp.=29.1%; Fig 1C). In the 3 communities for neg. or pos. voxels the highest PC located in the hippocampus and anterior cingulate, respectively. In both sexes, the RC involved the subcortex (neg.) and cortex (pos.). Correlated gene ontology terms are shown in Fig 1. **Conclusion:** Our results suggest different biological mechanisms related to sex-specific brain-behavior correlates of CVS, in which their networks suggest distinct key areas for inter-community communication.

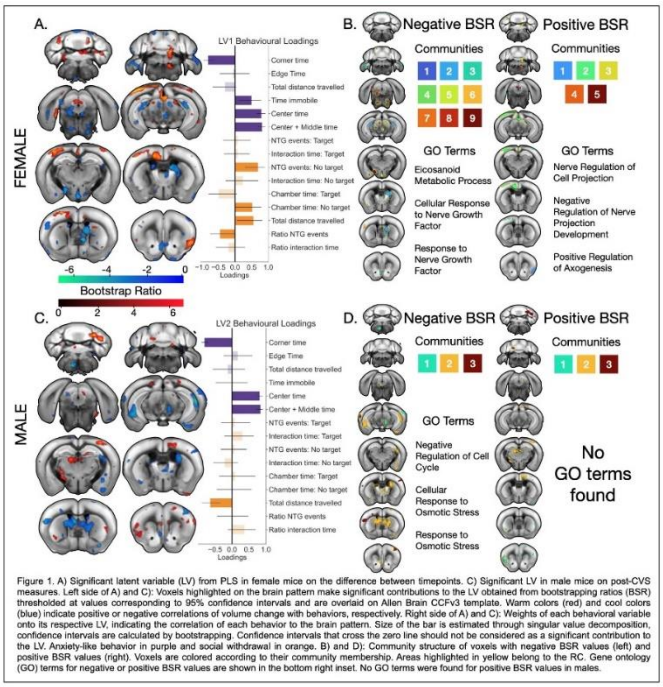

Figure 1. A) Significant latent variable (LV) from PLS in female mice on the difference between timepoints. C) Significant LV in male mice on post-CVS measures. Left side of A) and C): Voxels highlighted on the brain pattern make significant contributions to the LV obtained from bootstrapping ratios (BSR) thresholded at values corresponding to 95% confidence intervals and are overlaid on Allen Brain CCFv3 template. Warm colors (red) and cool colors (blue) indicate positive or negative correlations of volume change with behaviors, respectively. Right side of A) and C): Weights of each behavioral variable onto its respective LV, indicating the correlation of each behavior to the brain pattern. Size of the bar is estimated through singular value decomposition, confidence intervals are calculated by bootstrapping. Confidence intervals that cross the zero line should not be considered as a significant contribution to the LV. Anxiety-like behavior in purple and social withdrawal in orange. B) and D): Community structure of voxels with negative BSR values (left) and positive BSR values (right). Voxels are colored according to their community membership. Areas highlighted in yellow belong to the RC. Gene ontology (GO) terms for negative or positive BSR values are shown in the bottom right inset. No GO terms were found for positive BSR values in males.

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Poster

PSTR427. Animal Models of Mood Disorders: Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR427.03/NN15

Topic: G.05. Mood Disorders

Support: R01MH051399
R01MH129306
Hope for Depression Research Foundation

Title: Profiling Transcriptional Maladaptations in a Mouse Model of Treatment-Resistant Depression

Authors: *A. TORRES BERRIO[1], E. M. PARISE[2], M. ESTILL[4], T. M. GYLES[3], C. J. BROWNE[5], L. SHEN[4], E. J. NESTLER[2];
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Abstract: Major Depressive Disorder (MDD) is the most prevalent psychiatric disorder worldwide, representing a high level of global economic burden. Fluoxetine (FLX) and like

antidepressants have been widely used to treat MDD, nonetheless, ~50% of patients do not achieve full remission. Further, a subset of those afflicted are considered non-responsive to orally-available medications and are considered to have treatment-resistant depression (TRD). More recently, Ketamine (KET) has been shown to induce a rapid antidepressant response in ~50% of TRD patients, providing a novel therapeutic approach. However, the molecular mechanisms underlying TRD are poorly understood. This study was aimed at characterizing the transcriptional profile of successful vs. unsuccessful response to KET in chronically-stressed mice that failed to respond to an initial course of FLX as a model of TRD. We exposed adult male mice to chronic social defeat stress (CSDS), a validated mouse model of depression that identifies a spectrum of resilient vs. susceptible outcomes based on a social interaction test (SIT). Mice classified as susceptible underwent antidepressant treatment with FLX in their drinking water for 28 days or received water during the same period (water-treated). After FLX treatment, we identified a subset of mice (~35%) that continued to show reduced social interaction despite treatment (non-responders). FLX non-responders and water-treated mice were subsequently given a single injection of KET and assessed in the SIT 24 hr later. Transcriptome-wide changes in the prefrontal cortex (PFC) and nucleus accumbens (NAc) 48 hr after KET administration were profiled by RNA-sequencing. We found that ~50% of FLX-non-responder mice exhibited an antidepressant response to a single KET injection, a significantly greater response than that seen in susceptible mice treated with water (0%). We further identified a subset of treatment-resistant mice that failed to respond to consecutive FLX and KET treatment. Pattern analysis of the differentially expressed genes in the PFC and NAc revealed transcriptional profiles associated with the antidepressant-like actions of FLX and of KET as well as a series of genes that were unique to treatment resistance to both drugs. We developed a novel paradigm of treatment resistance in mice that allows the identification of potential mechanisms underlying TRD. The KET response rate in FLX-non-responders is similar to that seen in TRD patients, lending further validity to our model. Moreover, our findings suggest that prior unsuccessful antidepressant treatment induces a “priming effect” that increases the likelihood of successful response to KET.

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Poster

PSTR427. Animal Models of Mood Disorders: Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR427.04/NN16

Topic: G.05. Mood Disorders

Support: NIMH Grant 5R01MH106500-09

Title: Behavioral and translatome network profiling of socially stressed female mice

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Abstract: Stress impacts vulnerability for mental illnesses, including depression. Most behavioral paradigms attempting to model this phenomenon induce stress in manners that do not adequately reflect the human experience. Consequently, social stress models with higher ecological validity have become increasingly popular. Unfortunately, despite the higher rates of depression among females, most of these models only include male subjects. To address this deficit, we used the chronic witness defeat stress paradigm with D1-Cre- RiboTag (RT) and A2A-Cre-RT female mice. A social preference test with same-sex conspecifics was used to demonstrate a susceptible group that displays reduced social preference and a resilient group with social preference similar to controls. To obtain a more granular characterization of the behavior in this group of mice and male wildtype mice, video recordings from the social preference test were used to track animal behavior using the deep-learning based Python tool DeepLabCut. Spatiotemporal data extracted from DeepLabCut was then used to calculate kinetic variables based on mouse location, activity and interactions. Factor analysis was then employed using these variables to identify latent factors driving the observed behavior during the social preference test. To elucidate the molecular mechanisms underlying stress susceptibility and resilience in female mice, RNA-seq profiling of ribosome-associated mRNA from dopamine receptor 1 and 2 expressing medium spiny neurons (D1-MSNs and D2-MSNs) was performed. Weighted gene co-expression network analysis (WGCNA) was used to identify gene co-expression modules significantly associated with the stress groups. The susceptible and resilient mice were found significantly different in latent variables involving exploratory behavior, interaction with the cup containing the conspecific mice, surveillance behavior and chamber/cup preference. The gene co-expression networks that were differentially regulated between the stress groups involved protein synthesis and trafficking, neurite formation, synapse formation and maintenance and mitochondrial translation and activity in both D1- and D2-MSNs. Collectively, our studies are uncovering distinct behavioral characteristics and cell specific translatomes of socially stressed mice.

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Poster

PSTR427. Animal Models of Mood Disorders: Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR427.05/Web Only

Topic: G.05. Mood Disorders

Support: Quinnipiac University School of Health Sciences Faculty Scholarship Grants (to MMM)
Quinnipiac College of Arts and Sciences Faculty Scholarship Grants (to AJB)

Title: Microglia & Neuronal Dysfunction marker, PFKFB3, in Rodent Model of Chronic Unpredictable Stress

Authors: *M. M. MIRRIONE[1], C. FORD[1], C. WHITELOCK[2], K. RONALTER[2], K. JONES[1], A. J. BETZ[3];
[1]Biomed. Sci., [2]Hlth. Sci., [3]Psychology Department, Behavioral Neurosci. Program, Quinnipiac Univ., Hamden, CT

Abstract: The hippocampus undergoes significant atrophy (cell death) during chronic stress and depression. The underlying mechanisms of neuronal dysfunction and disrupted neuronal circuits are thought to involve altered function of microglia. Neurons and glia work together seamlessly in the healthy brain and are critical for maintaining proper health and function of neurons. However, it has been postulated that glial cells become altered during stress and may be causally linked to disrupted neuronal circuits in mood disorders. Here, we further explore a potential link between microglia morphology changes and neuronal metabolic dysfunction in the hippocampus, using the widely accepted mild Chronic Unpredictable Stress (CUS) model. Male, Sprague-Dawley rats were randomized to control (n=5) and CUS (n=5) groups on post-natal day 21. CUS animals were exposed to three weeks of randomized stressors, evaluated for sucrose preference, then sacrificed on post-natal day 44, brains excised, fixed in formalin, and sectioned at 30 microns for immunofluorescence microscopy analysis. We hypothesized that increased microglia activation and proliferation would be evident in the hippocampus following CUS compared to controls, and would correlate with metabolic damage in neurons measured using the marker 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3). PFKFB3 is glycolysis enzyme thought to accumulate with excitotoxicity, and could contribute to oxidative stress and cell death. Immunofluorescence was performed on the hippocampal tissue for microglia via an antibody for ionized calcium-binding adapter molecule 1 (Iba1) and an antibody for PFKFB3. In male rats, we quantified the amount of microglia and PFKFB3 within the dorsal and ventral hippocampus CA1, CA3, DG sub-regions using MatLab and CellProfiler. Our data reveal an increase in PFKFB3 in the ventral hippocampus CUS animals, and remained as a trend when normalizing to total number of neurons. We also measured changes in cell body size of microglia in CUS animals, across all subregions, but no change in total counts of microglia. Together our data suggests evidence of early stages of neuronal dysfunction in the hippocampus resulting from CUS exposure in adolescent animals, which may be associated with altered microglia function. Future experiments will evaluate the association between reduction in microglia soma size, and the number and complexity of processes, and altered neurotropic support for hippocampal neurons from CUS. Overall, our results may provide insight into the molecular mechanisms responsible for atrophy and neuronal dysfunction in limbic circuits related to stress and depression.

Disclosures: M.M. Mirrione: None. **C. Ford:** None. **C. Whitelock:** None. **K. Ronalter:** None. **K. Jones:** None. **A.J. Betz:** None.

Poster

PSTR427. Animal Models of Mood Disorders: Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR427.06/NN17

Topic: G.05. Mood Disorders

Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (National Council for Scientific and Technological Development)

Title: Investigation of transcriptome alterations (RNAseq) in neuronal population of ventral hippocampus in mice susceptible and resilient to chronic social defeat stress

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Abstract: Depression is a common and recurrent mental disorder impairing the daily life and well-beingof the individual. Social stress is already well established in the literature as a model ofdepression and, in this context, we can highlight the chronic social defeat stress model(CSDS).In this model, the social interaction test is used to assess social avoidance,considered a depressive-like behavior. Not all mice submitted to CSDS develop socialavoidance behavior and are called resilient. The ventral hippocampus is a well studiedstructure in the context of depression, mainly because of its neuroplasticity and relation withother limbic system structures. In addition, the ventral hippocampal cell layers play differentroles during behavior. Ventral CA1 inactivation increases avoidance of conflict cue behavior,while CA3 inactivation increases approach behavior to conflict cue. Furthermore,neurogenesis confers resilience to chronic stress by inhibiting granular cells from ventraldentate gyrus. Litterature shows different results depending on the specific hippocampal celllayer, making their separation very important. In this study we aimed to investigate thetranscriptome alterations in distincts ventral hippocampal cell layers after CSDS. For theCSDS 12 week old C57/BL6 mice were submitted to 10 minute aggression sessions against 6month old aggressor retired breeder Swiss mice for 10 consecutive days. One day after thelast aggression session we performed the social interaction test and, after 24 hours, mice wereeuthanized and the brains were collected and fresh-frozen in isopentane. All brains weresliced in a cryostat, flushed with violet cresyl, and microdissected using a laser-capturemicrodissection apparatus to separate ventral dentate gyrus and ventral CA1 celllayers.Following the social interaction test, we could classify 7 animals as resilient to CSDSshowing social interaction ratio (SIR)average 3.02, and 11 animals showing social avoidancebehavior (susceptible)with SIR average 1,03, in addition to 10 control mice, not submittedto CSDS protocol, with SIR average 2.32. Furthermore, 5 brains of each group werecollected, processed, and microdissected, where we separated the ventral dentate gyrus andCA1 cell layers from the hippocampus for further analysis. The CSDS protocol wassuccessfully performed in the present study, once we could observe and classify the mice aspresenting either susceptible or resilient phenotype. This is an ongoing study and

themicrodissected material from each animal will be submitted to transcriptome analysis ofCA1, and Dentate Gyrus cell layers.

Disclosures: G. Zanetti: None. **M.F. Pagliusi:** None. **A.S. Vieira:** None.

Poster

PSTR427. Animal Models of Mood Disorders: Molecular Mechanisms

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Topic: G.05. Mood Disorders

Support: Canadian Institutes of Health Research (CIHR, PJT - 173237, PI Silveira PP)
Hope for Depression Research Foundation (MJM)
JPB Foundation through a grant to the JPB Research Network on Toxic Stress: A Project of the Center on the Developing Child at Harvard University (PPS, MJM)

Title: Transcriptome profiles from mice hippocampal ventral dentate gyrus reveals a gene network associated with environmental enrichment exposure that predicts mental health in humans

Authors: *R. M. S. DE LIMA[1], B. BARTH[3], D. MAR ARCEGO[2,4], J. MAIRES HOPPE[2], B. ALBERRY[2], E. FITZGERALD[2], K. CRAIG[5], S. PATEL[6], I. POKHVISNEVA[6], C. PARENT[6], T.-Y. ZHANG[6], C. DALMAZ[7,8], P. PELUFO SILVEIRA[2,4,3], M. J. MEANEY[2,9];
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Abstract: Anxiety and major depressive disorders are highly prevalent worldwide, and strongly associated with exposure to stress. Individual differences in sensibility to the environment may explain why some individuals are at higher risk for the development of psychopathology. Exploring the molecular mechanisms of resilience is essential for informing targeted prevention and treatment investigations. Here, we tested whether the Environmental Enrichment (EE) paradigm promotes resilience in male mice, and we analyzed transcriptional networks involved in response to EE exposure in comparison to standard-housed animals (SD). We measured anxiety and depressive-like behaviors after exposing animals to 8 weeks of EE. We observed an anxiolytic effect of EE in comparison to SD (Novelty suppressed Feeding, $F_{1,39}= 14.34$, p=

0.001), without differences in the depressive-like phenotype between the groups (Forced Swimming Test: p>0.05). RNA sequencing of the hippocampal ventral dentate gyrus (N=9/10 group) was used to identify a functional gene network associated with EE through a weighted gene co-expression network analysis (r=0.57, p=0.01). Enrichment analysis revealed that this network is associated with mitochondrial function, and response to stress, especially oxidative. Human single nucleotide polymorphisms from these genes were weighted using the association between alleles and gene expression from GTEx hippocampus dataset, and used to compute an expression-based polygenic score (ePGS) in the UK Biobank. Variations in ePGS represent individual differences in the expression of the network. We investigated the interplay between ePGS and adult stress exposure on the development of psychopathology. The ePGS moderated the association between stress exposure and the International Classification of Disease-10 codes F40-F41, that comprise anxiety disorders (N=1240, B= -0.03, P= 0.025), in which higher stress is associated with higher risk for anxiety disorders in the low ePGS group (simple slope analysis: B= 0.25, P< 0.001). This suggests that variations in the expression of a network associated with EE in mice are also associated with mental health in humans. These findings offer insights for future research aimed at investigating the causal role of specific molecular pathways associated with the promotion of resilience.

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Poster

PSTR427. Animal Models of Mood Disorders: Molecular Mechanisms

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Topic: G.05. Mood Disorders

Support: Vulnerable Brain Project
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Title: C-fos expression and gad-immunoreactivity in brains of adolescent female mice undergoing activity-based anorexia, food restriction only, wheel running only or neither

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Abstract: Anorexia nervosa (AN) is a complex mental disorder characterized by significantly low body weight due to voluntary food restriction, persistent behaviors like excessive exercise that interfere with weight gain, and body dysmorphia (American Psychiatric Association, 2013). Activity-based anorexia (ABA) is an animal model that presents all critical symptoms of AN

except distorted body image. Specifically, ABA represents two main characteristics of AN: increased voluntary physical activity and increased restriction of food intake, leading to excessive body weight loss (Aoki, 2020, Animal Model of Eating Disorders). ABA animals increase wheel running activity during the dark phase and 2-3 hours before feeding time (Mistlberger, 1994, Neuroscience & Biobehavioral Reviews, 18(2), 171-195). This food anticipatory activity (FAA) has been shown to associate with anxiety levels (Wable et al., 2015, *Behav Neurosci*, 129:170), reward-seeking, and anticipation of palatable food stimuli (Mendoza et al., 2005, European Journal of Neuroscience, 22(11), 2855-2862). Many neuronal changes can lead to changes in feeding behavior and hunger-evoked hyperactivity. Altered neuroendocrine and brain reward circuit function is one hypothesized mechanism behind AN pathology. Previous study on ABA after the feeding period showed increased activation in the supraoptic nucleus, arcuate nucleus and locus coeruleus (Scharner et al., 2016, Frontiers in neuroscience, 10, 475). We addressed two questions that remained unanswered: 1) What is the brain state of ABA animals during food anticipatory activity (FAA) time, when they are hungry and running simultaneously? 2) What is the brain state of ABA animals during the re-feeding time? To answer these two questions, we created four groups of animals: food-restricted, exercising, ABA, and control with neither food restriction nor exercise. Brains were collected at two time zones: during FAA @6 pm and during re-feeding @8 pm. Brain regions we investigated included the prefrontal cortex, caudate putamen, nucleus accumbens, lateral hypothalamus, substantia nigra, VTA, dorsal raphe, habenula and insula. C-Fos marked neuronal activities, and GAD-immunoreactivity was used to identify whether they are GABAergic or not.

Disclosures: Y. Dong: None. **C.J. Aoki:** None.

Poster

PSTR427. Animal Models of Mood Disorders: Molecular Mechanisms

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Program #/Poster #: PSTR427.09/NN20

Topic: G.05. Mood Disorders

Support: NIH Grant Da055169

Title: catFISH analysis of medial preoptic area neuronal activation during maternal interactions with offspring of varying needs

Authors: *E. LOPEZ ROBINSON[1], K. COPELAS[2], A. GADEKAR[2], M. PEREIRA[2]; [1]Psychological Brain Sciences, Neurosci. and Behavior, [2]Psychological and Brain Sciences, Neurosci. and Behavior, Univ. of Massachusetts Amherst, Amherst, MA

Abstract: Maternal behavior that is sensitive to the needs of the offspring in everyday interactions is essential for healthy development and wellbeing in mammals. Postpartum depression (PPD) is a serious health problem that has a tragic impact on the mother's ability to sensitively care for her child, with life-long consequences for both the mother and her child. Our

prior work demonstrated that the medial Preoptic Area (mPOA), a critical node in the circuitry regulating maternal behavior, is required for this critical maternal ability (referred to here as maternal sensitivity). The objective of this study was to examine the contribution of mPOA cells to maternal sensitivity. To this aim, we leveraged the well-validated Wistar-Kyoto (WKY) rat model of depression and used cellular compartment analysis of temporal activity by fluorescence in situ hybridization (catFISH) technique to assess overlap between mPOA population of cells during maternal interactions with offspring with varying needs. WKY and control Sprague-Dawley (SD) mother rats experienced two 10-min interaction episodes, 20 min apart with normal vs increased needs pups. Mothers were perfused immediately after the second episode and their brains processed to quantify nuclear cfos pre-mRNA and cytoplasmic mRNA transcripts in the mPOA. Consistent with our prior results, WKY mothers exhibited similar caregiving behaviors regardless of their offspring needs, highly contrasting the sensitive parenting of SD mothers, indicative of deficits in maternal sensitivity. c-fos catFISH analysis revealed overlapping but distinct neuronal populations involved in adjustments of care that resolve the needs of the offspring. Together, this new work expands our understanding of the mPOA contribution to parenting.

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Poster

PSTR427. Animal Models of Mood Disorders: Molecular Mechanisms

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Program #/Poster #: PSTR427.10/OO1

Topic: G.05. Mood Disorders

Support: Dean's Undergraduate Research Fund

Title: Characterization of Mu Opioid Receptor Expression on Dopaminergic Axons of Nucleus Accumbens Shell in Relation to Wheel Running

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Abstract: Anorexia nervosa consists of primary symptoms that include excessive exercise, severe weight loss, and food restriction. Severe weight loss is a huge contributing factor to the high mortality rate of the disorder, which is the highest out of all psychiatric illnesses. My research primarily focuses on the excessive exercise component as it is the major contributor to weight loss. To study excessive exercise I work with the activity-based anorexia (ABA) model where mice are exposed to wheels, food restriction, or both within their adolescence to observe its impact on their exposure to a wheel in adulthood. The research focuses on why the mice who excessively exercise in response to food restriction do so in the first place. It could potentially be

attributed to the euphoric sensation produced during exercise known as “Runner’s High.” Endorphins and dopamine are two molecules suggested by popular press to be associated with this sensation but neurobiological verification of this idea is lacking. On the other hand, there is substantial evidence that synaptic plasticity involving dopamine in ventral striatum, also called nucleus accumbens, is associated with addiction. To determine if it's the euphoric sensation motivating this exercise symptom, I am quantifying the number of mu opioid receptors on dopaminergic axons in nucleus accumbens. Mu opioid receptors bind endorphins and suppress dopamine release. A large quantity of these receptors on dopaminergic axons would suggest the euphoric sensation is not attributed to the excessive running. In order to quantify the level of mu opioid receptors on dopaminergic axons, I’m using immune-electron microscopic staining to identify tyrosine hydroxylase-immunopositive axons and mu opioid receptors localized on them. I’m also running correlation analysis of these co-localizations and each animal’s wheel running. The same tissue will also be used to assess the extent to which mu opioid receptors occur at excitatory synapses and inhibitory synapses to learn whether mu opioid receptor-mediated modulation of these synapses correlate with the extent of wheel running.

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Poster

PSTR427. Animal Models of Mood Disorders: Molecular Mechanisms

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Program #/Poster #: PSTR427.11/OO2

Topic: G.05. Mood Disorders

Support: R21-MH128574

Title: Glyoxalase I inhibitors as novel fast-acting antidepressant drugs following chronic mild stress

Authors: M. ULIVIERI[1], **H. ROSBERG**[1], X. FANG[1], C. BUI[1], A. LEE[1], E. ALCANTARA[2], *S. C. DULAWA[3];
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Abstract: Depression is a complex mood disorder affecting millions of people of all ages worldwide. Common antidepressants usually require weeks of treatments and are inefficient in a wide range of patients (30-40%). Ketamine is the only fast-acting antidepressant compound currently approved for clinical use, but still lacks the effectiveness in approximately 30% of patients. We recently demonstrated that subchronic Glyoxalase I (GLO1) inhibition induces antidepressant effects in mice following chronic mild stress (CMS). GLO1 is a key enzyme mediating the catabolism of methylglyoxal (MG), an endogenous $GABA_A$ partial agonist. In our study, we aimed to demonstrate the fast-acting antidepressant effects of methyl gerfylin (MeGFN), a GLO1 inhibitor, following acute systemic administration in control and CMS

animals, investigating possible gender differences in susceptibility to depressive behavior and drug response. We used adult male and female Balb/cj mice and exposed them to a 6-week CMS protocol. At the end of the 6 weeks, animals were tested in several behavioral tests assessing depression-like behavior: frustration induced by reward omission, effort-related choice (ERC), social interaction, coat state, open field, forced swim test (FST). On the test day, animals were separated into four different treatment groups: MeGFN, ketamine (fast-acting antidepressant control group) or their respective vehicles. We found that CMS induces a progressive reduction of sucrose preference across the 6 weeks. A single systemic injection of MeGFN or ketamine, exerted fast-acting antidepressant (within 24-48 hours) by improving social interaction dysfunction (with no effects on social novelty), reduction of coat deterioration, restoring reward processing alterations, and reducing immobility time and increasing swimming time in the FST, without affecting locomotor activity. Therefore, our results show the impact of CMS on frustration induced by reward omission protocol, sociability, and ERC, as well as other depressive-like behaviors, and reveal fast-acting effects of acute MeGRF treatment. Overall, our study suggests that glyoxalase system, and particularly GLO1 enzyme, is a novel suitable target for novel fast-acting antidepressant drugs.

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Poster

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McGill University

Title: Social defeat stress in adulthood alters the Slit - Robo, but not the Netrin-1- DCC guidance cue system in the PFC of female mice

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Abstract: Elevated levels of the Netrin-1 guidance cue receptor gene deleted in colorectal cancer (*DCC)* in the adult prefrontal cortex (PFC) is a consistent trait of major depressive disorder (MDD) in humans and plays a causal role in susceptibility to chronic social defeat stress (CSDS) in adult male mice (Manitt, et al., 2013; Torres-Berrío et al., 2017). In contrast, the axonal guidance gene SLIT1 is downregulated in ventromedial PFCs (vmPFC) of female, but not male,

patients with MDD, and similarly, is reduced in vmPFCs of female and not male mice exposed to chronic variable stress (van der Zee et al., 2022). Although depression is twice more prevalent in women than men, the molecular mechanisms underlying this disparity remain unknown. Here we investigated if the Netrin-1/DCC and Slit/Roundabout (Robo) guidance cue systems are dysregulated in the PFC of adult female mice following CSDS. Adult (P75±15) C57BL/6J female mice were exposed to CSDS for 5 minutes each day for 10 days. To induce aggression from CD1 mice, urine of C57BL/6J male mmice was applied to the females. Control mice were housed with a different conspecific every day. Following CSDS, mice were assessed in the social interaction test (SIT) and 24h later, PFC tissue was collected to measure mRNA and miRNA expressions. Defeated females were segregated into resilient and susceptible groups based on the SIT. Compared to control mice, susceptible and resilient mice showed an increase in body weight and a deficit in the nestlet shredding test, a measure of self-care-like behaviour. In the PFC, there was no difference in mRNA levels among groups for either *Dcc* and *Netrin-1*, nor for *Unc5c*, another prominent Netrin-1 receptor gene. However, the expression of miR-218 – a microRNA which represses *Dcc*, *Robo1*, and *Robo2* and is intronically encoded in the *Slit2 and Slit3* genes – was slightly elevated in susceptible mice compared to control and resilient mice. Moreover, both susceptible and resilient mice show elevated *Slit2* and *Robo1* mRNA levels in the PFC. These data indicate that contrary to males, CSDS in females alters the Slit/Robo, but not the Netrin-1/DCC guidance cue system in the PFC, possibly via currently unidentified sexually dimorphic molecular processes involving microRNAs.

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Poster

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Topic: G.05. Mood Disorders

Support: Supported by The Charles E. Kubly Mental Health Research Center at Marquette University.

Title: Effects of Chronic Stress and Prefrontal Cortical REDD1 Overexpression on Attentional Set Shifting Behavior in Mice

Authors: *B. KURTOGLU[1], M. HEARING[2], J. MANTSCH[3];
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Abstract: Cognitive (behavioral) flexibility, the ability to adapt behaviors in response to changes in the environment is an essential element for everyday life, with deficits commonly observed in neuropsychiatric disease states and reducing resilience to negative life events such as stress. The

rodent prelimbic cortex (PrLC) plays a critical role in processing information necessary for optimal cognitive flexibility and is known to undergo structural and functional changes following prolonged stress exposure, thus PrLC dysfunction represents a likely substrate for stress-induced deficits in cognitive control. We have recently shown that chronic unpredictable stress (CUS) produces an enduring dysfunction in PrLC physiology and impaired cognitive flexibility using an operant-based attentional set shifting in male but not female mice, however what adaptations drive these deficits remains unclear. To gain more insight into this, our studies chose to focus on the protein REDD1 (regulated in development and DNA damage responses-1) (aka DDIT4, RTP801, Dig2) as it is increased in post-mortem dorsolateral prefrontal cortex (dlPFC) tissue from individuals diagnosed with depression. In line with these findings, we find that there is an increase in REDD1 expression and a decrease in Raptor phosphorylation, one of the key elements of the mTORC1 complex, in the PrLC after CUS, suggesting disrupted mTORC1 function. To determine if REDD1 overexpression is sufficient to produce deficits in attentional set shifting, we used a viral vector to overexpress REDD1 in the PrLC of male mice. Relative to control mice, REDD1 mice required more trials to pass the extradimensional shift testing criterion that was equivalent to that produced by CUS. Notably, REDD1 overexpression did not impact acquisition of lever training, or measures of motivation for non-drug reward. Furthermore, we also examined the effects of REDD1 overexpression on PFC pyramidal neuron physiology and found that there is a reduction in miniature excitatory post synaptic current (mEPSC) signaling. The observation that CUS and REDD1 overexpression produce deficits in attentional set shifting in male mice likely has relevance for understanding a number of stress related disorders. Future research will assess the cell-type localization of REDD1 increases following stress in males, determine whether female mice are similarly affected by REDD1 overexpression and/or is upregulated in females following CUS, chronic CORT effects on attentional set shifting and examine the necessity of disrupted mTORC1 for stress effects.

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Poster

PSTR427. Animal Models of Mood Disorders: Molecular Mechanisms

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Program #/Poster #: PSTR427.14/OO5

Topic: G.05. Mood Disorders

Title: Critical role of mPFC layer 1 interneuron activity in stress-induced depressive-like behavior

Authors: *C. SHEN, W. CUI, D. LEE, I. SANTIAGO, H. WANG, W. ZOU, W.-C. XIONG, L. MEI;
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Abstract: The prefrontal cortex (PFC) serves as a central hub for emotional processing and stress responses; its abnormal function has been implicated in major depression disorders

(MDD). Layer 1 of the medial PFC (mPFC) consists of inhibitory interneurons (L1INs) that coordinate cerebral functions by integrating inputs from various brain regions and regulating information flow within the mPFC. However, it remains unknown how the mPFC L1INs change their activity in depressive-like behaviors. To this end, we selectively expressed the calcium indicator GCaMP6f in L1INs of the mouse mPFC (via NDNF-Cre) and recorded calcium activities during the tail suspension test (TST). We found that 24.1% of L1INs displayed a higher calcium activity during immobility in naïve mice, suggesting that these L1INs may be involved in depressive states. In agreement, this proportion rose to 48.1% after chronic restraint stress (CRS, an established paradigm to cause depressive-like behaviors). Notably, the expanded proportion mainly consisted of neurons that were previously insensitive to immobility or struggle behaviors, indicating circuit-level activity alterations. On the other hand, 51.9% of L1INs showed a higher calcium activity during struggle behavior in naïve mice, which reduced to 38.0% following CRS. These findings provide evidence that the activity of L1INs changes in association with depression levels. By retrograde tracing, we found that the nuclei of the diagonal band (NDB) and the ventromedial thalamus (vmTH) as primary inputs to NDNF+ L1INs. CRS enhanced cFos expression in NDB neurons and their vesicle release probabilities, but reduced cFos expression in vmTH neurons and their vesicle release probabilities. Experiments are underway to examine whether circuit activity imbalances of mPFC L1INs could be a possible mechanism for depressive-like behaviors.

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Poster

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Topic: G.05. Mood Disorders

Support: NIH Grant R01 MH107508

Title: Increased adiposity due to maternal western-style diet consumption shapes serotonin innervation of the amygdala and anxiety behavior in adolescent nonhuman primate offspring

Authors: *G. DUNN[1], A. MITCHELL[2], J. GRAHAM[2], J. JACINTO-SCHREEDER[2], H. GUSTAFSSON[2], E. L. SULLIVAN[2];
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Abstract: Obesity is an epidemic that currently effects 40% of adults in the US. Further, about one-third of pregnant women are classified as obese. While many factors contribute to becoming obese, consumption of an obesogenic diet (e.g. high in saturated fat and sugar) is the largest predictor of increased adiposity. Previous research suggests that children born to obese mothers are at increased risk for developing neurodevelopmental and neuropsychiatric disorders. The

mechanisms underlying this increased risk are poorly understood. Utilizing a non-human primate model of maternal obesity, we hypothesized that maternal consumption of an obesogenic diet would increase anxiety-like behaviors in offspring through a reduction in serotonin innervation in the amygdala. We performed behavioral assessments in 34-month-old Japanese macaques (n=51; females = 26), born to either control or Western-style diet consuming mothers, and measured rates of anxiety-like behaviors. Further, we designed a fluorescent immunohistochemistry experiment to examine serotonin axonal projections into the amygdala in 36-month-old Japanese macaques (n=16, females = 8). In this study we utilized structural equation modeling for the simultaneous estimation of the relationship between maternal diet, metabolic state, offspring sex and offspring serotonin or behaviors measures. We found that maternal adiposity had a significant direct effect of a reduction in serotonin innervation in the amygdala ($\beta_{\text{Adiposity} \rightarrow \text{Offspring Serotonin}} = -0.482$, SE = 0.239, $p<0.05$). However, maternal diet was not found to have a significant effect on offspring serotonin outcomes ($\beta_{\text{Diet} \rightarrow \text{Offspring Serotonin}} = 0.153$, SE = 0.211, $p=0.470$). Similarly, we found an association between maternal adiposity ($\beta_{\text{Adiposity} \rightarrow \text{Offspring Serotonin}} = 0.363$, SE = 0.135, $p<0.01$), but not diet ($\beta_{\text{Diet} \rightarrow \text{Offspring Anxiety}} = -0.086$, SE = 0.120, p=0.473), on the rates of offspring anxiety behavior at 34 months. In summary, the current study provides support for reduced 5-HT innervation of the amygdala, due to maternal obesity, being a mechanism underlying the association between maternal WSD and increased anxiety behaviors in offspring.

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Poster

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Topic: G.05. Mood Disorders

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Title: Zinc deficiency induces proteomic changes, altered cytokine expression, and mtDNA damage in a mouse model of depression.

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Abstract: Background: Major depressive disorder (MDD) is a prevalent psychiatric disorder. Zinc deficiency is associated with an increased risk of MDD in humans and induces depressive-like behavior in rodents. Zinc homeostasis plays a crucial role in macromolecule synthesis,

signal transduction, redox homeostasis, DNA repair, and gene transcription. The prominent coexisting factors in depressive disorder are metabolic alterations and mitochondrial dysfunctions. In this study, we aimed to investigate the proteomic changes and mtDNA damage in the context of zinc deficiency-induced depressive disorder using a mouse model of stress. **Methods:** Male mice were fed either a standard diet (50 mg Zn/kg) or a zinc-deficient diet (3mg Zn/kg, ZnD) for 4 weeks. After one week, some mice from both groups were also subjected to chronic restraint stress (CRS) for 21 days. Bio-Plex Pro Mouse Cytokine 23-plex - Luminex multiplex technology was used to measure cytokine levels in the mouse prefrontal cortex (PFC), hippocampus (Hp), and serum. Proteomic analysis was performed using mass spectrometry to investigate protein alterations in the PFC and Hp. The LORD-Q PCR method was employed to assess mtDNA damage levels. **Results:** Our findings revealed numerous protein alterations in the PFC and Hp indicating disruptions in mitochondria and energy production, protein metabolism and translation, as well as RNA metabolism in the PFC. In the Hp, pathways related to mitochondrial function, axonal transport, mitochondria, energy production, GTPases of the Rho Family, and ATP synthesis showed differential regulation. The analysis of cytokine expression demonstrated increased levels of interleukin (IL)-5 (IL-5), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and monocyte chemoattractant protein-1 (MCP-1) in the PFC of ZnD mice. In the Hp, ZnD + CRS resulted in altered expression of IL-3, IL-4, IL-6, IL-9, IL-10, IL-12p70, eotaxin, G-CSF, GM-CSF, macrophage inflammatory protein-1 beta (MIP-1B), tumor necrosis factor-alpha (TNF-alpha), and interferon-gamma (INF-gamma). Moreover, increased mtDNA damage was observed in the PFC suggesting a potential role of reactive oxygen species in ZnD-induced mitochondrial dysfunction. **Conclusions:** These findings highlight the critical role of zinc homeostasis in the pathophysiology of major depressive disorder and suggest potential implications for metabolic alterations and mitochondrial dysfunctions. Further research is necessary to elucidate the underlying mechanisms and develop targeted therapeutic interventions.

Disclosures: B. Szewczyk: None. **L. Gasior:** None. **B. Pochwat:** None. **J. Turek:** None. **P. Mielczarek:** None. **J. Solich:** None. **A. Faron-Górecka:** None. **A. Tatarczuch:** None. **K. Kotarska:** None.

Poster

PSTR427. Animal Models of Mood Disorders: Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR427.17/OO8

Topic: G.05. Mood Disorders

Title: Mice lacking GPR171 display alterations in mood in a sex dependent manner

Authors: *M. C. RADDATZ[1], C. PORTER[2], M. STOTT[1], E. BOBECK[1];
[2]Biol., [1]Utah State Univ., Logan, UT

Abstract: GPR171 is a recently de-orphanized G protein-coupled receptor whose biological functions are still largely unknown. Thus far, studies have revealed a positive correlation between eating behaviors and GPR171 activity, anxiolytic effects of GPR171 antagonism, and synergistic effects of morphine and GPR171 agonism in acute pain treatment. However, the research regarding GPR171 functions is incomplete, with a considerable degree of disparity and ambiguity in what is currently known. Additionally, all the research thus far completed on GPR171 has relied almost exclusively on exogenous ligands which are only relatively selective and are subject to unknown pharmacodynamics and pharmacokinetics. In order to directly determine the functions of GPR171 and its necessity in several behaviors, we conducted an extensive phenotypic characterization of a novel GPR171 knockout mouse model. GPR171 knockout (KO), heterozygous (HET), and wild-type (WT) mice of both sexes were subject to a behavioral battery assay including tests assessing physiological functions, anxiety, depression, and locomotion. GPR171 KO did not alter general feeding behaviors or feeding after fasting ($F(52,416) = 1.286$, $p = .41$) and did not significantly impact overall weight ($F(2, 52) = .56$, $p = .58$). However, GPR171 knockout did affect mood, with a significant effect of genotype on anhedonia measured in the sucrose preference test ($F(2, 52) = 6.85$, $p = .002$). Post-hoc analyses indicate that male GPR171 KO mice displayed increased anhedonia compared to HET and WT males ($p = .02$ and $p = .006$ respectively) while females were unaffected by genotype. Interestingly, the knockout of GPR171 also resulted in decreased anxiety-like behaviors as measured in the elevated plus maze ($F(2, 93) = 3.615$, $p = .03$), without affecting total locomotion ($F(2, 93) = 0.66$, $p = .5$). These results indicate that GPR171 is necessary for normal mood regulation, particularly in male mice, with much smaller effects in females, potentially representing sexually dimorphic receptor actions. While GPR171 was originally proposed to be a feeding receptor, our results indicate that it is not necessary for normal physiological functions, likely due to redundancy in feeding mechanisms. Together, our results expand on the known functions of GPR171 while exploring sex differences which have historically been overlooked. GPR171 not only serves as a viable target for pharmacological manipulation, but also as a potential biomarker for different disease states, particularly those involving mood dysregulation.

Disclosures: **M.C. Raddatz:** None. **C. Porter:** None. **M. Stott:** None. **E. Bobeck:** None.

Poster

PSTR427. Animal Models of Mood Disorders: Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR427.18/OO9

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Womenmind Seed Grant

Title: Elevated MAOA and altered bioenergetics underlying elevated emotionality in depression

Authors: *R. ZHOU[1,2], R. LABEAU[3], R. C. LAISTER[4], J. H. MEYER[5,2], J.-P. GUILLOUX[3], T. TOMODA[5,2], E. SIBILLE[5,2];

[1]Univ. of Toronoto, Toronto, ON, Canada; [2]Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada; [3]Univ. Paris Saclay, Paris, France; [4]Univ. Hlth. Network, Toronto, ON, Canada; [5]Univ. of Toronto, Toronto, ON, Canada

Abstract: The Monoamine Oxidase A (MAOA) is an outer mitochondrial membrane protein that catalyzes the oxidative deamination of various monoamines, while producing hydrogen peroxide and electrons to fuel bioenergetic machinery in cells. In human brains, MAOA is widely expressed in both neurons and glia, and primarily responsible for metabolizing monoaminergic neurotransmitters, serotonin and norepinephrine, produced in the brain stem. Altered MAOA activity or expression levels in the brain are associated with changes in diverse behavioral traits, such as mood, cognition and personality, likely through deregulated monoaminergic neurotransmission. Notably, elevated MAOA expression in corticolimbic brain areas has been consistently reported in major depressive disorder (MDD) via positron emission tomography (PET) imaging studies. High MAOA level is thought to contribute to low brain serotonin levels and hence depressed mood, or resistance to conventional monoaminergic antidepressant treatments. However, the causal link of elevated MAOA to the pathobiology of MDD and/or treatment resistance has not been tested, and the mechanisms underlying these psychopathological conditions remain to be studied. To test this causality, we have developed a humanized model of elevated MAOA in mice, in which human *MAOA* gene is selectively expressed in relevant neuronal or glial cell types within corticolimbic areas, and studied the impacts on emotional behavior, neurochemistry and cellular functions, with particular emphasis on mitochondrial bioenergetic pathways. Current results show that the mice with increased MAOA expression in pyramidal cells of prefrontal cortex (PFC) exhibit elevated MAOA protein levels comparable to that seen in depression in PET studies, concurrent with reduced serotonin levels in PFC and altered emotional behavior. In addition, primary neurons and astrocytes with elevated hMAOA show reduced mitochondrial membrane potential, suggesting altered bioenergetics induced by MAOA expression. Studies are underway to further study the impact of elevated MAOA on mitochondrial functions (e.g., rate of ATP production, oxygen consumption rate, mitochondrial contents) using a series of functional tools, such as a redox-sensing probe, Seahorse ATP rate assays, pharmacological inhibitors for electron transfer complex components, along with the mitochondria-specific proteomic probes for proximity labeling. Through these studies, we aim to decipher the mechanisms mediating MAOA-induced bioenergetic and psychopathological consequences.

Disclosures: R. Zhou: None. **R. Labeau:** None. **R.C. Laister:** None. **J.H. Meyer:** None. **J. Guilloux:** None. **T. Tomoda:** None. **E. Sibille:** None.

Poster

PSTR427. Animal Models of Mood Disorders: Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR427.19/OO10

Topic: G.05. Mood Disorders

Support: MOST 110-2320-B-002-014-MY3

Title: Depletion of BLM-s causes decreased proliferation of adult neural progenitor cells at dentate gyrus by modulating cell cycle progression and mitochondrial dynamic

Authors: H.-R. LO[1], Y.-J. JHANG[2], C.-C. TSOU[2], H.-J. CHENG[1], ***P.-H. HUANG**[2]; [1]Inst. of Mol. Biol., Academia Sinica, Nankang, Taiwan; [2]Grad. Inst. of Pathology, Natl. Taiwan Univer, Taipei, Taiwan

Abstract: Mood disorders rank among the top ten causes of human disability and recent advances in genomic studies indicate that molecules involved in neurodevelopment are causally related to mood disorders. We have previously demonstrated that mice with null BLM-s (BCL-2 like molecule, small transcript isoform), a BH3-only apoptosis sensitizer/derepresser regulating apoptosis of postmitotic immature cortical neurons, exhibit depression and anxiety behaviors that is closely related with reduced dendritic complexity of hippocampal dentate gyrus (DG) granule cells and reduced ventral DG circuit connectivity (Huang et al. Transl Psychiatry 12:411, 2022). Our study of global *Blm-s*-KO mice and *Gli1::CreER^{T2};Blm-s$^{lox/lox}$;Rosa26R-CAG::tdTomato* mice further suggests that BLM-s deficiency results in decreased proliferation of adult DG neural stem cells (DG NSCs) in part via cell-autonomous interference with the activation and subsequent cell cycle progression of quiescent *Gli1*-responsive DG NSCs in adult mice. Via study of adult DG neurosphere cultured *in vitro*, we demonstrate that depletion of BLM-s causes prolonged G1-phase upon cell cycle progression in actively proliferating DG neurospheres. Additionally, adult *Blm-s$^{-/-}$* DG neurospheres harbor higher glycolytic flux, lower mitochondrial respiratory capability, and elevated intracellular reactive oxygen species (ROS) compared with wildtype DG neurospheres. The metabolic profile and mitochondrial morphology together suggest that BLM-s depletion confers DG NSCs in a more naïve primitive and inactivated RGL status.

Disclosures: H. Lo: None. **Y. Jhang:** None. **C. Tsou:** None. **H. Cheng:** None. **P. Huang:** None.

Poster

PSTR427. Animal Models of Mood Disorders: Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR427.20/OO11

Topic: G.05. Mood Disorders

Support: 4K00NS125774 - 02
Ernest E. Just Postdoctoral Fellowship in the Life Sciences

Title: Mrgprb4-lineage Social Touch Somatosensory Neurons Engage a Skin-Brain Pathway Mediating Stress Resilience

Authors: *S. FULTON[1], M. SCHAFFLER[3], M. JOHNSON[5], B. W. HING[6], P. KAHLER[5], I. HULTMAN[5], S. SRIVASTAVA[5], J. ARNOLD[1], J. A. BLENDY[4], R. HULTMAN[5], I. ABDUS-SABOOR[2];
[1]Zuckerman Inst., Columbia Univ., New York City, NY; [2]Columbia Univ., New York, NY; [4]Systems Pharmacol. and Translational Therapeut., [3]Univ. of Pennsylvania, Philadelphia, PA; [6]Psychiatry, [5]Univ. of Iowa, Iowa City, IA

Abstract: Tactile interaction between social mammals is a naturally rewarding stimuli, motivating social bonding and other adaptive behaviors. Socially valanced touch can also have anxiolytic effects and has been shown to attenuate stress-related pathophysiology. Importantly, a lack of social tactile stimulation, particularly during early life, can induce neurobiological changes associated with mood disorders. However, the neuronal circuits by which social touch mediates stress resilience and affective states are unknown. We have previously characterized a genetically defined subpopulation of social touch-responsive somatosensory neurons in the mouse dorsal root ganglion, Mrgprb4-lineage neurons. We found that activation of Mrgprb4-lineage neurons stimulates dopamine release, promotes CPP, and facilitates normal mating behaviors in female mice. Thus, Mrgprb4-lineage neurons appear to be part of a rewarding skin-brain touch circuit, but it is still unknown if these neurons also play a role in the anxiolytic effects of social touch. Here, we use mouse genetics and targeted neuronal manipulations in combination with behavioral, electrophysiological, and molecular phenotyping to demonstrate a critical role for Mrgprb4-lineage touch neurons in stress resilience. We found that early-life ablation of Mrgprb4-lineage touch neurons increases vulnerability to subthreshold stressors in adulthood for both male and female mice, including an increase in anhedonia-like and despair-like behaviors. Multi-unit *in vivo* neurophysiological recordings across seven brain regions revealed that Mrgprb4-lineage neuron ablation induces a unique pattern of electrical activity at the network-level, characterized by altered modulatory interactions between the Basolateral Amygdala (BLA) and the mesolimbic reward pathway (e.g. NAc, VTA), as well as specific changes in VTA-NAc signaling. We next leveraged molecular profiling in these altered regions (BLA, NAc) to define the cell-type specific transcriptomic changes associated with developmental loss of Mrgprb4-lineage neuron input to the CNS. Finally, we show that Mrgprb4-lineage neuron activation attenuates HPA reactivity, highlighting the therapeutic potential of targeting this somatosensory population in stress-related psychiatric disorders. Together, our data demonstrate that Mrgprb4-lineage neurons mediate skin-brain communication pathways that are critically important to stress resilience phenotypes.

Disclosures: S. Fulton: None. **M. Schaffler:** None. **M. Johnson:** None. **B.W. Hing:** None. **P. Kahler:** None. **I. Hultman:** None. **S. Srivastava:** None. **J. Arnold:** None. **J.A. Blendy:** None. **R. Hultman:** None. **I. Abdus-Saboor:** None.

Poster

PSTR427. Animal Models of Mood Disorders: Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR427.21/OO12

Topic: G.05. Mood Disorders

Support: NMIH Grant MH106460

Title: Understanding the mechanisms of how a ketogenic diet might treat bipolar disorder

Authors: *M. TSYGLAKOVA, C. A. MCCLUNG;
Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Bipolar disorder is a common and debilitating mood disorder. It affects a person's mood, concentration and energy levels, and it is characterized by aberrant GABAergic and dopaminergic signaling, as well as mitochondrial dysfunction and oxidative stress. There is recent interest in the ketogenic diet as a treatment for bipolar disorder with small case study reports of efficacy. However, the neurobiological mechanisms by which ketone bodies might ameliorate symptoms of bipolar disorder are yet to be determined. Here we investigate whether a ketogenic diet rescues manic-like behavior in *Clock*Δ19 mice, a mouse model of bipolar mania, and whether it leads to changes in gene expression in the ventral tegmental area (VTA) of the brain. To investigate the effects of ketogenic diet on behavior, homozygous *Clock*Δ19 and wild type (WT) male and female mice (n = 8-10/treatment, sex, genotype group) were treated with regular chow or a ketogenic diet AIN-76A-Modifled (Bio-Serv S3666) for two weeks and then throughout behavioral testing. The behavior testing battery consisted of the following tests: locomotor activity, open field, dark/light box, elevated plus maze and forced swim test. Following testing, mice were sacrificed, brains were rapidly extracted and flash frozen, punches from the VTA were taken and RNA isolated for quantitative PCR analysis. Our results show that the ketogenic diet normalized the abnormally high novelty seeking behavior in female *Clock*Δ19 mice, with no effect in the males in the light dark box. In comparison the diet produced an antidepressant-like response in both males and females in the forced swim test. Interestingly ketogenic diet increased locomotor activity in female ClockΔ19 mice and male WT mice only. In addition, we found that ketogenic diet led to a significant decrease in *tyrosine hydroxylase* (a rate-limiting enzyme in dopamine synthesis) expression in *Clock*Δ19 mice, suggesting that the diet might reduce their aberrant hyper dopaminergic transmission. Our findings suggest that the ketogenic diet affects mouse behavior relevant to bipolar disorder and may reduce the hyper dopaminergic synthesis in the VTA of *Clock*Δ19 mice. Funding: This work was supported by a grant from NMIH (MH106460) (CAM).

Disclosures: M. Tsyglakova: None. **C.A. McClung:** None.

Poster

PSTR428. Addictive Drugs: Sex Differences in Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR428.01/OO13

Topic: G.09. Drugs of Abuse and Addiction

Support: R03 DA049212

Title: Divergent effects of mGlu3 receptor activation on meth-seeking and meth-induced working memory deficits in male and female rats

Authors: *R. CONRAD, C. MODRAK, P. HAMOR, L. WU, M. SCHWENDT;
Univ. of Florida, Gainesville, FL

Abstract: Chronic methamphetamine (meth) abuse is associated with a spectrum of neurobehavioral deficits, including changes to working memory (WM). Studies suggest that impaired cognition predicts a higher risk for relapse and worsens treatment outcomes in chronic meth users. Moreover, both the severity of cognitive impairment and the intensity of meth craving undergoes dynamic changes after discontinuation of meth use, bringing about a high-risk period for recovering meth users. Critically, while females tend to engage in more robust meth-seeking behavior than males, sex differences in meth-induced WM deficits are unclear, and the neurobiology underlying these behavioral deficits remains poorly understood. Here, we introduce a translational animal model that allows for the concurrent evaluation of post-meth WM performance and meth-seeking, along with the effects of mGlu3 glutamate receptor activation on these variables. mGlu3 is a viable target for intervention, as studies show that dysregulation of mGlu2/3 receptors is associated both with WM deficit and persistent drug-seeking. Male and female Sprague-Dawley rats were first trained on the operant delayed match-to-sample (DMS) task and tested for 10 days to establish baseline WM across a range of delays (0-24s). Rats then underwent 21 days of meth self-administration followed by ~7 weeks of homecage abstinence, during which DMS was re-tested. We found that a post-meth WM deficit coincided with robust meth-seeking as far as 12 days into abstinence, with females more severely impaired than males. Next, treatment of indirect mGlu3 agonist 2-PMPA (30 mg/kg, i.p) rescued WM impairment at long delays in a subgroup of the most severely-impaired rats. Conversely, acute 2-PMPA administration failed to alter cue/context-primed meth-seeking nor had any delayed effects on extinction learning or WM. Finally, under a condition of high WM demand (24s delay only), 2-PMPA treatment partially improved WM performance, motivating further investigation of acute pro-cognitive effects of mGlu3 activation and its underlying effects with respect to meth-seeking and post-meth cognitive changes while considering sex as a variable.

Disclosures: R. Conrad: None. **C. Modrak:** None. **P. Hamor:** None. **L. Wu:** None. **M. Schwendt:** None.

Poster

PSTR428. Addictive Drugs: Sex Differences in Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR428.02/OO14

Topic: G.09. Drugs of Abuse and Addiction

Support: R03 DA049212

Title: Investigation of sex-specific and meth-seeking behaviors in rats with a history of predator scent stress.

Authors: *C. G. MODRAK, C. S. WILKINSON, L. KNACKSTEDT, M. SCHWENDT; Psychology, Univ. of Florida, Gainesville, FL

Abstract: Only a subset of trauma-exposed individuals go on to experience long-lasting behavioral impairments and receive a diagnosis of post-traumatic stress disorder (PTSD). Developing effective treatments for PTSD is complicated due to the heterogeneity of stress responses and comorbidity with other psychiatric disorders such as substance use disorder. As such, individual and sex-specific differences in stress responses have been observed in comorbid (and non-comorbid) populations, with females at a greater risk to develop PTSD after trauma exposure, and engaging in differential anxiety and fear-reactive behavior than males. Overall, PTSD patients are twice as likely to use methamphetamine (meth), and preclinical research shows that both early life stress and acute stress in adulthood augment meth-seeking, with females twice as likely to use meth with shorter abstinence periods. Unfortunately, animal research to date inadequately addresses the individual and sex-specific heterogeneity of stress responses. Thus, the present study sought to investigate the consequences of a trauma-like stressor on subsequent anxiety-like behavior and meth-seeking in male and female rats. Rats (n=40) were briefly exposed to either trimethylthiazoline (TMT) or a control scent (n=24) during the predator scent stress (PSS) task and evaluated for anxiety-like behavior one week later in the elevated plus maze (EPM) and acoustic startle response (ASR). Following re-exposure to the TMT context to assess conditioned fear response, rats underwent 21 days of meth self-administration, extinction training, and cue-primed reinstatement. Rats were perfused and evaluated for cFos expression throughout the prefrontal cortex, as prefrontal activation is linked to enhanced meth-seeking behavior. TMT-exposed rats exhibited elevated anxiety and fear behaviors as shown by increased freezing during re-exposure, hypolocomotion in EPM, and less time in the open arms during EPM compared to controls. This same group subsequently exhibited greater meth intake and significantly reinstated to meth-paired cues following extinction, suggesting elevated meth-seeking behaviors in response to stress. While there was no link between drug-seeking and cortical activity, there was a negative correlation between locomotion and time spent in the TMT quadrant during exposures and the prelimbic and infralimbic cortices, suggesting the possibility for a role of these regions in PSS-induced fear. Future directions are to use AI approaches to identify additional fear-reactive behaviors following PSS exposure.

Disclosures: C.G. Modrak: None. **C.S. Wilkinson:** None. **L. Knackstedt:** None. **M. Schwendt:** None.

Poster

PSTR428. Addictive Drugs: Sex Differences in Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR428.03/OO15

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA033436
NIH Grant DA045140

Title: Role of the prelimbic cortex projection to the nucleus accumbens core in the reinstatement of cocaine-seeking

Authors: *J. MESA, L. KNACKSTEDT;
Univ. of Florida, Gainesville, FL

Abstract: The prelimbic (PL) cortex to nucleus accumbens core (NAc) pathway is strongly implicated in the reinstatement of cocaine-seeking. Pharmacological or optogenetic inhibition of this pathway attenuates reinstatement, and reinstatement is accompanied by release of glutamate into the NA core. The antibiotic ceftriaxone reliably reduces the reinstatement of cocaine-seeking and the release of glutamate into the NA core. However, the majority of cocaine users also consume alcohol and in a rodent model of cocaine-alcohol polysubstance use (PSU), we found that glutamate release in the NA core no longer accompanies cocaine-reinstatement and ceftriaxone is unable to attenuate reinstatement. Here we continue to probe the role of this pathway in mediating reinstatement. Expt. 1 utilized the retrograde tracer cholera toxin B in combination with c-Fos immunohistochemistry to determine whether ceftriaxone attenuates the activity of neurons projecting from the PL-NAc. Expt. 2 used chemogenetics to determine whether inhibition of the PL-NAc pathway attenuates reinstatement in PSU rats. Sprague Dawley rats underwent surgery for jugular catheterization and received either intra-NAc infusion of CTb, or AAV6-cre into the NAc and AAV2-hSyn-DIO-hM4D(Gi) into the PL cortex. Rats next underwent 12 days of cocaine intravenous self-administration (IVSA; 2 hr/day; 1 mg/kg/infusion) followed by 2 weeks of daily instrumental extinction training. A subset of rats received 2-bottle choice alcohol/water access (6 hr/day) in the home cage following daily cocaine IVSA sessions. Rats underwent a 2 hr cue-primed reinstatement test and were immediately perfused. Brains were dissected and the PL and NA core were sliced and stained for c-Fos. In Expt. 1, rats were treated with either ceftriaxone (200 mg/kg IP) or vehicle for 5-7 days prior to reinstatement testing. We found that ceftriaxone attenuated cue-induced reinstatement but did not alter c-Fos expression in prelimbic cortex neurons overall and in those that project to the NA core. Expt. 2 data analysis is ongoing. The current results indicate that inhibition of neurotransmission along the PL-NAc pathway is not necessary to attenuate cocaine seeking in rats that consume cocaine alone.

Disclosures: J. Mesa: None. **L. Knackstedt:** None.

Poster

PSTR428. Addictive Drugs: Sex Differences in Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR428.04/OO16

Topic: G.09. Drugs of Abuse and Addiction

Title: Behavioral economic analysis of oxycodone-THC co-use in a rat model

Authors: *K. DRIVER[1], C. JONES-GOUCHER[1], A. SANCHEZ[1], B. SETLOW[2], M. SCHWENDT[1], L. A. KNACKSTEDT[1];
[1]Psychology, [2]Dept. of Psychiatry, Univ. of Florida, Gainesville, FL

Abstract: Opioid use disorder (OUD) lacks an effective, broad-spectrum treatment. Limited clinical evidence suggests that co-use of cannabinoids can reduce the rates of opioid dependence and severity of withdrawal, but potentially increases anxiety. Despite poor understanding of risks and benefits of opioid-cannabinoid interactions, OUD is currently considered a qualifying condition for the use of medical cannabis in several US states. Thus, well-controlled, translational animal models are necessary to investigate the neural and behavioral consequences of cannabinoid-opioid co-use. The current study investigated the effects of daily oral Δ9-tetrahydrocannabinol (THC) consumption on behavioral economic demand for intravenously self-administered (IVSA) oxycodone in male and female Sprague-Dawley rats. Rats were first trained to self-administer IV oxycodone or oral sucrose under a FR (fixed ratio)-1 schedule for 6 days followed by a FR-3 schedule for 6 days. After reaching stable intake during training, rats began economic demand procedures in which the FR requirement to earn a reinforcer was increased in quarter log unit increments on successive days until zero reinforcers were attained for a given FR. Throughout the demand procedures, rats also received access to unsweetened gelatin containing either THC or vehicle in the home cage for one hour following the oxycodone/sucrose session. Somatic signs of withdrawal were assessed, and the light-dark box test was used to assess anxiety-like behavior at 22 hr withdrawal. Following completion of economic demand procedures, self-administration at FR-3 was re-established prior to 14 days of home cage abstinence. On Day 15 of abstinence, rats underwent a cue-primed relapse test. Brains were collected to assess levels of mu opioid and CB1 cannabinoid receptor phosphorylation in select regions in response to an acute oxycodone challenge. No effects of sex were observed on oxycodone self-administration. THC consumption led to increased demand elasticity (i.e. reduced seeking upon FR increases) for oxycodone IVSA and decreased cue-primed relapse, with no effects on sucrose intake or somatic signs of withdrawal. The findings of this study provide first-ever preclinical evidence regarding the effects of THC on motivation to seek oxycodone, as well as assessment of corresponding receptor activity changes.

Disclosures: K. Driver: None. **C. Jones-Goucher:** None. **A. Sanchez:** None. **B. Setlow:** None. **M. Schwendt:** None. **L.A. Knackstedt:** None.

Poster

PSTR428. Addictive Drugs: Sex Differences in Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR428.05/OO17

Topic: G.09. Drugs of Abuse and Addiction

Title: Oxytocin reduces alcohol intake in a model of alcohol+opioid polysubstance use

Authors: *T. D. THOMPSON[1], C. S. WILKINSON[1], C. M. MODRAK[1], R. CONRAD[1], I. LEON[2], L. A. KNACKSTEDT[1];
[1]Univ. of Florida, Gainesville, FL; [2]San Diego State Univ., San Diego, CA

Abstract: Over two million Americas have both alcohol use disorder (AUD) and an illicit drug use disorder; 24.2% of those who misuse opioids report an AUD diagnosis. The neuropeptide, oxytocin, shows promise as a treatment for alcohol use disorder (AUD). Humans with AUD treated with intranasal oxytocin decrease alcohol craving, withdrawal, and intake; and preclinical research in rodents show oxytocin decreases alcohol intake. It is unknown whether oxytocin can reduce alcohol intake in the context of alcohol+opioid polysubstance use (PSU). To begin to examine this, and to determine the neurocircuitry of oxytocin's effects on alcohol intake with a focus on the basolateral amygdala (BLA), we developed a rodent model of oral alcohol+oxycodone PSU. Forty Sprague Dawley rats (half female) were assessed for baseline anxiety-like behavior on the elevated plus maze (EPM) and acoustic startle response (ASR) task, implanted with cannula in the BLA, and then divided into three groups based on future drug condition: alcohol-only (n = 16), oxycodone-only (n = 8) or PSU (n = 16). Rats that would later comprise the oxycodone-only and PSU groups were given daily 2-bottle choice (2-BC) access to an oxycodone solution (0.1 mg/ml) and water or water only for 6 hrs/day for 7 days. Next, a subset of rats that would later comprise the alcohol-only and PSU groups received intermittent access to alcohol (IAA) where rats received 2-BC access to alcohol (20% v/v) and water for five 24 hr periods separated by 24 hrs with no access. Next, PSU rats underwent 12 days of PSU sessions where they received 2-BC access to oxycodone and water for 3 hrs followed by 6 hrs 2-BC for alcohol and water. Monosubstance conditions continued to receive access to either oxycodone or alcohol according to the same timing. Oxytocin (0 or 1 mg/kg, IP or 0.6 nmol/0.25uL/side, intra-BLA) was administered 30 minutes prior to alcohol access on test day. Mean startle positively correlated with oral oxycodone intake. Oxycodone access, both prior to IAA and on the same day in PSU sessions, increased alcohol intake. Systemic oxytocin decreased alcohol consumption in single and polysubstance rats relative to basesline alcohol consumption. These results implicate baseline anxiety increases susceptibility to oxycodone and suggest oxytocin will remain effective in polysubstance users.

Disclosures: T.D. Thompson: None. **C.S. Wilkinson:** None. **C.M. Modrak:** None. **R. Conrad:** None. **I. Leon:** None. **L.A. Knackstedt:** None.

Poster

PSTR428. Addictive Drugs: Sex Differences in Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR428.06/OO18

Topic: G.09. Drugs of Abuse and Addiction

Support: F31DA057806

Title: Sex-specific effects of predator scent stress on oxycodone self-administration

Authors: *C. S. WILKINSON[1], S. BHUTANI[2], M. SCHWENDT[1], L. A. KNACKSTEDT[1]; [1]Psychology Dept., [2]Univ. of Florida, Gainesville, FL

Abstract: Post-traumatic stress disorder (PTSD) is often comorbid with substance use disorder (SUD). Animal models of psychiatric disorders are critical for the uncovering of relevant neurobiology. A widely used rat model of PTSD exposes outbred rats to predator scent stress once for 10 minutes and, weeks later, evaluates fear and anxiety-like behavior. Reliably, only a subset of rats show long-term susceptibility to such stress, and in our prior work, we found that susceptible rats displayed increased cocaine-seeking. The present study sought to use the same model to investigate the relationship between stress susceptibility and opioid-seeking. Eighty-four Sprague Dawley rats (half female) received a single 10-minute exposure to the fox pheromone 2,5-dihydro-2,4,5-triethylthiazoline (TMT) or unscented control condition in an inescapable chamber. Seven days later, rats were assessed for anxiety-like behavior using the acoustic startle response and elevated plus maze (EPM). Rats were implanted with jugular catheters and underwent self-administration of intravenous oxycodone (0.1 mg/kg/infusion) for 6 days on a fixed ratio-1 (FR-1) schedule of reinforcement followed by 6 days on an FR-3 schedule. Rats underwent extinction training and a single cue-primed reinstatement test. Tail blood was collected immediately before and after TMT/control exposure and after the reinstatement test for quantification of corticosterone (CORT) concentrations. TMT-exposed rats increased freezing during exposure and decreased time spent in the open arms of the elevated plus maze one week later. Female TMT-exposed rats decreased active lever presses for oxycodone and oxycodone intake compared to controls, while male TMT-exposed rats did not differ from controls. Corticosterone release was protective against anxiety-like behavior but was not related to the reinstatement of oxycodone-seeking. These findings indicate a sex-specific effect of stress on subsequent motivation to seek oxycodone and further analysis will elucidate the role of corticosterone and neuronal activation in these effects.

Disclosures: C.S. Wilkinson: None. **S. Bhutani:** None. **M. Schwendt:** None. **L.A. Knackstedt:** None.

Poster

PSTR428. Addictive Drugs: Sex Differences in Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR428.07/OO19

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant 1R01DA049470-02S1

Title: Evaluation of cannabidiol’s effects on oxycodone-induced antinociception and reward-related behavior

Authors: *A. C. BRICE-TUTT[1], W. MALPHURS[1], A. BEHNOOD-ROD[1], A. S. SENETRA[2], R. M. CAUDLE[3], M. FEBO[4], A. W. BRUIJNZEEL[5], A. SHARMA[2], B. SETLOW[5], N. P. MURPHY[1], J. K. NEUBERT[1];
[1]Orthodontics, [2]Pharmaceutics, [3]Oral and Maxillofacial Surgery, [4]Neurosci., [5]Psychiatry, Univ. of Florida, Gainesville, FL

Abstract: We evaluated the effect of chronic treatment with cannabidiol and oxycodone, alone or in combination, on pain- and reward-related behavior in male and female rats (N = 8-10/treatment/sex). Rats were trained to consume sweetened condensed milk solution under painful (44.5°C) and non-painful (37°C) conditions in an operant pain assay. They were then treated daily for 14 days with oxycodone (0.56 mg/kg, i.p.), cannabidiol (3.2 and 10 mg/kg, i.p.), or cannabidiol + oxycodone combinations, and operant pain responding was evaluated. Rearing behavior was also recorded to assess development of behavioral sensitization. Oxycodone alone increased operant responding in a sex- and temperature-dependent manner. Neither dose of cannabidiol alone altered responding. Combined with oxycodone, however, cannabidiol dose-dependently increased the effect of oxycodone only at the nociceptive (44.5°C) temperature. Oxycodone also produced locomotor sensitization, which was neither attenuated nor potentiated by cannabidiol. In a separate study, rats conditioned with oxycodone in a conditioned place preference paradigm developed a significant preference for the oxycodone-paired chamber, an effect that was not impacted by combination with cannabidiol during conditioning. These results suggest that while devoid of inherent analgesic and rewarding effects, cannabidiol potentiates the antinociceptive effects of oxycodone without affecting its rewarding properties under these experimental conditions. As such, cannabidiol may be useful as an opioid-sparing approach to treat pain.

Disclosures: A.C. Brice-Tutt: None. **W. Malphurs:** None. **A. Behnood-Rod:** None. **A.S. Senetra:** None. **R.M. Caudle:** None. **M. Febo:** None. **A.W. Bruijnzeel:** None. **A. Sharma:** None. **B. Setlow:** None. **N.P. Murphy:** None. **J.K. Neubert:** None.

Poster

PSTR428. Addictive Drugs: Sex Differences in Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR428.08/OO20

Topic:

Support: Knackstedt P0183697

Title: The effects of sex and orbitofrontal cortex inhibition on cocaine-seeking in a voluntary abstinence model

Authors: *C. JONES-GOUCHER, L. A. KNACKSTEDT;
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Abstract: Contingency management uses non-drug reinforcers to facilitate abstinence from drugs of abuse, effectively reducing drug seeking. Once discontinued, drug seeking often resumes. An animal model of contingency management is the voluntary abstinence model, in which after several weeks of intravenous drug self-administration, animals are provided a choice between drug and palatable food reinforcement in discrete trials. Voluntary abstinence produces a decrease in drug seeking that does not persist when access to palatable food is removed. Using such a model with male rats, we previously found that sucrose availability leads to a decrease in, but not cessation of, cocaine seeking and a differential relapse-induced c-Fos expression such that voluntary abstinence results in greater relapse-induced Fos expression in the ventral orbitofrontal cortex (vOFC). Here we sought to extend these findings to female rats and test the necessity of the vOFC in relapse, using intra-OFC AAV8-hSyn-hM4D(Gi)-mCherry or control AAV8-GFP vector and systemic DCZ administration. Male and Female Sprague-Dawley rats (n=50, half male) first self-administered sucrose pellets for five days and then intravenous cocaine for 12 days. Rats then underwent 14 days of voluntary abstinence during which time rats had the opportunity to choose between sucrose and cocaine reinforcement in discrete trials (20 trials/3 hr session). Next, a relapse test was conducted during which time only the cocaine-paired lever was available and delivered cocaine-paired cues. Both males and females exhibited greater choices on the cocaine lever during early abstinence than late abstinence, with the sucrose lever representing more than 50% of choices by Day 6. However, by Day 14, group-level analysis finds that male and female rats continued to choose the cocaine lever during 35% of trials. In the last five days of voluntary abstinence, 35% of females and 39% of males continued to choose the cocaine lever on the majority of trials. There were no sex differences in the number of presses on the cocaine lever during the relapse test. Analysis of vOFC inactivation and cortical c-Fos expression is ongoing. These results extend the use of the voluntary abstinence model to female rats and will potentially shed light into the role of the vOFC in relapse both sexes.

Disclosures: C. Jones-Goucher: None. **L.A. Knackstedt:** None.

Poster

PSTR428. Addictive Drugs: Sex Differences in Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR428.09/OO21

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R33DA045140

Title: Simultaneous intravenous cocaine and alcohol self-administration alters the motivation to seek cocaine and associated neurobiology

Authors: *A. SANCHEZ[1], C. JONES-GOUCHER[2], J. R. MESA[3], L. B. COTTLER[1], M. SCHWENDT[3], B. SETLOW[3], L. A. KNACKSTEDT[3];
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Abstract: An estimated 60-90% of cocaine users also use alcohol, but animal models of such polysubstance use (PSU) are lacking. In a recent epidemiological study from our research group, cocaine users reported using the two drugs simultaneously, for several hours at a time, approximately 7 days/month. Here we back-translated this real-world pattern of human PSU into a rat model of simultaneous cocaine+alcohol self-administration, to investigate the neurobiology of drug seeking in a PSU condition. Male and female Sprague-Dawley rats underwent intravenous self-administration (IVSA) of cocaine alone (n=24) or a cocaine+alcohol solution (n=25) for 2 hr/day for 5 consecutive days, then 6 hr/day, 2 days/week, for 5 weeks. Two doses of cocaine were tested (0.25 and 0.5 mg/kg/infusion) with and without alcohol (12.5 or 25 mg/kg/infusion). Control rats received yoked-saline infusions. At the conclusion of IVSA, rats were tested on a progressive ratio schedule for 2-3 days, followed by tests for cued cocaine-seeking (relapse) at 1 and 30 days of abstinence. Brains were collected and processed for fluorescent *in situ* hybridization for c-fos and dopamine 2 (D2) receptor mRNA expression. For the low dose of cocaine, the addition of alcohol to the intravenous solution had no effect on cocaine intake during self-administration, breakpoint for self-administration, or cued relapse after 30 days of abstinence. For the high dose of cocaine, the addition of alcohol to the intravenous solution resulted in increased cocaine intake during self-administration, increased breakpoint for self-administration, and increased cued relapse after 1 day of abstinence, with no sex differences observed. C-fos mRNA expression in both D2-positive and -negative cells was increased relative to yoked controls following the cued relapse test in the cocaine-only and cocaine+alcohol condition in the nucleus accumbens core and shell. In the prelimbic (PL) cortex, however, the number of c-fos/D2-positive cells was greater in the cocaine+alcohol condition relative to the cocaine-only condition. Current work is quantifying dopamine release in the PL and nucleus accumbens following intravenous cocaine and cocaine+alcohol administration. In conclusion, simultaneous cocaine+alcohol self-administration increases cocaine intake and the motivation to seek drug and drug-associated cues in a dose-dependent manner, with a possible role for dopamine signaling in the PL.

Disclosures: **A. Sanchez:** A. Employment/Salary (full or part-time):; University of Florida. **C. Jones-Goucher:** A. Employment/Salary (full or part-time):; University of Florida. **J.R. Mesa:** A. Employment/Salary (full or part-time):; University of Florida. **L.B. Cottler:** A. Employment/Salary (full or part-time):; University of Florida. **M. Schwendt:** A. Employment/Salary (full or part-time):; University of Florida. **B. Setlow:** A. Employment/Salary (full or part-time):; University of Florida. **L.A. Knackstedt:** A. Employment/Salary (full or part-time):; University of Florida.

Poster

PSTR428. Addictive Drugs: Sex Differences in Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR428.10/OO22

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R33DA045140

Title: Effects of concurrent cannabis on cocaine use and relapse in Sprague Dawley rats

Authors: *K. M. GONZALEZ[1,2], T. N. THOMSON[3,2], M. L. HALCOMB[3,2], L. B. COTTLER[4,2], L. A. KNACKSTEDT[5,2], B. SETLOW[3,2];
[1]Neurosci., [2]Ctr. for Addiction Res. and Educ., [3]Psychiatry, [4]Epidemiology, [5]Psychology, Univ. of Florida, Gainesville, FL

Abstract: Polysubstance use (PSU), or the ingestion of multiple drugs of abuse within a defined period, is inarguably commonplace, with cannabis and cocaine being one of the most prevalent drug combinations. To better understand this phenomenon, we developed a rodent model of cocaine + cannabis use based on the most common patterns of human PSU determined as part of a collaborative NIDA-funded project with UF epidemiologists. Thirty-two male and female Sprague Dawley rats (10 wks.) were implanted with chronic indwelling jugular catheters and received 3 days of either cannabis smoke, placebo smoke, or clean air exposure before undergoing 3 days of training in intravenous (IV) cocaine self-administration (SA) for 3 hours/day at a dose of 0.5 mg/kg/infusion, with each infusion accompanied by an audiovisual cue. Following these 3 days of baseline cocaine intake, they resumed exposure to cannabis smoke, placebo smoke, or clean air for 5 hours/day, 5 days/week for 5 weeks. During this time, rats continued to undergo cocaine SA sessions 1 day/week, immediately following exposure to smoke or clean air. All rats then underwent a 30-day abstinence period in which they were left undisturbed in their home cages, followed by a cued cocaine relapse test. During the 3 days of IVSA training, rats previously exposed to cannabis smoke self-administered more cocaine than controls, a pattern that reversed when cannabis exposure immediately preceded IVSA sessions. During the cued relapse test session, the cannabis smoke group exhibited elevated cocaine-seeking compared to the two control groups. Overall, the results show that although cannabis co-use attenuates cocaine intake acutely, it produces heightened cocaine seeking when cannabis is not onboard, both during self-administration and cued relapse. These findings emphasize the importance of working with animal models that reflect real-world combinations and patterns of drug intake.

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Poster

PSTR428. Addictive Drugs: Sex Differences in Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR428.11/OO23

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R33DA045140

Title: The role of dopamine and glutamate mediating sex differences in oxytocin's effects on intravenous oxycodone demand

Authors: *H. L. BLOUNT[1,2], C. S. WILKINSON[1,2], A. SANCHEZ[1], T. D. THOMPSON[1], M. SCHWENDT[1,2], L. A. KNACKSTEDT[1,2];
[1]Psychology, Univ. of Florida, GAINESVILLE, FL; [2]Ctr. for Addiction Res. and Educ., Gainesville, FL

Abstract: Oxytocin is an emerging treatment for a variety of neuropsychiatric disorders, including substance use disorders. Previously, our lab has found that systemic and intra-accumbal oxytocin decreases cocaine cue-primed reinstatement, via a mGlu2/3 receptor-dependent mechanism. Further, we have found that systemic oxytocin produces dopamine and glutamate efflux in the nucleus accumbens core (NA core) of cocaine-experienced male rats but only increases glutamate, and not dopamine, efflux in cocaine-experienced females. Here we assess the effects of chronic, systemic oxytocin on economic demand for intravenous oxycodone in male and female rats. Male (n=25) and female (n=25) Sprague Dawley rats were implanted with jugular catheters. A subset received bilateral microdialysis cannulae aimed at the NA core. Rats were trained to self-administer intravenous oxycodone (0.4 mg/kg/infusion for males; 0.32 mg/kg/infusion for females) for 3 hr/day on FR-1 for 6 days then FR-3 for 6 days. Rats then began economic demand procedures in which the FR requirement to earn a reinforcer was increased in quarter log unit increments every second day until zero reinforcers were attained for a given FR. Rats were treated with oxytocin (1 mg/kg, IP) or vehicle (saline) prior to demand sessions. In a subset of rats, microdialysis was conducted after reaching FR-18. During microdialysis procedures, rats were unilaterally probed with a microdialysis cannulae and baseline samples were collected for two hours, followed by oxytocin (1 mg/kg IP). Thirty minutes later, intravenous oxycodone (1 mg/kg) was administered and samples collected for 3 more hours. HPLC was conducted to determine glutamate and dopamine content. We found that in males, but not females, chronic oxytocin increases demand elasticity (alpha) to self-administer oxycodone. Also in males, oxytocin reduces P_{max}, or the maximum price (FR) rats are willing to "pay" for intravenous oxycodone. Analysis of the effects of oxytocin on NA core dopamine and glutamate efflux may explain the sex-difference in oxytocin's effects on oxycodone demand. These data provide further evidence that oxytocin is effective at reducing drug-seeking, but indicate important sex differences in its ability to do so in the context of opioid-seeking.

Disclosures: H.L. Blount: None. **C.S. Wilkinson:** None. **A. Sanchez:** None. **T.D. Thompson:** None. **M. Schwendt:** None. **L.A. Knackstedt:** None.

Poster

PSTR428. Addictive Drugs: Sex Differences in Neural Mechanism

Location: WCC Halls A-C

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Program #/Poster #: PSTR428.12/OO24

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant R01 DA053328

Title: Local infusion of the D3 receptor agonist pramipexole into the prelimbic cortex reduces sucrose self-administration in male rats.

Authors: *D. GONZALEZ, F. J. CANO, H. UDAYASHANKAR, V. K. DANG, K. K. SZUMLINSKI;
UC Santa Barbara, Santa Barbara, CA

Abstract: High sugar consumption activates the mesocorticolimbic dopamine system resulting in an increase in extracellular dopamine at various terminal regions in the brain, including the prefrontal cortex (PFC). Despite this, relatively little is known regarding the functional relevance of specific dopamine receptor subtypes within the PFC in regulating the rewarding and reinforcing properties of sugars or other palatable foods. Herein, we employed classic neuropharmacological approaches to bi-directionally regulate the function of dopamine D1 and D3 receptors within the medio-dorsal aspects of the PFC and examine for effects on the self-administration of sucrose. For this, adult male Sprague-Dawley rats were trained to lever-press for delivery of 45 mg banana-flavored sucrose pellets for 2 h/day until responding stabilized. Using a within-subjects, semi-randomized design, rats were microinjected with vehicle (VEH) or the following dopaminergic compounds: the D1 receptor agonist A-6890, the D1 receptor antagonist SCH 23390, the D3 receptor agonist pramipexole and the D3 receptor antagonist NG 2904 (each at 10 µg/side). Two-three days were allowed between microinjections to examine for carry-over effects. Of all the compounds tested, only the infusion of the D3 receptor agonist pramiprexole reduced sucrose reinforcement and intake. This pramiprexole effect was apparent in rats in which the prelimbic subregion of the PFC was targeted, with no effects observed in rats with the more dorsal anterior cingulate subregion targeted. Our results do not support a major role for D1 receptor activation within either the prelimbic or anterior cingulate cortices in gating sucrose reinforcement. Further, our results indicate an active, inhibitory, role for D3 receptors selectively within the prelimbic cortex in sucrose reinforcement. This latter finding aligns with the purported inhibitory effects of D3 receptor agonists on the self-administration of drugs of abuse, arguing a key role for prelimbic D3 receptors in regulating the reinforcing properties of both drug and non-drug/food rewards of relevance to the potential therapeutic utility of D3 receptor-selective agonists and partial agonists for the treatment of over-eating and food-craving disorders and the potential off-target effects of such compounds during the treatment of substance use disorders.

Disclosures: D. Gonzalez: None. **F.J. Cano:** None. **H. Udayashankar:** None. **V.K. Dang:** None. **K.K. Szumlinski:** None.

Poster

PSTR428. Addictive Drugs: Sex Differences in Neural Mechanism

Location: WCC Halls A-C

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Program #/Poster #: PSTR428.13/OO25

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA grant R01 DA053328

Title: Sucrose intake and craving is dissociated from mTOR activity or expression within the PFC

Authors: *F. J. CANO[1], C. J. E. DENNING[2], V. K. DANG[2], H. UDAYASHANKAR[2], D. GONZALEZ[3], K. MOHAMMADI[2], K. K. SZUMLINSKI[2];
[1]Psychological and Brain Sci., Univ. of California Santa Barbara, Goleta, CA; [2]Psychological and Brain Sci., Univ. of California Santa Barbara, Santa Barbara, CA; [3]Psychological and Brain Sci., UC Santa Barbara, Santa Barbara, CA

Abstract: The similar temporal profile of incubated food- vs. drug-seeking posits that these behavioral phenomena may involve common, time-dependent, biochemical adaptations within neural circuits governing motivated behavior. Notably, Everolimus, an FDA-approved mTOR inhibitor, dose-dependently blocks incubated cocaine-seeking during late withdrawal and this anti-incubation effect is associated with a reversal of withdrawal-induced protein changes in the prelimbic (PL) subregion of the prefrontal cortex. Herein, we conducted a series of studies to examine the relationship between mTOR activity and measures of sucrose reinforcement in adult Sprague Dawley rats. For the first study, male and female Sprague Dawley rats were trained to self-administer 45 mg of banana-flavored sucrose pellets for 6 h/ day for 10 days and then were gavage infused with either vehicle (VEH) or the maximally effective Everolimus dose from our published cocaine study (1.0 mg/kg), prior to a test for cue-reinforced responding on withdrawal days (WD) 1 or 30. Immunoblotting was then conducted to examine for Everolimus' effects on mTOR activation within PL and infralimbic (IL) PFC subregions. As mTOR is upregulated by simple sugars, a distinct cohort of male Sprague-Dawley rats were trained to lever-press for the same sucrose pellets for 2 h/day until responding stabilized, at which point, bi-lateral microinjections of VEH or Everolimus (100 ng/side) into the dorsomedial aspect of the prefrontal cortex were performed to examine for effects of local mTOR inhibition on sucrose intake. Despite lowering phospho-mTOR levels within the IL, Everolimus gavage infusions did not alter the magnitude of incubated sucrose-seeking exhibited by either male or female rats, relative to early withdrawal controls. Microinjections of Everolimus into the PL or the more dorsal anterior cingulate also did not change sucrose intake, relative to an infusion of vehicle. These findings indicate that, unlike incubated cocaine-craving, incubated sucrose-craving is not correlated with elevated mTOR activity within prefrontal cortex subregions, nor does it require mTOR activation to manifest. Likewise, mTOR activity within the PL and anterior cingulate does not impact sucrose intake. These data indicate that the incubation of drug- and sucrose-seeking involve distinct molecular adaptations within the prefrontal cortex. Related to this, our data to date indicate negligible off-target effects of both systemic and intracranial Everolimus on the consumption of palatable food of relevance to its side-effect profile in the treatment of cocaine use disorder.

Disclosures: F.J. Cano: None. **C.J.E. Denning:** None. **V.K. Dang:** None. **H. Udayashankar:** None. **D. Gonzalez:** None. **K. Mohammadi:** None. **K.K. Szumlinski:** None.

Poster

PSTR428. Addictive Drugs: Sex Differences in Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR428.14/PP1

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA053328

Title: A relatively brief history of oral methamphetamine self-administration is sufficient to induce some signs of negative affect: relation to mGlu1 receptor activity within vPFC

Authors: C. J. E. DENNING[1], R. A. CABRERA[1], L. E. MADORY[1], J. N. HERBERT[1], *K. SZUMLINSKI[2];
[1]Univ. of California, Santa Barbara, Santa Barbara, CA; [2]Univ. California-Santa Barbara, Santa Barbara, CA

Abstract: Anxiety is one of the most common comorbid neuropsychiatric conditions with methamphetamine (MA) use disorder and the abrupt discontinuance of MA following chronic exposure induces withdrawal symptoms in humans consisting of depression and anxiety. As negative emotional states can drive drug-seeking and relapse, we are working to develop procedurally facile mice model of high-dose oral MA self-administration to examine the effects of a relatively brief history of oral MA upon brain and behavior. For this, adult male and female C57BL/6J (B6) mice were trained to orally self-administer water or one of three MA concentrations (0.8, 1.6 and 3.2 g/L) under a fixed ratio 1 reinforcement schedule for 1 h/day for 7 consecutive days. Then, mice were subjected to a 1-day behavioral test battery for negative affect. Immunoblotting was then conducted on tissue from ventral and dorsal prefrontal cortex (vPFC and dPFC). Females consumed more MA than and males and mice of both sexes consumed equivalent amounts of MA from the 1.6 and 3.2 g/L solutions. A prior brief history of 3.2 g/L significantly elevated the number of marbles buried and reduced the number of exploratory dips in the elevated plus-maze exhibited by both male and female mice, but also increased the number of entries into the open arms. The 3.2 g/L MA concentration also increased the latency of females to first enter the light-side of the light-dark shuttle box. A less statistically robust effect of the 3.2 g/L MA concentration was detected for the number of novel object contacts. No MA effects were detected for acoustic startle, while a prior history of 1.6 g/L MA reduced the expression of active coping (i.e., swimming) in the forced swim test. Immunoblotting detected a significant increase in mGlu1 expression within the vPFC of mice with a history of 1.6 g/L MA, with a less robust increase observed also in mice with a history of 3.2 g/L MA. No changes in mGlu1 were detected in the dPFC. As mGlu1 antagonists can exert anxiolytic effects, we conducted a pilot study in MA-experienced mice (7 days of 3.2 g/L MA) in which mice were pretreated systemically with 5 mg/kg of the mGlu1 antagonist JNJ16259685 30 min prior to anxiety testing. The JNJ compound reduced the number of marbles buried by MA-experienced mice but did not alter behavior in the light-dark box or elevated plus-maze. Taken together, these data indicate that a relatively brief history of oral MA is sufficient to induce some signs of anxiety-like behavior during early withdrawal that may reflect, at least in part, a MA-induced increase in mGlu1 receptor activity within vPFC.

Disclosures: **C.J.E. Denning:** None. **R.A. Cabrera:** None. **L.E. Madory:** None. **J.N. Herbert:** None. **K. Szumlinski:** None.

Poster

PSTR428. Addictive Drugs: Sex Differences in Neural Mechanism

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Program #/Poster #: PSTR428.15/PP2

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA grant R01DA053328

Title: Cocaine-craving elicited by extended-access self-administration procedures is dissociated from changes in PFC AMPA and NMDA receptor expression

Authors: *L. L. HUERTA SANCHEZ, H. H. T. DOAN, S. V. VO, M. G. TADROS, T. L. LI, P. B. JAMES, K. K. SZUMLINSKI;
UC Santa Barbara, Santa Barbara, CA

Abstract: The incubation of craving is a term coined to describe a behavioral phenomenon wherein cue-elicited craving intensifies during drug abstinence. Our laboratory has previously demonstrated an increase in extracellular glutamate within the medial prefrontal cortex (mPFC) in rats that exhibited an incubation of craving, which is theorized to activate AMPA and NMDA ionotropic glutamate receptors (iGluRs). However, the role for iGluRs within mPFC subregions in regulating incubated cocaine-craving is unexplored. As a first step towards this understanding, we examined for AMPA and NMDA receptor subunit expression in rats expressing incubated cocaine-seeking following two different short-access self-administration procedures and failed to detect any cocaine-related changes in iGluR subunit expression. To determine whether our prior negative results reflected the amount of cocaine experience of the rats, the present study examined for iGluR changes within the mPFC following a long-access cocaine self-administration procedure more typical of incubated cocaine-craving studies. For this, adult, male, Sprague-Dawley rats were trained to self-administer IV cocaine for 6-hr/day for 10 days where each cocaine delivery was paired with light-tone stimulus complex. Then, at 3 or 30 days withdrawal, rats underwent a 2-hr cue test for cue-reinforced responding and tissue from the prelimbic cortex (PL) and infralimbic cortex (IL) was collected for immunoblotting for AMPA and NMDA receptor subunits. While our long-access self-administration procedures did elicit incubation of craving, we detected no changes in AMPA or NMDA subunit expression either subregion of the mPFC using our conventional immunoblotting procedures. While these results indicate that the incubation of cocaine-craving is dissociated from changes in the total protein expression of iGluR, they do not preclude the possibility that incubated cocaine-craving is associated with an intracellular redistribution of AMPA and/or NMDA receptor subunits and/or post-translational modifications of specific receptor subunits that influence the functional status of iGluR receptors within PFC subregions to impact the magnitude of cue-elicited cocaine-craving.

Disclosures: **L.L. Huerta Sanchez:** None. **H.H.T. Doan:** None. **S.V. Vo:** None. **M.G. Tadros:** None. **T.L. Li:** None. **P.B. James:** None. **K.K. Szumlinski:** None.

Poster

PSTR428. Addictive Drugs: Sex Differences in Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR428.16/PP3

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA
NIAAA

Title: A relatively brief history of oral methamphetamine self administration is sufficient to induce cognitive impairment in C57BL6J mice.

Authors: *R. A. CABRERA, *R. CABRERA, C. J. E. DENNING, J. N. HERBERT, K. K. SZUMLINSKI;
Univ. of California - Santa Barbara, Santa Barbara, CA

Abstract: A relatively brief history of oral methamphetamine self-administration is sufficient to impair reversal learning in C57BL/6J mice and lower vPFC expression of NMDA receptor subunits.
R.A. Cabrera, C.J.E. Denning, J.N. Herbert, K.K. Szumlinski
Methamphetamine (MA) use disorder is characterized by cognitive impairments that impede treatment prognosis and recovery. In humans with MA use disorder, cognitive dysfunction is theorized to reflect glutamate-related neurotoxicity within frontal cortical regions. However, relatively little is known regarding the psychobiological consequences of subchronic MA exposure, more typical of recreational MA use. To that end, we worked to develop a mouse model of high-dose oral MA self-administration under operant-conditioning procedures to examine the effects of a relatively brief history of oral MA upon brain and behavior. For this, adult male and female C57BL/6J (B6) mice were trained to orally self-administer water or one of three MA concentrations (0.8, 1.6 and 3.2 g/L) under a fixed ratio 1 reinforcement schedule for 1 h/day for 7 consecutive days. Then, spatial learning and memory were determined in a Morris water maze, followed by testing for reference and working memory in a water version of the radial arm maze. Immunoblotting was then conducted on tissue from ventral and dorsal prefrontal cortex (vPFC and dPFC) for glutamate receptor expression. Females consumed more MA than and males and mice of both sexes consumed equivalent amounts of MA from the 1.6 and 3.2 g/L solutions. No effects of MA were detected for our measures of visually cued spatial navigation, spatial learning or memory in the Morris water maze. However, females with a history of 3.2 g/L MA exhibited deficits in reversal learning upon platform relocation. In the radial arm maze, mice with a history of 1.6 g/L MA exhibited more working memory incorrect errors and more “chaining” (a non-spatial navigation strategy). Immunoblotting revealed significantly lower expression the obligatory GluN1 subunit of the NMDA receptor in the vPFC.

These data indicate that a relatively brief history of oral MA is sufficient to induce some signs of cognitive dysfunction that may reflect impaired NMDA receptor function within vPFC. Funding provided by NIDA grant R01 DA053328.

Disclosures: R.A. Cabrera: None. **R. Cabrera:** None. **C.J.E. Denning:** None. **J.N. Herbert:** None. **K.K. Szumlinski:** None.

Poster

PSTR428. Addictive Drugs: Sex Differences in Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR428.17/PP4

Topic: G.09. Drugs of Abuse and Addiction

Title: Sex differences in oxycodone self-administration after PKMζknockout

Authors: *A. KNIFFIN[1], M. KNOUSE[1], M. NENOV[1], L. BIRMINGHAM[2], L. A. BRIAND[3]; [2]Psychology & Neurosci., [3]Psychology and Neurosci., [1]Temple Univ., Philadelphia, PA

Abstract: Opioid addiction is increasingly prevalent and characterized by high rates of relapse and subsequent drug induced overdose. Although currently males exhibit higher rates of opioid use disorder and drug overdose, the gender gap is closing, with females exhibiting faster transition from use to abuse. As the patterns of use and abuse exhibit sex differences, understanding how opioid exposure impacts the underlying neurobiological systems in both males and females is critical. The constitutively active form of protein kinase C, PKMζ, is exclusively expressed in the brain and is increased after exposure to opioids. In the absence of PKMζ, mice consume more alcohol and engage in higher rates of cocaine seeking. However, it is not known whether PKMζ plays a role in opiate reward. Therefore, in the current experiments we examined the effect of constitutive PKMζ deletion on oxycodone self-administration in both sexes. At the 0.25mg/kg/inf dose of oxycodone, both male and female mice exhibit an increase in oxycodone taking on an FR1 schedule of reinforcement, while only male mice appear to exhibit an increase in motivation for oxycodone on a progressive ratio schedule. In contrast, at the 0.125mg/kg/inf dose, only female mice exhibit an increase in both oxycodone taking and seeking. Taken together, these data suggest that PKMζ deletion leads to dose- and sex-dependent alterations on oxycodone self-administration. Slice electrophysiology studies are underway to examine how PKMζ deletion impacts synaptic plasticity within the prefrontal cortex in both drug-naïve and oxycodone-experienced mice.

Disclosures: A. Kniffin: None. **M. Knouse:** None. **M. Nenov:** None. **L. Birmingham:** None. **L.A. Briand:** None.

Poster

PSTR428. Addictive Drugs: Sex Differences in Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR428.18/PP5

Topic: G.09. Drugs of Abuse and Addiction

Title: Adolescent social isolation leads to sex-specific increases in microglia proliferation in the reward system of adult mice.

Authors: *B. RHOADS, M. PINIERO, B. ARMSTRONG, N. STOREY, A. MCGRATH, L. BRIAND;
Temple Univ., Philadelphia, PA

Abstract: Adolescence is a critical developmental period during which insults can have persistent effects on mental and physical health. The absence of social reward during adolescence can lead to widespread neuronal alterations, impacting behavior and brain function. Social isolation during adolescence increases the motivation for cocaine and leads to structural and synaptic plasticity within the reward system. As both stress and drugs of abuse have been shown to influence microglia and microglia play a critical role in plasticity, we hypothesized that adolescent social isolation would impact microglia in the reward system. The goal of this study was to analyze the impact of adolescent social isolation stress on microglia proliferation and morphology. Male and female C57/Bl6J mice were either socially isolated or group housed at weaning (PND21) and remained in these housing conditions until adulthood. At PND63-105, mice were euthanized, and brains were collected for immunohistochemical analysis of the microglial marker, Iba1, within reward-related brain regions. Results from whole cell count analysis revealed a sex-specific effect of social isolation on microglia proliferation across brain regions in the limbic system. Specifically, socially isolated male mice exhibited increased microglia cell counts across the prefrontal cortex, nucleus accumbens, hippocampus, and basolateral amygdala. No significant effect of social isolation was seen in female mice. Analysis of non-limbic regions is ongoing, but preliminary analysis of microglia cell counts in the barrel cortex suggests the increase in microglia proliferation may be brain wide. Additional studies are being conducted to determine the impact of adolescent social isolation on microglial morphology using confocal microscopy and IMARIS imaging software. Overall, it was seen that there are sex-specific alterations in microglia proliferation across all brain regions studied with socially isolated males demonstrating an increase in microglia cell counts as compared to all other conditions.

Disclosures: B. Rhoads: None. **M. Piniero:** None. **B. Armstrong:** None. **N. Storey:** None. **A. McGrath:** None. **L. Briand:** None.

Poster

PSTR428. Addictive Drugs: Sex Differences in Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR428.19/PP6

Topic: G.09. Drugs of Abuse and Addiction

Title: Sex differences in nucleus accumbens synaptic plasticity: involvement of PKMζ

Authors: *M. NENOV, M. C. KNOUSE, L. A. BRIAND;
Psychology and Neurosci., Temple Univ., Philadelphia, PA

Abstract: The nucleus accumbens (NAc) is part of the corticolimbic reward system. Impairments in NAc circuits are associated with development of various psychiatric disorders including substance use disorder, depression, and anxiety. Epidemiological sex differences in these disorders are well characterized and understanding mechanistic sex differences within the NAc may shed light on these disorders. The current study examined sex differences in the main form of synaptic plasticity within the NAc, long term depression (LTD), using slice electrophysiology. We found that a 5-min low frequency stimulation protocol elicited LTD in the NAc of male mice but had no effect in female mice. However, when the duration of the stimulation protocol was increased to 10 min, LTD was evoked in both male and female mice to a similar extent. Next, we examined if sex differences in LTD induction could correspond to the pre- or postsynaptic changes in NAc medium spiny neurons. We found significantly higher AMPA/NMDA ratios and readily releasable pool size in female mice compared to male mice. This increase in readily releasable pool size was accompanied by a decrease in release probability in female mice. Finally, we tested whether these sex differences could be mediated, in part, by the atypical PKC, PKMζ. We found that constitutive deletion of PKMζ resulted in robust LTD induction in female mice at the shorter induction protocol, while disrupting LTD in male mice. Strikingly, there was no effect of PKMζ KO at the longer LTD induction protocol. As PKMζ plays a role in the insertion of AMPARs post-synaptically, studies are underway to determine whether PKMζ deletion alters the sex differences in AMPA/NMDA ratio seen in wildtype mice. Further, we will examine whether PKMζ deletion effects presynaptic transmission, including the size of the readily releasable pool and release probability. Overall, our results indicate that sex differences in NAc LTD are mediated by sex differences in synaptic strength that may be mediated by PKMζ.

Disclosures: **M. Nenov:** None. **M.C. Knouse:** None. **L.A. Briand:** None.

Poster

PSTR428. Addictive Drugs: Sex Differences in Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR428.20/PP7

Topic: G.09. Drugs of Abuse and Addiction

Title: Impact of adolescent social isolation on microglial expression in male and female mice

Authors: *N. L. STOREY[1], B. J. ARMSTRONG[2], B. T. RHOADS[2], L. A. BRIAND[3]; [1]Temple Univ. Grad. Neurosci. Program, Philadelphia, PA; [3]Psychology and Neurosci., [2]Temple Univ., Philadelphia, PA

Abstract: Adolescence is a critical period for the development of multiple circuits within the brain, including the brain's reward circuitry. Social isolation during adolescence alters the development of this reward circuit, which can lead to long term detrimental changes in behavior, including increased drug seeking. Adolescence is a period of increased vulnerability to substance use disorder (SUD) and stressors like social isolation during this time can increase this vulnerability. Previous research in our lab demonstrates that adolescent social isolation increases motivation for cocaine as well as cocaine seeking in both male and female mice. However, the underlying mechanisms driving this stress-induced increase in vulnerability is not well understood. Both stress and drugs of abuse lead to an increase in microglial activation within the limbic system. As microglia play a critical role in synaptic plasticity, we hypothesized that adolescent social isolation would impact microglia with the reward circuit. Data from adult mice suggest that adolescent social isolation leads to sex-specific alterations in microglial proliferation, but it is not clear if these differences in adulthood are persistent changes that occur during adolescence. Therefore, the present research specifically examines microglial expression 2 weeks following post-weaning (PND21) social isolation. Analysis of cell counts within the prelimbic and infralimbic cortices indicate that microglial expression is higher in socially isolated mice compared to group housed controls. This increased expression appears to be driven by an effect in the male mice. In contrast, females are not exhibiting large effects of the social isolation on microglial expression in the prefrontal cortex. These sex-specific alterations in microglial proliferation are consistent with what we have seen in adult mice following adolescent social isolation, suggesting that these effects persist. Analysis of additional regions in the reward pathway including the nucleus accumbens, hippocampus, and basolateral amygdala are ongoing and will be presented at the meeting. The current findings suggest that adolescent social stress can lead to persistent, sex-specific alterations in microglial proliferation that could impact the response to future insults in adulthood including drugs of abuse. Future studies will examine how the effects of adolescent social isolation impact drug-induced changes in microglia.

Disclosures: N.L. Storey: None. **B.J. Armstrong:** None. **B.T. Rhoads:** None. **L.A. Briand:** None.

Poster

PSTR428. Addictive Drugs: Sex Differences in Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR428.21/PP8

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant DA053443
NIAAA Grant AA006420
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NIAAA Grant AA028549
NIAAA T32 Training Grant AA007456

Title: Suvorexant, an FDA-approved dual orexin receptor antagonist, effectively reduces oxycodone self-administration and conditioned reinstatement in male and female rats

Authors: *J. ILLENBERGER, F. J. FLORES-RAMIREZ, A. MATZEU, B. J. MASON, R. MARTIN-FARDON;
The Scripps Res. Inst., La Jolla, CA

Abstract: Amid the opioid crisis, effective strategies to prevent prescription opioid use disorder (OUD) are urgent. The orexin system is recruited by drugs of abuse and blockade of orexin receptors prevents drug-seeking behaviors. This study sought to determine whether repurposing suvorexant (SUV)-- a dual orexin receptor antagonist marketed as Belsomra® (Merck) for insomnia treatment-- can treat two features of prescription OUD: exaggerated consumption and relapse. Wistar rats (males and females; n=16/group) were trained to self-administer oxycodone (0.15 mg/kg, i.v., 8h/day) in the presence of a discriminative stimulus (S^D). The capacity to decrease self-administration with SUV (0-20 mg/kg, p.o.) was tested. Next, the rats underwent extinction training and the effect of SUV (0 and 20 mg/kg, p.o.) to prevent S^D-induced reinstatement of oxycodone-seeking behavior was tested. Repeated measures ANOVAs analyzed self-administration and reinstatement data. Withdrawal scores were analyzed with Friedman nonparametric tests. Males ($F(14,196)= 7.09$, $p< 0.001$) and females ($F(1,13)= 74.80$, $p< 0.001$) acquired oxycodone self-administration and exhibited physical signs of opioid withdrawal (males: $F_r= 36.23$, $p< 0.001$; females: $F_r= 37.24$, $p< 0.001$). Females consumed twice as much oxycodone (48.55 ± 5.01 mg/kg) vs. males (22.49 ± 4.83 mg/kg). At 20 mg/kg SUV decreased oxycodone self-administering during the first hour in males ($F(2,26)= 5.05$, $p< 0.05$) and females ($F(2,26) = 4.77$, $p < 0.05$). The oxycodone S^D reinstated oxycodone-seeking behavior with more efficacy in females ($t(9)= 4.10$, $p < 0.01$). Suvorexant reversed oxycodone seeking in males to extinction level (Bonferroni $p> 0.05$ vs. EXT; $F(3,35)= 6.72$, $p< 0.01$). In females while a reduction was measured, a substantial reinstatement persisted (Bonferroni $p < 0.01$ vs. 0 mg/kg; $F(3,13)=10.15$, $p< 0.001$). The findings support targeting orexin receptors for the treatment for prescription OUD and repurposing SUV as pharmacotherapy for prescription OUD.

Disclosures: J. Illenberger: None. **F.J. Flores-Ramirez:** None. **A. Matzeu:** None. **B.J. Mason:** None. **R. Martin-Fardon:** None.

Poster

PSTR428. Addictive Drugs: Sex Differences in Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR428.22/PP9

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH 1R15DA055201-01A1

Title: Exploring sex differences in naloxone conditioned place aversion

Authors: *B. BRADY[1], H. H. CHAMSEDDINE[3], D. VAN DYK[2], L. I. PERROTTI[4];
[1]Univ. of Texas at Arlington, Mansfield, TX; [2]Univ. of Texas at Arlington, Arlington, TX;
[3]Univ. of Texas At Arlington, Benbrook, TX; [4]Psychology, UT Arlington, Arlington, TX

Abstract: Exploring sex differences in naloxone conditioned place aversionHouda H. Chamseddine, Blake N Brady, Davis Van Dyk, Linda I. Perrotti
Substance use disorder is a chronic condition characterized by cycles of intoxication, withdrawal, and relapse. Negative affective and somatic symptoms commonly accompany cessation of substance use. These symptoms can be incredibly aversive, to the extent that they can be classically conditioned to be associated with cues or environments. Interestingly, women have been shown to demonstrate greater cue-reactivity to drug-associated stimuli. Women also progress from casual drug use to abuse at an accelerated rate compared to men. Human and animal literature also demonstrate elevated vulnerabilities in females associated with circulating ovarian hormones. Unfortunately, but surprisingly, studies investigating sex differences and hormonal influences during opioid withdrawal (OW) and its motivational influences are severely lacking, and the few that address sex as a variable report disparate findings. Much of what is currently known about OW has been derived from studies in exclusively men or male animals, and extrapolated to women, with most withdrawal scales having been developed using male subjects. Thus, the objective of the present study was to investigate sex differences and hormonal influences in the motivational consequences of opioid withdrawal using a conditioned place aversion paradigm. Specifically, we sought to determine (1) the degree to which gonadally intact, adult male and female rats displayed aversion to environments associated with an acute naloxone precipitated withdrawal after morphine pretreatment, (2) the degree to which ovariectomized female rats pretreated with estradiol benzoate or a peanut oil vehicle developed conditioned place aversion, and (3) the differences in aversion after administering varying morphine and naloxone doses in intact females. Overall, the results of this study demonstrated a robust naloxone-conditioned place aversion that seemed to be relatively unaffected by sex, ovariectomy, or estradiol treatment.

Disclosures: B. Brady: None. **H.H. Chamseddine:** None. **D. Van Dyk:** None. **L.I. Perrotti:** A. Employment/Salary (full or part-time):; Department Chair.

Poster

PSTR428. Addictive Drugs: Sex Differences in Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR428.23/PP10

Topic: G.09. Drugs of Abuse and Addiction

Support: R33 DA041883 03

Title: Sex-specific role of CNIH3 in fentanyl IV self-administration

Authors: *T. LINTZ[1], H. E. FRYE[2], E. C. NELSON[3], J. D. DOUGHERTY[4], J. MORON-CONCEPCION[5];
[1]Washington Univ. in St. Louis Neurosci. PhD Program, St. Louis, MO; [2]Washington Univ. in St. Louis, Saint Louis, MO; [3]Dept. of Psychiatry, Washington Univ. Sch. of Med., Saint Louis, MO; [4]Washington Univ. Sch. of Med., St. Louis, MO; [5]Anesthesiol., Washington Univ., Saint Louis, MO

Abstract: Cornichon homolog-3 (CNIH3) is an AMPA receptor (AMPAR) auxiliary protein that traffics AMPARs to the postsynaptic membrane and potentiates AMPAR signaling. AMPARs are key components of hippocampal synaptic plasticity, which is important in opioid-seeking behavior. A previous genome-wide association study (GWAS) comparing humans that used opioids occasionally to those that used daily has shown that single nucleotide polymorphisms in CNIH3 provide significant protection against the development of opioid use disorder, particularly in women, however, the role of CNIH3 in opioid-seeking behavior has yet to be elucidated. To investigate sex differences in the role of CNIH3 in opioid use, we assessed fentanyl consumption using an operant intravenous self-administration (IVSA) approach in male and female wildtype and CNIH3 KO mice. Our results indicate that CNIH3 KO impairs the acquisition of fentanyl IVSA in female mice and prevents the increased consumption of fentanyl per session in males observed over time in control mice. Furthermore, CNIH3 KO dampens drug-seeking during cue-induced reinstatement in male CNIH3 KO mice. Additionally, we use viral approaches to determine the necessity of CNIH3 in the dorsal hippocampus for opioid-seeking behavior. This study, the first to identify sex-specific effects of the AMPAR auxiliary protein CNIH3 on opioid-seeking, begins to uncover the role of CNIH3 on sexually dimorphic AMPAR-dependent behavior and hippocampal synaptic plasticity.

Disclosures: T. Lintz: None. **H.E. Frye:** None. **E.C. Nelson:** None. **J.D. Dougherty:** None. **J. Moron-Concepcion:** None.

Poster

PSTR428. Addictive Drugs: Sex Differences in Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR428.24/PP11

Topic: G.09. Drugs of Abuse and Addiction

Title: Oxytocin attenuates stress-induced responding for oxycodone under a progressive ratio schedule in male rats

Authors: *T. Q. YATES, E. C. MCNEELY, R. J. RAGER-AGUIAR, E. LORENZ, K.-C. LEONG;
Psychology, Trinity Univ., San Antonio, TX

Abstract: Instances of Opioid Use Disorder (OUD) are both prevalent in occurrence and significant in impairment caused. Stress is a major component in the expression of OUD,

increasing drug use, craving, and likelihood of relapse. Oxycodone is a commonly abused prescription opioid and use is highly susceptible to stress effects. Therefore, pharmacological interventions need to be explored to combat the negative effect of stress on oxycodone abuse. The neuropeptide oxytocin has been shown to be a potent anxiolytic compound and may be utilized to diminish the effect of stress on drug reward. The present study will investigate the ability for oxytocin to attenuate the effect of the pharmacological stressor yohimbine on oxycodone reward salience and motivation of oxycodone-seeking behavior. To better mirror the use of oral oxycodone in human abuse, male rats were trained to lever respond in operant conditioning chambers for oral self-administration of oxycodone. After stabilization of behavioral response for at least two sessions, the effect of stress on oxycodone-seeking was measured through systemic administration of the pharmacological stressor yohimbine (2 mg/kg; i.p.) 30 minutes prior to a progressive ratio (PR) test. Our results found that yohimbine increased lever responding and break point for oxycodone in male rats, thus demonstrating stress-induced motivation of oxycodone-seeking. Concurrent oxytocin, administered systemically (1 mg/kg; i.p.) attenuated the effect of yohimbine, reducing lever pressing and breakpoint. Subsequent experiments investigated the role of the central amygdala (CeA) in mediating this attenuating effect of oxytocin. Overall, our results demonstrate the potential therapeutic effect of oxytocin in diminishing the negative effect of stress on oxycodone addiction behavior.

Disclosures: T.Q. Yates: None. **E.C. McNeely:** None. **R.J. Rager-Aguiar:** None. **E. Lorenz:** None. **K. Leong:** None.

Poster

PSTR428. Addictive Drugs: Sex Differences in Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR428.25/PP12

Topic: G.09. Drugs of Abuse and Addiction

Support: R01 DA037897

Title: Sex-specific efficacy of a dual agonist of GLP-1Rs and Y2Rs in opioid-dependent male and female rats

Authors: *A. CAFFREY[1], E. LAVECCHIA[1], H. D. SCHMIDT[2];
[1]Univ. of Pennsylvania, Philadelphia, PA; [2]Univ. of Pennsylvania Sch. of Med., Univ. of Pennsylvania Sch. of Med., Philadelphia, PA

Abstract: Fatal opioid overdoses remain one of the leading causes of preventable death in the United States. The number of deaths caused by synthetic opioids continues to rise year-over-year. Current FDA-approved medications for opioid use disorder (OUD) are limited by high relapse rates. Therefore there is an urgent need to develop new pharmacotherapies to treat OUD. An emerging literature indicates that dual agonists of glucagon-like peptide-1 receptors (GLP-1Rs) and neuropeptide Y2 receptors (Y2Rs) have additive effects on opioid-mediated behaviors

compared to GLP-1R or Y2R monotherapies alone. Furthermore, GLP-1R/Y2R dual agonists are well-tolerated and do not produce emesis as seen with GLP-1R monotherapies. The goal of this study was to determine the efficacy of GEP12, a novel dual GLP-1R/Y2R agonist, to reduce fentanyl self-administration and reinstatement in both male and female rats. Rats were allowed to self-administer fentanyl (2.5 μg/kg, i.v.) for 21 days on a fixed-ratio 5 (FR5) schedule of reinforcement. Rats were then pretreated with vehicle or GEP12 (1.57 or 12.53 μg/kg) before the beginning of fentanyl self-administration test sessions. We found that systemic administration of GEP12 significantly reduced fentanyl self-administration in male rats at a dose that did not affect food intake or produce pica. These effects were sex-specific as GEP12 pretreatment did not alter fentanyl consumption in female rats. Opioid taking was then extinguished by replacing the fentanyl solution with saline. Opioid seeking during abstinence was elicited using an acute priming injection of fentanyl (45 μg/kg, i.p.). Prior to reinstatement test sessions, rats were pretreated with vehicle or GEP12 (1.57 or 12.53 μg/kg). Our preliminary findings indicate that GEP12 reduces fentanyl seeking during abstinence in both male and female rats. Taken together, these results indicate that GEP12 reduces voluntary opioid and taking. Future studies will explore the neural mechanisms underlying the sex-specific effects of GEP12 on opioid taking and work to expand the efficacy of dual agonists in opioid-mediated behaviors.

Disclosures: A. Caffrey: None. **E. Lavecchia:** None. **H.D. Schmidt:** None.

Poster

PSTR428. Addictive Drugs: Sex Differences in Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR428.26/PP13

Topic: G.09. Drugs of Abuse and Addiction

Support: This study was supported by seed funds from Rowan University School of Osteopathic Medicine.

Title: Effects of Oxycodone Exposure and Withdrawal on NAc Opioid Receptor Expression in Male and Female Rats and Across the Estrous Cycle

Authors: *B. PATEL[1], A. KRISHNAN[2], J. LOWETH[3];
[1]Mol. Cell Biol. and Neurosci., Rowan Univ., Stratford, NJ; [2]Mol. Cell Biol. and Neurosci., Rowan Univ. Sch. of Osteo. Med., Stratford, NJ; [3]Mol. Cell Biol. and Neurosci., Rowan Univ. Sch. of Osteo. Med., stratford, NJ

Abstract: Effects of Oxycodone Exposure and Withdrawal on NAc Opioid Receptor Expression in Male and Female Rats and Across the Estrous Cycle
Bhumi P. Patel, Anuradha Krishnan, Jessica A. LowethGraduate School of Biomedical Sciences, Department of Cell Biology & Neuroscience, Rowan University School of Osteopathic Medicine
Prescription opioid misuse and abuse in the past two decades has significantly contributed to the current opioid epidemic. Although both men and women misuse prescription opioids, women are

more frequently prescribed opioids for pain, experience more intense opioid craving after chronic use, and transition more rapidly to substance use disorder compared to men. One likely factor contributing to these sex differences is fluctuations in ovarian hormone levels across the reproductive cycle. However, recent studies from our lab have found a significant and long-lasting dysregulation of the rat reproductive (estrous) cycle during extended-access oxycodone self-administration (6 h/day for 10 days, 0.1 mg/kg/infusion) and the first several weeks of withdrawal, preventing an accurate assessment of potential estrous cycle-dependent changes in oxycodone seeking behavior during earlier withdrawal time-points. Taking this estrous cycle dysregulation into account, we assessed the effects of estrous cycle fluctuations on cue-induced oxycodone seeking following prolonged withdrawal (~44 days), a time period when estrous cycle lengths and stages have returned to normal. Interestingly, we observed a significant reduction in cue-induced oxycodone seeking in females in the estrus stage of the estrous cycle (Estrus Females) compared to both females in diestrus and males following prolonged abstinence or withdrawal. One potential mechanism mediating these effects could be interactions between ovarian hormones and opioid receptor signaling within the mesolimbic reward circuitry, as ovarian hormones can influence opioid receptor expression and function which, in turn, can influence responding to opioid-associated cues. To begin to investigate this, we assessed surface and total protein expression levels of opioid receptors (mu, delta and kappa) in the nucleus accumbens core and shell in males and females and across the estrous cycle following prolonged withdrawal from saline or oxycodone self-administration. Together these findings will begin to identify potential neuroadaptations contributing to estrous cycle-dependent changes in oxycodone seeking and relapse vulnerability.

Disclosures: **B. Patel:** None. **A. Krishnan:** None. **J. Loweth:** None.

Poster

PSTR428. Addictive Drugs: Sex Differences in Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR428.27/PP14

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: R01 DA052386

Title: The Effects of Transitioning from Morphine to Buprenorphine During Pregnancy on Offspring Development in a Translational Rodent Model

Authors: *A. M. MYERS[1], L. M. RICHARDSON[1], J. DURAN[1], S. A. PERRINE[2], S. E. BOWEN[1], S. BRUMMELTE[1];
[1]Psychology, [2]Psychiatry and Behavioral Neurosciences, Wayne State Univ., Detroit, MI

Abstract: Opioid use during pregnancy has increased drastically in the last two decades. Pregnant women who use opioids are often prescribed Medications for Opioid Use Disorder (MOUDs), including buprenorphine (BUP) to mitigate negative effects on the fetus. However,

BUP exposure during pregnancy is not without risk as preclinical studies have shown a negative impact on maternal care behavior and offspring neurodevelopment. Further, it is not known how the transition from an opioid of abuse (i.e., morphine) to a MOUD (i.e., BUP) during gestation affects offspring outcomes. In the current study, we used a translational rodent model to investigate acute offspring neurodevelopmental outcomes following either the transition from morphine to BUP or continued use of morphine or BUP from preconception (drug administration began 7 days prior to mating) to the early postpartum period. Dams were assigned to one of 5 experimental groups: BUP continued ("BC", 1mg/kg, s.c.), morphine continued ("MC", 3 - 10 mg/kg, s.c.), morphine to BUP ("MB"; 3 - 5mg/kg morphine until Gestational Day (GD) 5, then 1.0 mg/kg BUP; s.c.), morphine to vehicle ("MV"; 3 - 5mg/kg morphine until GD5, then 1.0 mL/kg saline; s.c.), or saline continued ("VEH", 1.0 mL/kg, s.c.). MB and MV groups switched to BUP or saline (respectively) on GD5, to roughly mimic the time in humans when a woman would discover she is pregnant (i.e. ~ 6-8 weeks of pregnancy). All dams and their litters were sacrificed on postnatal day (PN) 2. Our results reveal that continuous exposure to BUP or morphine and the transition from morphine to BUP results in higher pup mortality, lower pup body weight, smaller pup body length, and fewer milk bands as compared to the VEH or MV groups (p's < .05). Importantly, preliminary evidence suggests that the BC, MC, and MB dams had lower nesting quality and partook in less pup-directed maternal care behaviors than drug-naïve dams, potentially contributing to the high pup mortality rate. Pup brains collected on PN2 will be analyzed using High-Performance Liquid Chromatography to determine monoamine concentrations in the striatum, prefrontal cortex, and hypothalamus. These results suggest that (1) the transition from morphine to BUP in early pregnancy and continued use of BUP or morphine negatively impacts offspring outcomes and (2) opioid-induced maternal care deficits appear to contribute to offspring neurodevelopmental outcomes following opioid exposure *in utero*.

Disclosures: **A.M. Myers:** None. **L.M. Richardson:** None. **J. Duran:** None. **S.A. Perrine:** None. **S.E. Bowen:** None. **S. Brummelte:** None.

Poster

PSTR429. Alcohol Induced Changes in Neural Circuits and Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR429.01/PP15

Topic: G.09. Drugs of Abuse and Addiction

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Title: Adolescent binge drinking alters prelimbic somatostatin circuitry in mice

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Abstract: Somatostatin (SST)-expressing neurons are a population of inhibitory neurons implicated in stress and neuropsychiatric illnesses. Our lab recently showed that binge drinking in adult mice can disrupt the function of SST neurons in the prelimbic (PL) region of the prefrontal cortex. However, the consequences of alcohol exposure during critical developmental windows, including adolescence, for SST neurons and associated circuitry have not been characterized. Here, we use electrophysiology and behavioral studies to investigate the effects of adolescent binge drinking on PL SST neurons. We used the Drinking-in-the-Dark (DID) paradigm to model binge drinking in male and female SST-Cre:Ai9 reporter mice and SST-Cre:Ai32 mice expressing channelrhodopsin from postnatal days (PND) 28 to 52. This age range spans the bulk of the rodent adolescent period. Patch-clamp electrophysiology was performed 24 hr after the end of adolescent DID. SST neurons were identified based on the presence of fluorescence, while pyramidal and non-pyramidal neurons were identified by morphology and membrane properties (i.e. cell capacitance and membrane resistance). We found that SST neurons become hyperexcitable following adolescent DID, while pyramidal neurons became hypoexcitable. However, there was no change in SST-mediated GABA transmission onto other PL neurons at this timepoint. Ongoing experiments seek to characterize the impact of adolescent DID on subsequent binge drinking in adulthood and typical development of PL SST neurons during adolescence.

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Poster

PSTR429. Alcohol Induced Changes in Neural Circuits and Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR429.02/PP16

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant AA025677
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Title: Extended amygdala neuronal excitability and alcohol drinking: differential effects of hypocretin/orexin receptor signaling in CRF neurons

Authors: *Y. MA, H. SARDAR, A. R. MORNINGSTAR, M. E. BENABOU, R. N. FAJARDO, I. F. KANDIL, A. C. Y. YU, J. A. KAUER, W. J. GIARDINO;
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Abstract: Alcohol use disorder (AUD) patients experience negative emotions and hyperarousal during withdrawal, which perpetuate the progression of addiction. These negative emotional features involve neurocircuitry of the extended amygdala and stress-related neuropeptides such as corticotropin-releasing factor (Crf) and hypocretin (Hcrt). The bed nucleus of stria terminalis (BNST) in the extended amygdala is a sexually dimorphic brain region that regulates negative valence, stress, and alcohol consumption. We hypothesized that signaling through two Hcrt receptor subtypes, HcrtR1 and HcrtR2, in Crf neurons may differentially impact alcohol intake and anxiety-like behaviors, and that neuronal excitability changes in the BNST may mediate these effects. To test these hypotheses, we deleted either HcrtR1 or HcrtR2 from Crf neurons by crossing Crf-Cre mice with floxed HcrtR mouse lines. The Crf-HcrtR1 and Crf-HcrtR2 cohorts along with Cre negative wild-type littermates underwent 8 weeks of two-bottle choice intermittent access alcohol drinking. To determine the impact on anxiety-like behaviors, the elevated plus maze and open field test were performed before alcohol access, and in the last week of alcohol drinking during acute withdrawal. Intrinsic excitability of BNST neurons was measured in brain slices collected after behavioral testing, and in age-matched alcohol-naive controls. Electrophysiological recordings indicated that the BNST neurons in females were more excitable, but 8 weeks of alcohol exposure blunted sex differences in BNST neuronal excitability. Deletion of HcrtR1 in Crf neurons significantly reduced alcohol intake in both male and female mice; however, no differences in intrinsic excitability of BNST neurons were observed that might account for these differences. By contrast, deleting HcrtR2 from Crf neurons revealed a trend toward sex-dependent effects on alcohol intake, with male Crf-HcrtR2 knockout mice displaying greater alcohol intake compared to wild-type littermates. Crf-HcrtR2 deletion also reduced baseline anxiety-like behaviors in male mice prior to alcohol drinking. These findings suggest that HcrtR1 signaling in Crf neurons is critical for excessive alcohol drinking in both males and females, and that HcrtR2 signaling in Crf neurons may play a sex-dependent role in alcohol intake and anxiety-like behavior. The differential effects of HcrtR1 and HcrtR2 deletion in Crf neurons provide valuable insights for developing potential AUD treatments that target these neuropeptide systems using subtype-selective HcrtR modulators.

Disclosures: Y. Ma: None. **H. Sardar:** None. **A.R. Morningstar:** None. **M.E. Benabou:** None. **R.N. Fajardo:** None. **I.F. Kandil:** None. **A.C.Y. Yu:** None. **J.A. Kauer:** None. **W.J. Giardino:** None.

Poster

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Program #/Poster #: PSTR429.03/PP17

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH/NIDA R01 DA042737

Title: Chronic exposure to alcohol disrupts inflammatory protein marker abundances in the striatum.

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Abstract: Alcohol misuse is the third leading preventable cause of death in the world. The World Health Organization currently estimates that 1 in 20 deaths are directly alcohol-related. One of the ways in which consuming excessive levels of alcohol can both directly and indirectly affect human mortality and morbidity, is that it can produce chronic inflammation. Chronic inflammation, and neuroinflammation specifically, is one of the strongest risk factors for later developing a neurodegenerative disease. Recently, studies have suggested a link between increased alcohol use and the incidence of neurodegenerative disease. However, the mechanism in which alcohol potentially accelerates neurodegenerative processes remains unknown. To further elucidate how alcohol-induced neuroadaptations may drive neuroinflammation, we assayed changes in the inflammatory proteome after mice were exposed to voluntary chronic alcohol use. Here we show that mice chronically exposed to alcohol display unique alterations in their inflammatory proteome in multiple striatal brain subregions commonly associated with heavy drinking and chronic alcohol use. Using mass spectrometry following voluntary alcohol self-administration in mice we show that inflammatory protein marker abundances and signaling pathways are disrupted from chronic exposure to alcohol compared to water drinking control mice across striatal regions. Further, in mice that were allowed to experience abstinence from alcohol compared to mice that were non-abstinent, the inflammatory proteome and signaling pathways showed additional differences, suggesting that the inflammatory response evoked by chronic alcohol exposure is dependent on alcohol use history. These alterations may drive increased neurodegenerative pathophysiology in mice susceptible to these diseases. These findings suggest that proinflammatory responses driven by chronic alcohol use may be accelerating neurodegenerative diseases. Further, specific proinflammatory proteins that we found with largely upregulated abundances may serve as targets for intervention to reverse the adverse effects of alcohol-induced inflammation, and thus decrease the probability of developing a neurodegenerative disease due to excessive alcohol consumption.

Disclosures: B. Duffus: None.

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PSTR429. Alcohol Induced Changes in Neural Circuits and Neurophysiology

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Program #/Poster #: PSTR429.04/PP18

Topic: G.09. Drugs of Abuse and Addiction

Support: AA027915
Miami University

Title: Interactive influences of chromosomal sex and gonadal sex on alcohol consumption in Four Core Genotypes Mice

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Abstract: Alcohol Use Disorder (AUD) is a persistent worldwide issue, with increasing rates in recent years. Clinical evidence has shown sex differences in onset and duration of AUD. Preclinical research also reveals an influence of biological sex on alcohol consumption, with rates of voluntary consumption consistently higher in female vs. male rodents. However, the neural mechanisms underlying these differences have yet to be understood. The Four Core Genotypes (FCG) mouse model provides a unique opportunity to investigate the influences of both chromosomal sex (i.e., XX vs. XY chromosome complement) and gonadal sex (i.e., ovaries vs. testes) on drinking behaviors. In this model, four different genotypes are tested: XX/Sry-, XX/Sry+, XY/Sry-, and XY/Sry+. In previous studies, we have uncovered influences of chromosomal sex on alcohol consumption using a continuous access drinking task (XX > XY) and a limited access Drinking in the Dark (DiD) paradigm (XY > XX in Sry+ mice with testes). Here, we determined the influence of chromosomal sex on intermittent 24-h alcohol consumption and explored interactions between chromosomal and gonadal sex in female FCG mice using ovariectomy. For Experiment 1, intermittent access (IA), mice were presented with two bottles of 20% ethanol and water solution for 24 hours every-other day (MWF) for 4 weeks. Bottles were weighed to calculate consumption and two water bottles were available on intervening days. In Experiment 2, XX/Sry- and XY/Sry- mice underwent surgical ovariectomy (OVX vs. SHAM) 4 weeks before the commencement of DiD. Mice received two bottles (15% ethanol and water) for 4 hours during their active (dark) cycle for 15 sessions. Aversion resistance was assessed by adding increasing levels of quinine to the ethanol bottle. Results from Experiment 1 (IA) showed that XY mice consume more ethanol than XX mice, similar to what has previously been observed with limited access, but not continuous access, consumption. These effects of chromosomal sex were dependent on gonadal sex and exposure, such that XY/Sry+ mice consumed more ethanol than XX/Sry+ mice in the first two weeks of drinking while differences in XY/Sry- vs. XX/Sry- mice only emerged after 4 weeks. We also observed that mice with ovaries (Sry-) consumed more water than mice with testes (Sry+). Data collection for Experiment 2 is ongoing and will clarify the role of chromosomal sex and potential interactions with gonadal sex in aversion-resistant drinking. Together, these studies reveal that gonadal and chromosomal sex interact to produce sex differences in alcohol drinking behaviors and call for further study of how sex chromosome genes influence alcohol consumption.

Disclosures: R.A. Zegarelli: A. Employment/Salary (full or part-time):; Miami University. **K.D. Ream:** A. Employment/Salary (full or part-time):; Miami University. **E.P. Eccles:** None. **D.P. Underwood:** None. **A.K. Radke:** A. Employment/Salary (full or part-time):; Miami University.

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PSTR429. Alcohol Induced Changes in Neural Circuits and Neurophysiology

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA Grant T32AA007474
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Title: Astrocytes and their role in mediating the increase in intrinsic excitability of lateral orbitofrontal cortex pyramidal neurons following chronic alcohol consumption

Authors: *A. KASTNER, M. OKAS, J. WOODWARD;
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Abstract: Alcohol use disorder (AUD) is characterized by the progression from recreational drinking to uncontrollable and excessive consumption resulting in a myriad of social and neurobiological complications. The mechanisms underlying the dependence-induced escalation in drinking are not completely understood, however a key brain region disrupted in individuals with AUD is the orbitofrontal cortex (OFC). Studies from the Woodward laboratory show that acute ethanol inhibits action potential firing of lateral orbitofrontal (lOFC) cortex pyramidal neurons in a glycine dependent manner via reversal of the astrocyte GlyT1 transporter. Following repeated cycles of chronic intermittent ethanol (CIE) exposure, lOFC neurons become hyperexcitable and are no longer inhibited by acute ethanol. These effects are blunted in mice expressing a calcium exporter (PMCA) selectively in lOFC astrocytes. However, whether dampening lOFC astrocytic calcium also prevents the CIE-induced escalation in drinking is unknown. Male (n=8-9/group) and female (n=4-5/group) C57/Bl6J mice (~9 weeks of age) were infused with either an astrocyte specific PMCA (AAV2/5-GfaABC1D-hPMCA2w/b-mCherry) or control (AAV2/5-GfaABCD1-LCK-GFP) virus in the lOFC. Following recovery, they underwent 4 weeks of baseline drinking where Mon-Fri, they had free access to 15% ethanol or water for 2 hours beginning 3 hours into the dark cycle. Mice were then counterbalanced into CIE or Air groups based on alcohol consumption during baseline and then exposed to cycles of CIE or Air exposure followed by a test week of homecage drinking. Each CIE or Air exposure consists of 4 days of vapor exposure (16 hrs on, 8 hrs off) and a 3-day withdrawal period. Results from preliminary studies show that PMCA had no effect on baseline drinking in male or female mice. Following CIE exposure, both GFP control and PMCA male mice increased their ethanol consumption. No differences in drinking were observed after CIE exposure in GFP control or PMCA female mice. Next, we investigated the impact of expressing PMCA in lOFC astrocytes on lOFC neuronal excitability. Female C57/Bl6J mice expressing PMCA or LCK-GFP underwent 2-4 weeks of CIE or Air exposure and electrophysiology recordings were conducted 72 hours following the last exposure. Results from these ongoing studies suggest that PMCA may prevent the increase in evoked action potentials following CIE exposure. Together, these findings indicate a potential dissociation in the role of astrocytes in the increase in lOFC excitability and the escalation in drinking that follows CIE exposure.

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIA 1R01AG0702255

Title: Characterization of human synaptic $GABA_A$ receptors sensitivity in Alcohol Use Disorder

Authors: *J. M. GRANCHI[1], B. MILLER[1], T. MEYER[2], A. TEIXEIRA[2], L. STERTZ[2], C. WALSS-BASS[2], A. LIMON[1];
[1]Grad. Sch. of Biomed. Sci., Univ. of Texas Med. Br., Galveston, TX; [2]Faillace Dept. of Psychiatry and Behavioral Sci., The Univ. of Texas Hlth. Sci. Ctr., Houston, TX

Abstract: Previous research highlights epigenetic changes made to $GABA_A$ Receptors following chronic alcohol use which may be related to synaptic changes of GABAergic signaling in individuals with Alcohol Use Disorder (AUD). Because $GABA_A$Rs play an integral role in alcohol pharmacodynamics and GABAergic signaling, AUD may lead to changes in their sensitivity to the endogenous GABA agonist. However, $GABA_A$Rs has not been electrophysiologically characterized in native human tissue of individuals with AUD. In this study, we used two electrode voltage clamp electrophysiology and microtransplantation synaptic membranes (MSM) methods to determine potential changes of $GABA_A$Rs affinity to GABA in AUD. We used postmortem dorsolateral prefrontal cortex samples from 6 control subjects and 6 subjects diagnosed with AUD (mean age of 53 ± 7 years) that were collected by the University of Texas Health Science Center at Houston Brain Collection. Ion current responses values were integrated with label-free proteomics datasets from the same brain region. We built concentration-response curves for GABA to determine EC_{50} and pEC_{50} values for each control and AUD sample. Additionally, we compared protein expression to find proteins that significantly correlate with AUD diagnosis, EC_{50}, and pEC_{50} for further analysis in Gene Ontology Pathways. Our results directly measuring the activity of native human $GABA_A$Rs complexes found a trend of lower affinity for GABA in AUD compared to controls ($p = 0.072$, t-test) and lower ion currents responses at 100 mM GABA, but not other concentrations. Initial one-way ANOVA analysis of the proteome by diagnosis revealed 124 proteins differentially expressed in AUD, among those GABBR2, a $GABA_B$ receptor protein, and GRIA3, a subunit of AMPA receptors, were expressed more in AUD samples. Response screenings analysis using the statistical software JMP 16Pro., found 116 proteins that were significantly correlated with EC_{50}, and 165 proteins significantly correlated with pEC_{50}. Gene ontological analysis with a background list of all 4,612 proteins from the original proteomic data collection indicates that the GNAI1-GNB5-GNB2 complex and proteins involved in GDP metabolic processes are significantly correlated with pEC_{50} values while proteins involved in valine, leucine, and isoleucine degradation are expressed differently based on diagnosis. These results suggest that GABA receptors in a subset of AUD subjects require higher GABA concentration than those of controls for activation.

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Topic: G.09. Drugs of Abuse and Addiction

Support: NRF Grant 2022R1A6A1A03054419

Title: The inhibitory effects of ginsenoside Rb1 and Rg1 on alcohol-induced addictive-like behaviors in rodents

Authors: *W.-A. KOOK, Y. LEE, D. KIM, H.-W. MIN, S.-Y. LEE, C.-G. JANG; Sungkwunkwan Univ., Suwon, Korea, Republic of

Abstract: Chronic alcohol consumption leads to alcohol dependence, which is accompanied by neuroadaptive modulations in the brain that ultimately lead to serious health complications. In previous studies, ginsenosides, major components of Korean Red Ginseng (KRG), alleviate alcohol-induced hyperactivity and cognitive impairment and inhibit morphine-induced hyperactivity and reinforcement in mice. However, there is no evidence that ginsenosides inhibit alcohol-induced psychological behaviors. Therefore, we evaluate the inhibitory effects of major protopanaxadiol (Rb1) and protopanaxatriol (Rg1) components of KRG on alcohol-induced addictive-like behaviors in mice. Male C57BL/6J 7 weeks old mice were used for all behavior experiments including oral self-administration (SA), conditioned place preference (CPP). Ginsenoside Rb1 and Rg1 (25, 50 and 100 mg/kg) were dissolved in saline and administered intraperitoneally at 10 ml/kg. Ethanol solutions (for SA, diluted to 10% v/v in distilled water and for CPP, diluted to 20% v/v in physiological saline) were used to form an alcohol addiction model. Statistical significance was determined using an ANOVA followed by a Fisher's LSD for all behavior tests. In SA studies, treatment with ginsenoside Rb1 and Rg1 significantly reduce alcohol induced self-administration on a fixed-ratio 4 and progressive ratio schedule of reinforcement. In CPP studies, treatment with ginsenoside Rb1 and Rg1 significantly inhibit alcohol-induced CPP. We identified the inhibitory effects of ginsenoside Rb1 and Rg1 on alcohol-induced reinforcement, motivational dependence, and reward. Our finding reflects that ginsenoside Rb1 and Rg1 are responsible for therapeutic effect of KRG, and they have therapeutic potential as a treatment for preventing alcohol-induced psychological dependence and reinforcement.

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PSTR429. Alcohol Induced Changes in Neural Circuits and Neurophysiology

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Topic: G.09. Drugs of Abuse and Addiction

Support: Alcohol Research Center 46-832-50 P64

Title: Converging effects of chronic pain and binge alcohol consumption on anterior insular cortex neurons projecting to the dorsolateral striatum

Authors: *Y. YIN, D. L. HAGGERTY, S. ZHOU, B. K. ATWOOD, P. L. SHEETS;
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Abstract: Chronic pain and alcohol use disorder (AUD) are highly comorbid. Patients with chronic pain are more likely to meet the criteria for AUD. Studies suggest that the disruptions in the underlying neural circuitry for chronic pain and AUD are similar, yet this connection remains understudied. The anterior insular cortex (AIC) is a brain region that is involved in both chronic pain and AUD. However, many of the previous studies focused on local lesion or excitation/inhibition, which lack circuit specificity. One major innervation target of AIC is the dorsolateral striatum (DLS). Binge-like alcohol drinking has been shown to alter synaptic transmission at AIC-DLS synapses. However, disruption of AIC-DLS circuits by chronic pain has never been studied. The goal of this work is to study the converging effects of chronic pain and binge alcohol consumption on AIC neurons that send projections to the DLS. We performed intracranial injections of retrograde beads into the right DLS of mice to label AIC-DLS neurons. We subsequently performed the spared nerve injury (SNI) surgery to induce chronic pain behavior. Both sham (control) and SNI mice then underwent 3 weeks of drinking-in-the-dark (DID) paradigm to model binge-like alcohol drinking. Following DID, whole-cell patch-clamp recordings in acute brain slices were performed to measure intrinsic and synaptic properties of AIC-DLS neurons. Our results show that SNI mice with no prior alcohol exposure to injury consumed less alcohol compared to sham mice. Electrophysiological analyses showed that AIC-DLS neurons from SNI-alcohol mice displayed increased neuronal excitability and increased frequency of miniature excitatory postsynaptic currents. However, exposing mice to 3 weeks of alcohol DID prior to injury eliminated differences in both alcohol intake and AIC-DLS neuronal activity observed in mice with no alcohol pre-exposure. Together, our data suggest that chronic pain and alcohol drinking interact to have a direct effect on both intrinsic excitability and synaptic transmission of AIC-DLS neurons in mice, which may be critical in altering motivated behaviors associated with pain and alcohol use.

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Poster

PSTR429. Alcohol Induced Changes in Neural Circuits and Neurophysiology

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Topic: G.09. Drugs of Abuse and Addiction

Support: AA024109

Title: Neural activity in anterior insula at drinking onset and licking predicts compulsive alcohol drinking

Authors: *P. STARSKI[1], M. MORNINGSTAR[4], S. N. KATNER[2], R. M. FRASIER[5], T. DE OLIVEIRA SERGIO[6], S. WEAN[1], C. LAPISH[1], F. W. HOPF[3];
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Abstract: Compulsion-like alcohol drinking, where intake persists despite adverse consequences, can contribute strongly to alcohol use disorder (AUD). Previous work has shown that anterior insula cortex (AIC) is important for promoting compulsion-like drinking, but AIC activity patterns promoting pathological intake remain unknown. In 15 adult male Wistar rat, we used 32-wire arrays to record in vivo single unit AIC activity during alcohol-only intake and during compulsive-like drinking with moderate or higher challenge (low vs high quinine adulteration), which would help uncover AIC patterns associated successfully maintaining compulsion-like intake. Compulsive-like drinking intake level was predicted by a specific lick-synchronized firing pattern in AIC, while another lick-associated pattern related to greater intake across conditions. Also, many AIC neurons had session-long plateau firing changes, but only cells with firing increases at drinking onset had greater activity under compulsive-like drinking conditions, in agreement with previous findings that rats quickly assess drinking context and adjust action strategy. AIC did not show firing elevations with saccharin intake, congruent with AIC inhibition not altering such consumption. We provide an integrated model where some aspects of AIC activity relate specifically to compulsive drinking, while others would promote alcohol drinking more generally. Further, similar changes under moderate and higher challenge suggest that one critical role of AIC is to maintain commitment to respond regardless of challenge level. Thus, we provide novel insights into AIC role in compulsive-like drinking and point to central neural mechanisms promoting this key facet of AUD.

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Poster

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Title: Corticolimbic hyperreactivity to alcohol cues among people at elevated risk for alcohol use disorder

Authors: *R. U. COFRESI[1], S. UPTON[1], A. A. BROWN[2], T. M. PIASECKI[5], B. D. BARTHOLOW[3], B. FROELIGER[4];
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Abstract: Low sensitivity (LS) to alcohol is a risk factor for developing alcohol use disorder (AUD). Compared to peers with high sensitivity (HS), LS individuals drink more, report more alcohol-related problems, and exhibit amplified alcohol cue reactivity (ACR) in the form of cue-directed attention and approach, cue-evoked craving, and a larger amplitude P3/LPP response, an event-related potential (ERP) index of incentive salience attribution, to alcohol-related cues. This amplified ACR suggests LS confers risk for AUD *via* over-attribution of incentive salience to alcohol and its cues. Given that incentive salience attribution is strongly tied to activity in the mesocorticolimbic system, we hypothesized that alcohol cues would elicit greater activation in the mesocorticolimbic system among LS compared to HS individuals. To test this hypothesis, we conducted an fMRI pilot study of ACR with LS and HS individuals who had previously completed an event-related EEG-ERP ACR task as part of a different study. Participants (N=32, M_{age}=20.3) recruited based on their Alcohol Sensitivity Questionnaire scores (HS: n=16; LS: n=16; 9 females/group) completed an event-related fMRI ACR task. General linear modeling of the fMRI BOLD response was conducted to identify regions showing significant ACR within a mesocorticolimbic mask (FWE, p<.05). ACR beta coefficients from 5-mm spheres around peak voxels were extracted. Recent alcohol use and craving were assessed. Archival P3/LPP ACR task amplitudes also were obtained for each participant. ACR in the fMRI-BOLD response was observed in left medial orbitofrontal cortex and left ventrolateral prefrontal cortex (vlPFC). vlPFC ACR was significantly greater for LS than HS, Welch's t(29.89)=2.46, p=.010. vlPFC ACR correlated with the frequency and intensity of past-week alcohol craving, r_{sp}≥.385, p≤.030, which was greater for LS than HS, U=41, p<.001. vlPFC ACR also correlated with ACR in the EEG-ERP P3/LPP response, r_{sp}=.355, p=.047, which was significantly greater for LS than HS, t(28)=1.71, p=.049. These findings are consistent with LS conferring risk for AUD *via* over-attribution of incentive salience to alcohol and its cues. Findings suggest that amplified ACR in LS people may stem from corticolimbic contributions to incentive salience attribution. These preliminary findings provide a compelling rationale for future fMRI studies in larger samples aimed at identifying the neurobiological loci and mechanisms underlying LS-based risk for AUD.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant AA027989

Title: Involvement of the basolateral and central amygdala in stress-induced increase of alcohol intake in female mice

Authors: *V. GARCIA-RIVAS, M. A. THOMAS, A. R. SOARES, Y. S. MINEUR, M. R. PICCIOTTO;
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Abstract: Recent data show that, compared to men, women are more likely to drink alcohol to cope with stress. Previous studies show that the basolateral (BLA) and central amygdala (CeA) mediate stress responses, while chronic ethanol exposure changes circuit function in both structures. It remains unknown whether these changes in amygdalar function could prime for sex-specific maladaptive responses to stress, which may increase voluntary alcohol intake to cope with stress. To test this hypothesis, we first developed a behavioral model that captures sex differences in stress-induced alcohol drinking. Male (n=24) and female (n=24) C57BL/6J mice were first trained to drink alcohol in their home cage through the introduction of 2 sippers (10% ethanol and water) for 2 hours daily for at least 10 days. Mice were then split into control (n=12 per sex) and 'stress' (n=12 per sex) subgroups. On sessions 12-13 and 21-22, 'stress' mice underwent foot shock stress sessions (120 shocks over 1 hour) at least 4 hours prior to the drinking session for that day, while control animals stayed in their home cage. 'Stress' female mice drank more alcohol in the 2 days following the stress session (day 13), when compared to all other subgroups. We then tested whether this female-specific phenomenon involved changes in amygdalar circuits, with a focus on principal, GABA and cholinergic neurons. We used dual-color fiber photometry to simultaneously record the activity of GABA and principal neurons (PN) in the CeA of freely moving mice drinking alcohol before or after stress (n=15 per sex). We found that repeated stress does not drastically change the pattern of activity of GABA and PN in the CeA of female mice during alcohol drinking bouts. Since we previously showed that limiting cholinergic signaling in the BLA decreases stress-related phenotypes, we then tested if chemogenetic inhibition of cholinergic terminals in the BLA could blunt stress-induced alcohol drinking. 24 hours after a first stress exposure, we observed that female mice reduced their alcohol intake after the inhibition of their BLA cholinergic terminals with a Gi DREADD. After a second stress exposure, this effect was lost in females but males showed an unexpected *increase* in drinking when inhibiting BLA cholinergic terminals. Collectively, we show that stress can promote an increase in alcohol intake in females, but not in males. Neither stress nor alcohol appear to modify the circuitry of the CeA in ways that would explain this effect. In contrast, restricting the cholinergic inputs to the BLA decreased stress-induced drinking in

females, indicating that acetylcholine in the BLA may be a mediator of stress-related alcohol intake in females

Disclosures: V. Garcia-Rivas: None. **M.A. Thomas:** None. **A.R. Soares:** None. **Y.S. Mineur:** None. **M.R. Picciotto:** None.

Poster

PSTR429. Alcohol Induced Changes in Neural Circuits and Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR429.12/PP26

Topic: G.09. Drugs of Abuse and Addiction

Support: P30 DA013429

Title: The effect of ethanol consumption and cigarette smoke exposure in adolescent mice on the neuroimmune system

Authors: D. STERN[1], *E. UNTERWALD[2], J. SHINKLE[1], B. KOSMIDER[3], Z. DAVIS-LUIZER[3], H. HAYEK[3], K. BAHMED[3];
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Abstract: Ethanol exposure can produce harmful effects by activation of neuroinflammatory responses in the brain. When high concentrations of ethanol interact with the neuroimmune system it can activate signaling cascades that amplify the expression of proinflammatory cytokines. Many drugs of abuse create this excessive inflammatory response, although some studies indicate that nicotine has anti-inflammatory effects. The current study measured the expression of the cytokines, TNF-a and IL-1b, and the chemokine CCL2 in the brains of mice that were exposed to either ethanol, cigarette smoke, or both during the adolescent period. Male and female C57/BL6 mice were exposed to cigarette smoke or air 2 hr/d, 5 d/wk for 4 weeks starting on postnatal day 28. Concomitantly, mice underwent intermittent two-bottle choice ethanol drinking; ethanol and water or 2 water bottles were provided for 21 hr beginning after smoke exposure 3 d/wk. Brains were obtained 20 hours after the last exposure to cigarette smoke. qRT-PCR was performed to measure the expression of TNF-a, IL-1b, and CCL2 mRNA in the prefrontal cortex (PFC). Results demonstrate higher expression of IL-1b mRNA in the PFC of male mice exposed to both smoke and ethanol compared to smoke only, ethanol only, or controls. Female mice that drank ethanol exhibited higher expression of IL-1b compared to controls. Male and female mice that drank ethanol had higher levels of TNF-a mRNA compared to mice exposed to ethanol and smoke, or water and smoke which were not different from controls. CCL2 showed sex-dependent regulation. Ethanol exposed male mice (with or without cigarette smoke) displayed lower expression of CCL2 mRNA compared to mice without ethanol. However, female mice that consumed ethanol without smoke exposure had higher expression of

CCL2 mRNA compared to no ethanol females. The results for IL-1b and TNF-a were consistent with the hypothesized stimulation of neuroinflammation due to ethanol exposure; the downregulation of pro-inflammatory chemokine CCL2 in male mice was unexpected. Additionally, the TNF-a regulation suggests the potential of nicotine being a protective factor when interacting with alcohol, which needs further study to establish.

Disclosures: **D. Stern:** None. **E. Unterwald:** None. **J. Shinkle:** None. **B. Kosmider:** None. **Z. Davis-Luizer:** None. **H. Hayek:** None. **K. Bahmed:** None.

Poster

PSTR429. Alcohol Induced Changes in Neural Circuits and Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR429.13/PP27

Topic: G.09. Drugs of Abuse and Addiction
R01AA023183
R01DA037294
T32AA007456
U01DA055017

Title: 3d brain-wide profiling of neuronal ensembles reactive to relapse-promoting vs. relapse suppressing cues in rats.

Authors: H. NEDELESCU[1], ***S. SRINIVASAN**[2], N. CONNOR[4], B. EASTWOOD[5], G. WAGNER[2], A. C. THAN[2], H. C. ZHANG[2], Z. MIKKULSKI[6], F. WEISS[7], J. R. GLASER[5], N. SUTO[3];
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Abstract: Drug addiction is a chronically relapsing brain disease. In rats, while environmental stimuli that signal drug availability (S+) promote relapse, we found that those signaling drug omission (S-) can suppress relapse. This bidirectional modulation of relapse is regulated by two functionally distinct neuronal ensembles (engram cells) of infralimbic cortex (IL) neurons, with each ensemble selectively reactive to S+ or S- (Fos protein). The neuroanatomical source of afferents that activate these neurons, however, remains unknown. Furthermore, procedures for automated brain-wide profiling are available for mice, but not rats, which is the preferred animal model for studying more complex models of drug addiction. To address both issues, in rats trained to self-administer cocaine/alcohol, we conducted a brain-wide analysis to identify neuronal ensembles that send axonal projections (AAV2retro-GFP) to IL. We developed an automated brain-wide 3D profiling procedure for rats, where image data and cell counts were registered using NeuroInfo to the Waxholm rat atlas (ref: https://www.nitrc.org/projects/whs-sd-atlas). Briefly, (1) whole slide images of serial brain sections (60 µm) were captured by a ZEISS

Axioscan slide scanner microscope, (2) these 2D images were aligned and registered using NeuroInfo to the Waxholm rat atlas coordinate system, (3) Fos- and GFP-positive IL-projecting neurons were detected with single cell resolution in NeuroInfo using deep learning methods with anatomic specificity conferred by matching image data to the atlas coordinate space. This method provides superior cellular tagging (staining) and image resolution - especially for the larger rat brains - than similar methods using brain clearing and light-sheet microscopes. Our new NeuroInfo tool can be used to identify brain-wide neuronal networks reactive to relapse-promoting vs. relapse-suppressing stimuli. In short, this study provides a new tool for automated 3D brain-wide profiling of rats and expands our knowledge of brain circuitry mediating environmental modulation of drug relapse.

Disclosures: H. Nedelescu: None. **S. Srinivasan:** None. **N. Connor:** None. **B. Eastwood:** None. **G. Wagner:** None. **A.C. Than:** None. **H.C. Zhang:** None. **Z. Mikkulski:** None. **F. Weiss:** None. **J.R. Glaser:** None. **N. Suto:** None.

Poster

PSTR429. Alcohol Induced Changes in Neural Circuits and Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR429.14/PP28

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R00 AA025991

Title: Acute and chronic ethanol effects on dorsal striatal cholinergic signaling

Authors: C. C. LEVY[1], L. E. SLADE[1], Y. MATEO[2], D. M. LOVINGER[2], *A. G. SALINAS[1,2]; [1]Pharmacol., LSU Hlth. Sci. Ctr. at Shreveport, Shreveport, LA; [2]Natl. Inst. on Alcohol Abuse and Alcoholism, Rockville, MD

Abstract: Alcohol use disorder (AUD) has adverse health and socioeconomic impacts totaling over $240 billion annually. Despite this, FDA approved medications for AUD are limited. Thus, further understanding of neurobiological mechanisms in AUD is required for new treatments. Given its role in cognition and decision-making processes, both of which are adversely affected in AUD, acetylcholine (ACh) signaling is an attractive pharmacotherapeutic target. Therefore, this study aimed to determine how ethanol affects ACh signaling in the dorsal striatum (DS). We previously showed that chronic ethanol treatment in mice and macaques results in a deficit in the cholinergic contribution to dopamine (DA) release. Similarly, we show here that acute ethanol (50 mM) depresses optogenetically evoked ACh-driven DA release measured with fast-scan cyclic voltammetry (FSCV). To more directly assess the effects of ethanol on striatal ACh, we used cell-attached electrophysiological recordings of cholinergic interneurons (CINs) and found that acute ethanol (40 mM) depressed CIN firing rate, supporting our FSCV results. To further evaluate ethanol effects on striatal ACh release, we used the iAChSnFR, a biosensor that increases in fluorescence intensity upon binding ACh. We used AAVs to express iAChSnFR in

DS of C57BL6J mice. After at least 4 weeks, brain slices containing iAChSnFR expression were used for slice photometry experiments. Similar to our previous results, we found that acute ethanol (40 mM) depressed ACh release. To determine if these effects applied in vivo, we used fluorescence fiber photometry with iAChSnFR and found a similar acute ethanol (2 g/kg) induced depression of ACh in DS. To assess chronic ethanol effects on ACh release, mice were injected with iAChSnFR as before. Following recovery, they underwent chronic intermittent ethanol vapor exposure (CIE) which consisted of ethanol vapor for 16 hours per day for four consecutive days followed by 72 hours of withdrawal. This cycle was repeated for four weeks. Following 72-96 hours of withdrawal, slice photometry was performed. CIE treated mice displayed deficits in ACh release compared to controls. Given the withdrawal period following the final ethanol exposure, this deficit is not likely due to any potential lingering acute ethanol effects. To determine the cause of this ACh deficit, we examined whether CIE resulted in a loss of CINs. We performed stereological counting of CINs in control and CIE mice and found no difference between groups. Thus, further work is required to determine the cause of the ACh deficiency following CIE. Altogether, our results show acute and chronic ethanol depresses ACh signaling in DS.

Disclosures: C.C. Levy: None. **L.E. Slade:** None. **Y. Mateo:** None. **D.M. Lovinger:** None. **A.G. Salinas:** None.

Poster

PSTR429. Alcohol Induced Changes in Neural Circuits and Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

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Topic: G.09. Drugs of Abuse and Addiction

Support: PNSD2019I015, National Plan on Drug abuse, Ministerio de Sanidad of Spain
ISCIII-Redes de Investigación Cooperativa Orientadas a Resultados en Salud (RICORS), Red de Investigación en Atención Primaria de Adicciones (RIAPAd; grant RD21/0009/0013)

Title: Inhibition of Phosphacan regulates ethanol-induced changes in perineuronal nets and PV+ neurons in a sex-dependent manner during adolescence: Implications for ethanol consumption

Authors: M. GALÁN-LLARIO, M. RODRÍGUEZ-ZAPATA, T. FONTÁN-BASELGA, E. GRAMAGE, M. VICENTE-RODRÍGUEZ, J. ZAPICO, B. DE PASCUAL-TERESA, ***G. HERRADON**;
Fac. of Pharmacy, CEU San Pablo Univ., Alcorcon, Spain

Abstract: Perineuronal nets (PNNs) are extracellular matrix structures surrounding neurons that are mainly formed during adolescence, when the brain is highly plastic and more vulnerable to the effects of drugs of abuse. Ethanol, which is frequently consumed by young adults in a binge-

like manner, alters the intensity of PNNs in different brain areas. However, little is known about possible sex differences in the regulation of the intensity of PNNs induced by ethanol. In addition, there is evidence showing the modulation of the effects of drugs of abuse through manipulation of PNNs, suggesting that pharmacological regulation of components of PNNs may be a novel therapeutic strategy in addictive disorders. One key constituent protein in PNNs is Phosphacan (Receptor Protein Tyrosine Phosphatase (RPTP) β/ζ). In the present work, we have assessed the capacity of MY10, a compound inhibitor of Phosphacan, to regulate ethanol consumption and ethanol-induced changes in PNNs in an Intermittent Access to Ethanol (IAE) model in adolescent mice. The data demonstrate that MY10 significantly decreases ethanol consumption in male mice, not in females. In general, ethanol consumption during the 4-week IAE paradigm induced a decrease in the intensity of PNNs in different cortical and hippocampal areas of male and female mice. We found a significant correlation between ethanol consumption and ethanol-induced alterations in the intensity of PNNs in the Dentate Gyrus (DG). Very interestingly, treatment with MY10 prevented this correlation in the DG of males and even reverted it in CA1 from both sexes, suggesting a connection between the capacity of MY10 to modulate the intensity of PNNs in the hippocampus and its ability to reduce ethanol consumption. In the insular cortex, a brain area that contains highly condensed PNNs around parvalbumin (PV)-expressing neurons, we found that inhibition of Phosphacan with MY10 potentiated the ethanol-induced increase of PV+ cells in male mice, whereas in females did the opposite. In conclusion, we have demonstrated that pharmacological inhibition of Phosphacan decreases ethanol consumption during adolescence only in male mice, which may be related to a sex-dependent regulation of ethanol-induced changes in the intensity of PNNs and number of PV+ cells in the hippocampus and the insular cortex.

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Poster

PSTR429. Alcohol Induced Changes in Neural Circuits and Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR429.16/QQ2

Topic: G.09. Drugs of Abuse and Addiction

Support: R01AA028228

Title: The kappa-opioid receptor in the nucleus accumbens shell: Subregional, sex-related, and intake-dependent effects on ethanol drinking

Authors: *B. E. PIRINO[1], B. A. CARPENTER[1], A. HAWKS[2], P. G. CANDELAS[2], A. T. GARGIULO[2], G. R. CURTIS[2], A. N. KARKHANIS[3], J. R. BARSON[4];
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Abstract: The kappa-opioid receptor (KOR) and its endogenous ligand, dynorphin, have been implicated in alcohol use disorder and are densely expressed in the nucleus accumbens (NAc) shell. Within the NAc shell, KORs are located on terminals of neurons originating from the ventral tegmental area (VTA), and the activity of the VTA to NAc shell pathway has been shown to suppress high-level ethanol drinking . Surprisingly, studies thus far have not found KOR activation in the NAc shell to affect ethanol drinking. Thus, we allowed male and female Long-Evans rats to drink under the intermittent access model, to investigate the effects of KOR manipulation in the NAc shell. Using microinjections of the KOR agonist, U50,488, we found that, although KOR stimulation in the middle subregion of the NAc shell had no effect on 20% ethanol drinking, KOR activity in the caudal NAc shell promoted ethanol drinking in males and higher-drinking females, while KOR activity in the rostral NAc shell instead decreased intake in males and lower-drinking females and enhanced intake in higher-drinking females. Conversely, blockade of KORs in the rostral NAc shell with the antagonist, nor-binaltorphimine, stimulated ethanol drinking in lower-drinking rats. To determine if these effects on ethanol drinking were substance-specific, we injected U50,488 into the rostral or caudal NAc shell of 2.5% sucrose-drinking rats and found no effect on sucrose intake. To determine if the differential effects of KOR manipulations could be due to ethanol-induced changes in gene expression, we performed quantitative real-time PCR on NAc shell tissue from ethanol-experienced and ethanol-naïve female rats. While there was no effect of ethanol on dynorphin gene expression, a history of ethanol drinking led to upregulated levels of KOR mRNA in the rostral NAc shell. To investigate if the effects of KOR manipulation could be due to KOR-mediated inhibition of VTA input, we injected an excitatory designer receptor exclusively activated by designer drugs (DREADDs) virus into the VTA and then injected CNO and U50,488 into the rostral NAc shell. We found that activation of the VTA to rostral NAc shell pathway suppressed ethanol drinking in high drinkers, and that U50,488, which otherwise enhanced ethanol drinking, reversed this DREADD-induced suppression. These findings demonstrate that (1) the KOR can affect ethanol drinking; (2) the direction of this effect depends on the level of intake, sex of the drinker, and subregion of the NAc shell; and (3) the ability of KOR activity in the rostral NAc shell to promote ethanol drinking in higher drinkers, which have upregulated KOR levels, may be due to its ability to suppress VTA input activity.

Disclosures: B.E. Pirino: None. **B.A. Carpenter:** None. **A. Hawks:** None. **P.G. Candelas:** None. **A.T. Gargiulo:** None. **G.R. Curtis:** None. **A.N. Karkhanis:** None. **J.R. Barson:** None.

Poster

PSTR429. Alcohol Induced Changes in Neural Circuits and Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR429.17/QQ3

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant AA026638

Title: Sex differences in stress-related neuropeptide signaling components and interaction with alcohol in GABAergic neurons of the lateral central amygdala in rats

Authors: *E. MOSS, E. BATSAIKHAN, D. KIRSON;
The Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Alcohol use disorder (AUD) is characterized by compulsive seeking and consumption of alcohol. AUD involves the recruitment of brain stress systems leading to the negative affective states in withdrawal; relief of which drives further drinking. The central amygdala (CeA) is an important brain region that serves as a neuropeptidergic hub of stress, anxiety, and addiction processing, and CeA GABA transmission is involved in the regulation of alcohol consumption. The CeA consists of the lateral (CeL) and medial (CeM) subdivisions. The majority of neurons in the CeA are GABAergic, including both GABAergic projection neurons and local interneurons. Overall, studies show that acute alcohol application increases GABAergic transmission in the CeM, decreases glutamatergic transmission in the CeM, and increases spontaneous glutamatergic transmission in the CeL. However, the acute effects of alcohol on GABAergic transmission in the CeL are currently unknown. Stress related neuropeptides, such as oxytocin and CRF, have been shown to alter CeA GABA signaling in rodents, and modulating these systems can affect alcohol intake and withdrawal symptoms. In addition, we have previously shown sex-specific effects of alcohol and stress related neuropeptides on CeM GABAergic signaling. However, oxytocin receptors and CRF are primarily expressed in CeL neurons. In this study, we used whole cell patch clamp electrophysiology to examine the synaptic effects of alcohol and oxytocin on CeL GABAergic signaling in *ex vivo* brain slices from male and female Wistar rats. Additionally, we used *in situ* hybridization via RNAscope technology to identify differences in mRNA expression of these neuropeptide systems in the CeL of naïve and alcohol dependent male and female rats. These experiments provide important insight into sex differences in CeA neuronal functions that may contribute to the development of alcohol dependence.

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Poster

PSTR429. Alcohol Induced Changes in Neural Circuits and Neurophysiology

Location: WCC Halls A-C

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Program #/Poster #: PSTR429.18/QQ4

Topic: G.09. Drugs of Abuse and Addiction

Support: R01AA027213
P50DA044123
U01NS115587

Title: High-dimensional neural activity in the anterior insular cortex during natural and drug reward consumption

Authors: *S. JUNG[1], A. LAWRENCE[2], S. KOUKUNTLA[1], A. R. GRAVES[1,3,2], T. D. HARRIS[1,3,5], P. H. JANAK[2,4,3];
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Abstract: The anterior insular (AI) cortex plays a major role in interoception where it mediates consummatory behaviors based on the current physiological state (Livneh and Andermann, 2021). Previous studies have shown that the insular cortex encodes expectation and consumption of natural and drug-related rewards and its chemogenetic modulation impacts their intake (Livneh et al., 2020; Jaramillo et al., 2018). Nevertheless, it is unclear how different reward-related variables are encoded by the population activity of AI neurons. In the current study, we recorded >100 neurons per session from three alcohol pre-exposed adult Long Evans rats (1 male and 2 females) using high-density Neuropixels 2.0 probes unilaterally implanted in the AI. We simultaneously recorded licks from rats as they freely drank from a bottle for 30 minutes containing either sucrose, alcohol, or a mixture of both. Kilosort 2.5 and manual curation were used to isolate single units. The modulation of these high-quality single units from the AI was assessed pre, during, and post each lick bout (defined as a continuous train of licks, with an inter-lick interval of ~ 0.16 sec and separated by > 0.5 sec) per recording session. Principal component analysis (PCA) was performed to investigate whether the tastant type or licking activity explained the variability in the population neural activity. We first observed that population activity in the AI is high dimensional (>7 principal components (PC) to explain ~80% of variance). We also found that the first two PCs discriminate licking from non-licking behavior. Furthermore, we found that PCs 3-7 encoded tastant identity, i.e., alcohol or sucrose. More specifically, projecting neural activity at the beginning and end of each lick bout onto PCs 3-7 showed a clear separation of activity by tastant. The separation persisted when we analyzed only the anticipatory neural activity (2s pre-bout), suggesting that these PCs encode tastant identity rather than licking patterns or perceived taste. These findings reveal moment-by-moment encoding of both general consummatory behavior and alcohol- and natural reward-specific variables including tastant anticipation and identity by simultaneously-recorded neuronal populations in the AI.

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Poster

PSTR429. Alcohol Induced Changes in Neural Circuits and Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR429.19/QQ5

Topic: G.09. Drugs of Abuse and Addiction

Support: R01AA023288
T32AA007474

Title: The effect of chronic alcohol exposure on neuronal excitability and synaptic transmission in the nucleus reuniens: potential implication for cognitive dysfunction

Authors: *K. L. LINDQUIST, J. A. RINKER, P. J. MULHOLLAND;
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Abstract: Chronic alcohol use can cause cognitive deficits, particularly working memory, which persists during alcohol withdrawal and abstinence, increasing the odds of relapse. The extent to which alcohol exposure disrupts the circuitry underlying cognition is not fully understood. The nucleus reuniens (RE) is a ventral midline thalamic nucleus that plays a key role in cognitive function, especially spatial working memory. The RE is bidirectionally connected to the medial prefrontal cortex and the hippocampus, which have been highly studied in alcohol use disorder and cognition. Despite this, the RE remains highly understudied within the alcohol field. These studies aim to determine how chronic alcohol exposure alters intrinsic excitability and synaptic transmission within the RE in an exposure model that causes cognitive deficits. Alcohol dependence was induced using chronic intermittent ethanol (CIE) vapor exposure (4 cycles) in adult male and female C57BL/6J mice. Following CIE, mice were either tested on a T-maze delayed alternation spatial working memory task or sacrificed for whole cell patch clamp electrophysiology to measure intrinsic excitability or synaptic transmission in the RE. Mice used in the T-maze task were trained prior to CIE and tested following 2 and 4 cycles, to track cognitive decline across the development of dependence. Both male and female mice exhibited significant deficits in performance following CIE. Despite this similar behavioral phenotype, CIE had divergent effects on intrinsic excitability based on sex. CIE significantly increased spike firing in the females and significantly decreased spike firing in the males. Interestingly, in the absence of alcohol exposure, RE intrinsic excitability appears to be sex-dependent, where males have significantly greater excitability than females. Spontaneous inhibitory and excitatory post-synaptic currents are currently under analysis to determine how chronic ethanol impacts synaptic transmission in the RE. These results indicate that the RE could be an exciting new target for the study of chronic alcohol-induced cognitive deficits. Future studies will interrogate this RE in vivo during performance on the spatial working memory task following CIE exposure.

Disclosures: K.L. Lindquist: None. **J.A. Rinker:** None. **P.J. Mulholland:** None.

Poster

PSTR429. Alcohol Induced Changes in Neural Circuits and Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR429.20/QQ6

Topic: G.09. Drugs of Abuse and Addiction

Title: Heavy adolescent drinking potentiates neuronal alcohol response later in life: involvement of GIRK and activin receptor signaling

Authors: *F. ZHENG, S. STÜRZENBERGER, N. BÜLOW, C. ALZHEIMER;
Univ. of Erlangen-Nürnberg, Erlangen, Germany

Abstract: Excessive ethanol consumption during adolescence is regarded as a risk factor for the development of alcoholism later in life, but the pathophysiological mechanisms that render the adult brain susceptible to alcohol are largely unknown. G protein-gated inwardly rectifying potassium (GIRK) channels are among the prime targets of alcohol to regulate neural activity. Previous work from our lab has demonstrated that activin A, a member of the TGF-β family, affects GIRK currents in mouse hippocampus. We asked here, (i) if hippocampal cells from adolescent and adult mice respond differently to alcohol exposure, and how GIRK channels are involved, (ii) how adolescent drinking affects alcohol response upon re-exposure in adulthood, and (iii) if activin plays a role as an adaptive factor. In whole-cell voltage-clamp recordings from dentate gyrus granule cells (GCs) in dorsal hippocampal slices, ethanol dose-dependently induced outward currents, which were significantly stronger in slices from adolescent compared to adult mice. Ethanol-evoked currents reversed near -90 mV, were largely diminished by the GIRK-inhibitor tertiapin Q, and were fully suppressed by low Barium. Unexpectedly, the effects of recombinant activin A on ethanol responses were stage-dependent, being potentiated in adolescence, but inhibited in adulthood. The inverse was true when ethanol responses were examined in GCs from adolescent and adult transgenic mice expressing a dominant-negative activin receptor IB mutant, which disrupts activin receptor signaling. These data underscore the essential role of endogenous activin signaling in determining the neural impact of alcohol consumption at different stages of life. Compared to alcohol-naive mice, sustained heavy drinking in the dark (DID, 20% alcohol) between postnatal days 32 and 45 produced a long-lasting sensitization so that ethanol-induced currents in adulthood were consistently potentiated, including its tertiapin Q-sensitive component. Consequently, the adolescent drinking paradigm enhanced the silencing of GC firing during ethanol exposure. Our results show that heavy adolescent drinking exerts a lasting impact on how GIRK channels react upon alcohol re-exposure later in life and that this process might possibly involve activin receptor signaling.

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Poster

PSTR429. Alcohol Induced Changes in Neural Circuits and Neurophysiology

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Program #/Poster #: PSTR429.21/QQ7

Topic: G.09. Drugs of Abuse and Addiction

Support: P60 AA010760
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F31AA030908
F32 AA028686
VA I01 BX004690
Andrews Genomics Fund
ARCS Foundation (Oregon Chapter)

Title: Whole brain activity changes in male and female C57BL/6J mice following binge-like ethanol drinking

Authors: *A. E. CHAN[1,2], K. B. GRIGSBY[1,2], J. Q. ANDERSON[1,2], B. E. JENSEN[2], A. R. OZBURN[1,2];
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Abstract: The nucleus accumbens core (NAcc) is an important regulator of binge-like drinking. Male and female mice exhibit distinct transcriptional responses in the NAcc following limited access binge-like drinking. Moreover, chemogenetic manipulations of the NAcc results in opposite effects on binge drinking in male and female mice (inhibition decreased intake in males, but increased intake in females; stimulation decreased intake in females, with no change in males). We used whole-brain fluorescent imaging for the immediate early gene, c-Fos, and a viral retrograde green fluorescent protein (GFP) tracer to determine whether whole-brain activity and NAcc brain circuitry were differentially engaged in males and females during binge-like ethanol drinking [using the Drinking in the Dark (DID) assay]. We hypothesize males have greater c-Fos levels in excitatory regions that project to the NAcc, and females have greater c-Fos levels in inhibitory or peptidergic regions that project to the NAcc. C57BL/6J mice (n=15-16/sex/fluid) underwent stereotaxic surgery to infuse 0.5uL AAVrg-hSyn-eGFP bilaterally into the NAcc, recovered, and then underwent a 4-day DID (2hr on days 1-3, 4hr on day 4) with water or 20% ethanol. Immediately following DID, blood was collected for determination of blood ethanol concentration (BEC). Brains were collected and processed for whole-brain clearing, immunolabeling for c-Fos, GFP, and NeuN, and light-sheet imaging. Image atlas registration and cell detection was conducted using SmartAnalytics software. There was no effect of sex on 4hr ethanol intake, BEC, or water intake. Principal component analysis of c-Fos cell density reveal that sex, fluid, and a sex by fluid subgroups are associated with significant amounts of the total variance ($p<0.05$, one-way ANOVA). The peptidergic Edinger-Westphal nucleus (known sensitivity to ethanol) and subfornical organ (hypothalamic nucleus involved in osmoregulation) were engaged in both sexes following ethanol intake [$p<0.05$, with > 0.5 log fold change (lfc); compared to water drinking mice]. Ethanol drinking females had a greater number of regions engaged following DID than males, including the excitatory/glutamatergic claustrum, infralimbic and somatosensory cortices, and GABAergic/peptidergic pallidum, globus pallidus, and hypothalamic nuclei. In total, 94 regions had $p<0.05$, >0.5 lfc in female ethanol, compared to male ethanol drinkers. Analysis of c-Fos and GFP co-labeled neurons is underway. These findings indicate that binge-like ethanol drinking significantly impact whole-brain activity in a sex-specific manner, as measured by c-Fos induction.

Disclosures: **A.E. Chan:** None. **K.B. Grigsby:** None. **J.Q. Anderson:** None. **B.E. Jensen:** None. **A.R. Ozburn:** None.

Poster

PSTR429. Alcohol Induced Changes in Neural Circuits and Neurophysiology

Location: WCC Halls A-C

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Program #/Poster #: PSTR429.22/QQ8

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH NIAAA R00 AA027774
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Title: Determining the role of insula-S1 neurons in mediating affective behaviors during alcohol abstinence

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Abstract: Alcohol Use Disorder (AUD) is a growing problem in our nation and worldwide. COVID-19 has led to a significant rise in alcohol use, with many people relapsing due to the negative affective states correlated with abstinence. An understanding of the functional connectivity network of abstinence-induced negative affect is needed. Previous work in our lab has elucidated parts of this network, identifying an insula-BNST (bed nucleus of the stria terminalis) pathway activated during stress and negative affect in abstinence. The current study focuses on elucidating the control network for insula-BNST pathway mediation of abstinence-induced negative affect. We mapped 2nd order inputs into this pathway and identified the somatosensory cortex (S1) as a dense population of neurons that project to the insula-BNST pathway. As the S1 is involved in processing tactile information arising from external stimuli, we hypothesize the S1 serves in this pathway as a gateway between the external environment and the internal state. To test this potential pathway in alcohol abstinence, we injected anterograde AAV1.Cre into both S1 and DIO.hM3Dq into both insulae in female mice to isolate specific insula cells that receive projections from S1. We then subjected the mice to a chronic alcohol drinking forced abstinence (CDFA) model. After 6 weeks of drinking and 2 weeks of abstinence, mice were subjected to multiple behavioral tests (Novelty Suppressed Feeding Test (NSFT), Acoustic Startle, Foot Shock Startle, and Air Puff Startle). Chemogenetic activation of these insula^{S1} neurons causes a decreased startle response at lower shocks in alcohol abstinent negative affective behavior in the Foot Shock Startle test. Incorporating multiple measures of negative affect and normalizing them across tests is important to create a more holistic view of an animal's affective state in alcohol abstinence. To this end, we began using "emotionality scores" to normalize and combine affective states across tests, statistically determining an emotionality score for each subject based on validated measures of negative affect for each test (e.g., latency to bite on NSFT). Preliminary data using this "emotionality" method demonstrates chronic ethanol drinking increases negative emotionality (i.e., negative affective state) in mice in protracted abstinence. Follow up studies are looking at the opposite side of this pathway, isolating specific S1 cells that project to the insula; along with incorporating emotionality data

after behavioral assays, to show that the S1 is a critical regulator of the insula to BNST pathway, and an overall contributor to alcohol abstinence-induced negative affect.

Disclosures: **A. Salazar:** None. **J.R. Little:** None. **S.W. Centanni:** None.

Poster

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Title: Inhibiting CRF and NPY1R+ Projections From the Central Amygdala to Lateral Hypothalamus Blunts Binge-Like Ethanol Drinking in Male, but Not Female Mice

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Abstract: Corticotrophin releasing factor (CRF) and Neuropeptide Y (NPY) are a critical 'pro-stress' and 'anti-stress' neuropeptides modulating binge-like ethanol intake in the central amygdala (CeA) and the lateral hypothalamus (LH). Both brain regions express these peptides, and are known to be particularly impacted by ethanol consumption. The CeA is an important region in relation to alcohol use disorder as it integrates stress and reward aspects to form behavioral responses. The LH is a very heterogeneous brain area, with a variety of neuropeptide and neurotransmitter systems which have been implicated in sleep regulation, food intake, and recently drug reward. Previous data from our lab indicates that CRF and NPY co-modulate ethanol intake, so we wanted to specifically investigate the role of the CRF and NPY systems in the CeA to LH pathway. To assess the role of these anxiety and reward pathways, chemogenetic approaches were used in male and female CRH-cre and NPY1R-cre expressing mice. The "drinking in the dark" behavioral model was used for two consecutive cycles of 20% ethanol solution, and 3% sucrose solution. Preliminary results showed interesting sex-differences regarding the CeA-LH pathway. Placing the Gi DREADD into the CeA, and micro-injecting CNO into the LH, we found a sex-specific reduction in ethanol drinking in male, but not female mice for both CRF and NPY1R+ projections. Silencing this CRF or NPY1R+ pathway had no effect on sucrose drinking in either male or females, suggesting a specific effect for ethanol. The present findings are in line with our prior hypothesis about the NPY system working, in opposition to CRF, as a protective mechanism against ethanol consumption. Interesting sex trends emerged in CeA to LH pathway, such that both CRF and NPY1R+ neurons in this

pathway only appear to modulate ethanol consumption in male, and not female mice. Supported by NIH: AA022048 & AA013573.

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Poster

PSTR429. Alcohol Induced Changes in Neural Circuits and Neurophysiology

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Topic: G.09. Drugs of Abuse and Addiction

Support: Zap Surgical Systems, Inc. San Carlos, CA, USA.
MOST 109-2320-B-002-014-MY3

Title: Non-ablative focal irradiation restores the functional connectivity between the nucleus accumbens and the dorsal anterior cingulate cortex in miniature pigs with long history of alcohol consumption

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Abstract: Excessive alcohol drinking is a globally prevalent condition and is linked to many negative health and social consequences. One essential site where ethanol acts directly on neurons is the nucleus accumbens (NAc) - one of the critical structures related to alcohol craving. However, most contemporary treatment options do not achieve a long-term reduction in alcohol usage. In this study, we investigated how the functional connectivity between the NAc and the dorsal anterior cingulate cortex (dACC) was affected by long-term alcohol consumption, and how radiation-based neuromodulation might affect the NAc-dACC functional connectivity and animals' alcohol-drinking behavior. Eight Lee Sung miniature pigs were included in the study: five were trained with operant procedures to voluntarily consume ethyl alcohol solution over 2-3 years, and three served as non-drinking controls. An Achieva 3.0 T scanner (Philips) with an 8-channel body surface coil was used as the receiver for magnetic resonance imaging (MRI) of the brain to obtain images, including T1- and T2-weighted, fast gray matter acquisition T1 inversion recovery (FGATIR) for structural identification, and echo planar imaging (EPI) for resting-state *f*MRI time series. Seed-based functional connectivity analysis was implemented by calculating correlation voxel-wisely throughout the brain with the time series retrieved from the NAc. Preliminary results showed that the NAc-dACC functional connectivity of alcohol-

consuming animals was lower than that of non-drinking controls. We irradiated the NAc using a cross-firing stereotactic radiosurgery approach in three highly motivated alcohol-seeking animals (Dmax = 30 Gy with a 5mm collimator). The selected dose has been shown to elevate metabolic activity at the target without evidence of tissue destruction (Yeh et al., 2021). Thereafter, voluntary alcohol consumption was shown to be significantly reduced in the three irradiated animals. Preliminary analysis of functional connectivity showed an increase in the NAc-dACC connectivity six months after radiosurgery. Overall, we have demonstrated a viable larger animal model of Alcohol Use Disorder, with behavioral measures of motivation that reasonably approximate "craving." NAc-dACC functional connectivity reduction may be a characteristic of excessive alcohol consumption, and the restoration in connectivity strength may be a restorative result of focal neuromodulation using non-ablative radiation. These results suggest that non-ablative focal radiation has the potential to be an effective neuromodulation tool for the treatment of chronic alcohol-seeking behaviors.

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Poster

PSTR429. Alcohol Induced Changes in Neural Circuits and Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH grant DA051922

Title: Drinking severity mediates the relationship between hypothalamic connectivity and rule-breaking/intrusive behavior in young adults

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Abstract: Background
Hypothalamic circuits play a key role in supporting motivated behavior. Problem drinking may compromise hypothalamic circuit functions, as in altered hypothalamus-pituitary-adrenal axis response to stress. Here, we investigated how alcohol use severity may impact hypothalamic resting-state functional connectivities (rsFC) and how the latter inter-relate the severity of drinking and other problem behaviors.

Methods
We curated the data of the Human Connectome Project S1200 data and identified 870 subjects with both physiological data and rs fMRI data meeting "scrubbing" criteria. Alcohol use severity was quantified by the first principal component (PC1) of PCA of all drinking measures, as in our published work. All participants were assessed with the Achenbach Adult Self Report syndrome scales, including rule-breaking and intrusive subscales. Imaging data were processed with published routines and evaluated at a corrected threshold.
Results
Whole-brain linear regression of the hypothalamus rsFCs against PC1 revealed across all subjects a cluster in the paracentral lobule (PCL, x, y, z = -4, -32, 66; Z = 4.49, 2440 mm3) and, in women alone, a cluster in the left temporo-parietal junction (left TPJ, x, y, z = -58, -54, 4; Z= 5.33, 824 mm3) in positive correlation with PC1, at voxel $p<0.001$, uncorrected, and cluster $p<0.05$, FWE-corrected. The β's of hypothalamus-PCL rsFC were significantly correlated with rule-breaking score in all ($r = 0.088$, $p = 0.009$) and in men ($r = 0.152$, $p = 0.002$), but not in women in linear regressions with age as a covariate. The β's of hypothalamus-left TPJ rsFC were significantly correlated with intrusive score in women ($r = -0.127$, $p = 0.006$), but not in all or men. In mediation analyses PC1 mediated the correlation between hypothalamus-PCL rsFC and rule-breaking in men and between hypothalamus-left TPJ rsFC and intrusive problems in women, both bidirectionally.
Conclusions
Hypothalamus-parietal cortical connectivities associate problem drinking and rule-breaking and intrusive problems each in men and women.

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Poster

PSTR429. Alcohol Induced Changes in Neural Circuits and Neurophysiology

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Topic: G.09. Drugs of Abuse and Addiction

Support: P50 AA17531
R37 AA26117
K01 AA30081

Title: Interneuron properties in the ventral subiculum of male and female rats

Authors: *E. BACH, O. ORTELLI, S. PITCAIRN, J. WEINER;
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Abstract: Inhibitory neurons play an essential role in maintaining normal physiological function throughout the brain, and a wide array of pathological conditions have been linked to aberrant inhibitory synaptic processes or associated excitatory-inhibitory imbalance. The ventral

subiculum (vSub) of the hippocampus has received increased attention for its role in acute anxiety, anxiety disorders as well as comorbid alcohol and substance use disorders. Our prior studies using models of alcohol dependence and vulnerability to alcohol use disorders revealed both sex-dependent and sex-independent adaptations of inhibitory input onto pyramidal neurons in the vSub. This finding prompted our interest in better understanding plasticity mechanisms in GABAergic neurons that ultimately dictate inhibitory tone within this brain region. One challenge in studying GABAergic neurons is their identification, particularly when studying functional adaptations using electrophysiological techniques. Relying on identification approaches such as action potential firing patterns or protein expression profiles can limit the types of interneurons being studied, the animal model being utilized and/or the types of electrophysiological studies that can be conducted. More recently the discovery of a specific GABAergic enhancer element (mDlx+) in a broad array of GABA neurons has allowed interneurons to be virally targeted for fluorescent identification or optical/chemogenetic manipulation. Here, we used this viral approach to fluorescently label and conduct electrophysiological recordings in mDlx+ vSub neurons of adult rats. We found that these mDlx+ neurons have shorter spontaneous EPSC (sEPSC) rise and decay times, but not amplitude differences, relative to vSub pyramidal neurons. Across sexes our results reveal that vSub mDlx+ neurons of female rats have a higher sEPSC frequency than males; a finding that could begin to explain the pathological sex-dependent adaptations seen in our animal models. Additional studies are underway to determine the GABAergic subtype(s) (protein expression identity) seen in mDLX+ neurons of the vSub.. Future studies aim to use our findings to integrate electrophysiological and molecular approaches and confirm the presence of distinguishing kinetic features of GABAergic neurons in young animals. This confirmation will allow us to circumvent limitations beset by viral expression windows and enable us to study the developmental progression in the broader vSub GABAergic neuronal population in our animal models of alcohol dependence and vulnerability to this disorder.

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Poster

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Title: Tip60 histone acetyltransferase regulates PDF neuropeptide expression and alcohol-tolerance acquisition in Drosophila melanogaster

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Abstract: Alcoholism is a condition characterized by behavioral and physiological changes, which can significantly impact an individual's health and interpersonal relationships. After prolonged alcohol consumption, the organism induces neuroadaptations that lead to tolerance, dependence, and a series of alterations in sleep patterns and circadian rhythms. Drosophila melanogaster has emerged as an effective model for studying alcohol neuroadaptations due to its behavioral responses to alcohol that closely resemble those observed in humans. Here, we explore the role of the histone acetyltransferase (Tip60) in the regulation of alcohol responses through its modulation of the ventrolateral neuronal system (LNv) of Drosophila. These neurons are known to regulate the sleep/wake cycle in Drosophila. We hypothesized that Tip60 would have a major role in the capacity of Drosophila melanogaster to acquire ethanol tolerance. Using the UAS-Gal4 system to knockdown Tip60 expression in LNv neurons (pdf-Gal4/UAS-Tip60-RNAi) we record the activity of flies during alcohol exposure and measure alcohol sensitivity and tolerance, against age-matched adult female flies. In parallel, we assessed the expression of the neuropeptide PDF to study the involvement of Tip60 in regulating PDF expression and LNv branching pattern. We found that Tip60 knockdown flies display increased alcohol sensitivity and reduced alcohol tolerance. Moreover, we found that Tip60 knockdown in LNv neurons leads to a significant reduction in the LNv branching pattern and that alcohol exposure exacerbates this reduction in pdf-Gal4/UAS-Tip60-RNAi flies, decreasing overall LNv neuronal branching. These results suggest that Tip60 is involved in the regulation of alcohol tolerance acquisition and brain branching patterns of LNv neurons. Understanding the molecular and cellular mechanisms underlying ethanol neuroadaptations can potentially lead to the identification of new therapeutic targets for alcohol-induced disorders.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH grant AA020919
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Title: Involvement of connexin-36 gap junctions in the activity of ventral tegmental area neurons

Authors: *A. J. PAYNE[1], S. SANDOVAL-PISTORIUS[2], A. B. AMENDOLARA[1], J. OBRAY[3], S. B. WILLIAMS[4], S. STEFFENSEN[4];
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Abstract: Gap junction proteins facilitate transmission of ions and other small molecules across electrical synapses. Vertebrate gap junctions are comprised of connexin subunits that join together to form a hemichannel known as a connexon. We have previously demonstrated that connexin-36 (Cx36) is expressed in the mature nervous system in a subpopulation of neurons in the ventral tegmental area (VTA). Cx36 has been evidenced previously to have a role in brain stimulation reward, and it has also been observed that Cx36 expression levels change in response to cocaine and methamphetamine self-administration. We hypothesize that these Cx36+ gap junctions play a role in alcohol reward and/or dependence. Here we customize a Gq-coupled Designer Receptor Exclusively Activated by Designer Drugs (DREADD) viral vector (AAV8.hCx36.hM3D(Gq)-mCherry.WPRE.rBG) with expression targeted to Cx36+ neurons. This vector or a control vector (AAV9.CB7.CI.mCherry.WPRE.rBG) was injected into the VTA of CD-1 GAD67 GFP transgenic mice and Wistar rats. *In vivo*, intraperitoneal administration of clozapine-N-oxide (CNO, 3 mg/kg), the ligand for the DREADDs, significantly increased VTA GABA neuron single-unit activity in anesthetized rats. *Ex vivo*, similar increases in VTA GABA neuron activity were observed but with a concomitant increase in VTA dopamine (DA) neuron activity. These *ex vivo* increases were dose-dependent and greater in amplitude in VTA GABA neurons than in VTA DA neurons. We furthermore utilized the calcium-sensitive fluorophore GCaMP6f to evaluate the impact of CNO and ethanol on calcium dynamics in the VTA. These two viruses were combined, aliquoted and flash frozen for storage. They were then co-injected into the VTA of C57BL/6J wild type mice. Following an incubation period, we sectioned the brains and measured the calcium-dependent fluorescence of many VTA neurons simultaneously in the presence of CNO, ethanol, NMDA, or combinations of these. High pass and low pass filters were applied to the mean fluorescence intensity of each region of interest to separate the fast calcium transients from the slow calcium swell instigated by the bath application of CNO. Neurons were categorized based on their response to CNO, including subpopulations whose calcium transient activity was increased, decreased, or unaffected by the presence of CNO. This work suggests that activation of Cx36+ neurons in the VTA may play a modulatory role of mesolimbic circuitry. Additional work is warranted to elucidate the connection between these effects and alcohol reward and/or dependence.

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Poster

PSTR429. Alcohol Induced Changes in Neural Circuits and Neurophysiology

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Topic: G.09. Drugs of Abuse and Addiction

Support: R01 DA035943
R01 AA026306
P50 DA044123

Title: Real-time Dopaminergic Encoding of Reward Consumption in the Prelimbic Cortex

Authors: *L. CASTELL[1], Y. CHENG[1], R. MAGNARD[1], A. DRISSI[1], Z. H. CAMHI[1], P. H. JANAK[2];
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Abstract: The prelimbic cortex (PrL) is implicated in both ethanol seeking and drinking (Khoo et al., 2019; Rinker et al, 2023; Siciliano et al., 2019; Timme et al, 2022). Ethanol stimulates the activity of midbrain dopaminergic neurons, however, the role of PrL dopamine in the control of alcohol drinking is unclear. The aim of this study was to decipher whether changes in dopamine release within the PrL cortex mediate cued reward consumption of an ethanol and/or natural reward within a task that allows separation of the seeking and consummatory phases of behavior. We used *in vivo* fiber photometry with DLight (AAV5-hSyn-dLight1.2) in the PrL cortex in freely-moving male (n=5) and female (n=8) rats to assess dopaminergic activity across task acquisition. Rats received pre-exposure in the home-cage of ethanol 15% (w/v) or water for 4 weeks, followed by daily behavioral training and dopamine signal recording. In each 40 min behavioral session, reward delivery (0.2ml/delivery of either 15% ethanol w/v or 3% sucrose w/v) was contingent upon port entry during an auditory cue presentation (interstimulus interval mean 78+/-45 sec), with a maximum of 21 rewards per session. Dopamine signals were obtained over at least two weeks of task acquisition; after stable acquisition with one reward (ethanol or sucrose), rats received test sessions with alternative rewards (sucrose or water). Bootstrapped confidence intervals (CI 95%, 1000 permutations) were used to determine statistically significant changes from baseline fluorescence as in Jean-Richard-Dit-Bressel et al. (2019). We found a consistent and sustained increase in dopamine during ethanol and sucrose consumption. This increase initiated before rats entered the drinking port and terminated at port exit, and the magnitude of this signal correlated with the relative value of the reinforcer with strongest signals for sucrose, intermediate for ethanol, and weakest for water. Our findings suggest PrL dopamine release scales with reward value. These data complement prior neural activity recordings (Amarante et al, 2014; Horst and Laubach, 2013) showing changes in PrL neuronal activity just prior and during reward intake and implicate dopamine in the modulation of this process.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA Grant AA020501

Title: Neuronal ensemble in the medial orbitofrontal cortex as a brake to excessive bing alcohol drinking.

Authors: *P. GIMENEZ GOMEZ[1], T. LE[1], M. ZINTER[1], S. MOLAS[2], P. M'ANGALE[1], T. FREELS[1], J. POPOOLA[3], T. THOMSON[1], A. TAPPER[1], D. SCHAFER[1], G. MARTIN[1]; [1]Univ. of Massachusetts Chan Med. Sch., Worcester, MA; [2]Univ. of Colorado, Boulder, CO; [3]The Catholic Univ. of America, Washington, DC

Abstract: Alcohol abuse is the seventh leading risk factor for both death and the burden of disease. While several drugs have been approved to treat this disorder, their efficacy remains modest due in part to their lack of specificity. In this study we identified a group of neurons (neuronal ensemble) in the medial prefrontal orbital cortex (mOFC) that shows activity when the mice reach an elevated blood ethanol content (BEC). Also, we have characterized this neuronal ensemble and its implications for alcohol consumption. We are using “Targeted recombination in active populations” (TRAP2) mice (males and females), a mouse line that in presence of 4-hydroxitamoxifen allows permanent genetic access to neurons activated by a specific experience. In TRAP2 mice subjected to the drinking in the dark paradigm of alcohol consumption we performed fiber photometry, single cell RNA-seq, electrophysiology and light-sheet microscopy to understand the characteristics of the neuronal ensemble (cellular type, intrinsic and extrinsic properties, layer location, areas of projection or transcriptional pattern among others) and then optogenetics to manipulate the ensemble and measure the effect of both inhibition and activation in the amount of alcohol consumed. We found that about 5% of mOFC neurons are activated by alcohol as it reaches intoxication level (i.e., BEC higher than 80mg/dL). Moreover, those neurons don’t show activity if the mice don’t reach that level of intoxication Single cell RNAseq shows that the neuronal ensemble is GABAergic and shows a decrease in transcripts related to ion channels. Calcium recording with fiber photometry while the mice drink shows a strong peak of calcium only at the end of the episode of consumption with no response at the beginning of the episode of consumption. Moreover, we show that this response is dependent on alcohol concentration since when they reach an intoxication level at the beginning of consumption (using a pharmacological approach) the neurons show also early activity indicating that those neurons acts in response to intoxication. Finally, using optogenetics we modulated the neuronal ensemble showing that an activation using Channelrhodopsin produces a strong decrease in the consumption while their inhibition using Halorhodopsin renders the opposite effect. Our data demonstrate that binge alcohol drinking recruits a neuronal ensemble with a unique set of properties and whose role is to act as a brake reducing drinking when the mice reach intoxication level.

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Poster

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Program #/Poster #: PSTR430.01/QQ18

Topic: G.09. Drugs of Abuse and Addiction

Title: Assessing behaviors associated with discontinuation of subchronic methamphetamine administration in C57Bl/6J mice using a novel, automated machine learning technology

Authors: *B. MENARCHEK[1,2,3], A. WHITE[1,2,3], C. MELVIN[1], V. SETOLA[1,4,2], D. REYNOLDS[5,6], Z. WRIGHT[6];
[1]Neurosci., [2]Rockefeller Neurosci. Inst., [3]Physiol. and Pharmacol., [4]Behavioral Med. and Psychiatry, [5]Lane Dept. of Computer Sci. and Electrical Engin., West Virginia Univ., Morgantown, WV; [6]SwiftSCIENCE, Pittsburgh, PA

Abstract: Methamphetamine (METH) use is on the rise, and the drug is quickly becoming one of the most commonly misused substances in the United States. The devastating effects of METH appear not only through the short- and long-term physical and psychological damage of the person engaging in misuse, but also from the subsequent addiction elicited from the drug-taking behavior. Given individual and societal harm caused by METH, there is a pressing need to identify features amenable to screening various treatments for the potential to alleviate the unpleasant, relapse-predisposing symptoms associated with discontinuation of chronic methamphetamine. Although the current literature describes neurochemical, neuroanatomical, electrophysiological, and locomotor effects associated with METH discontinuation, these types of measurables are labor-intensive to compare in studies surveying potential pharmacotherapeutics. In that regard, we sought to identify quantifiable behaviors associated with discontinuation of subchronic METH administration to C57BL/6J mice. We hypothesize that such behaviors might represent a useful index for our and others' studies of potential interventions to prevent/treat METH withdrawal symptoms in humans. To identify METH-discontinuation-associated behaviors, mice were treated with METH (2.5 mg/kg) for ten days, after which they were observed under no treatment for seven days. Video footage was collected during both the administration and discontinuation phases, and recordings were scored by blinded experimenters. We found that, during the first day of METH discontinuation, mice that were administered METH showed a significant three-fold increase in digging behavior compared to mice administered saline. Other behaviors identified in this study included a control behavior (or a behavior that was identical between both groups throughout both phases) and two behaviors associated with METH treatment but not affected during the discontinuation phase. In an effort to increase throughput for eventual treatment screening, experimenter-scored videos were used to train a machine learning algorithm to identify digging behavior. In a time-efficient manner, the

machine learning algorithm was able to replicate manual scores with greater than 90% accuracy. The discovery of a behavioral biomarker associated with methamphetamine discontinuation coupled with the ability to apply automated behavioral scoring to this paradigm could offer a new, moderate-to-high-throughput method for screening interventions for potential efficacy in human METH withdrawal.

Disclosures: B. Menarchek: None. **A. White:** None. **C. Melvin:** None. **V. Setola:** None. **D. Reynolds:** None. **Z. Wright:** None.

Poster

PSTR430. Amphetamines, Neural Plasticity, and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR430.02/QQ19

Topic: G.09. Drugs of Abuse and Addiction

Title: Sex differences in compulsive methamphetamine intake and relapse to drug seeking during abstinence

Authors: *A. DAIWILE, J. SUBRAMANIAM, B. LADENHEIM, M. T. MCCOY, M. JOB, J. L. CADET;
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Abstract: Sex differences in compulsive methamphetamine intake and relapse to drug seeking during abstinence Atul P. Daiwile*, Subramaniam Jayanthi, Bruce Ladenheim, Michael T. McCoy, Martin Job, and Jean Lud CadetMolecular Neuropsychiatry Research Branch, NIDA-IRP, Baltimore, MD 21224
Methamphetamine (METH) is an extremely addictive drug that continues to wreak havoc in the lives of individuals who suffer from METH use disorder (MUD). There is evidence for sex differences in patterns of abuse, amounts of METH taken, as well as in relapse rates among METH users who meet DSM criteria for MUD. We are actively investigating the behavioral differences between compulsive and non-compulsive METH self-administration (SA) by rats in the presence of an electric shock barrier. In the present study, we have used female and male Long Evans rats that were trained to self-administer METH (0.1 mg/kg/infusion, IV) on an FR-1 schedule for 22 days using a pattern of three 3-h sessions/day. After 22 days of METH SA, rats continued to self-administer METH in the presence of contingent footshocks (0.18mA to 0.36mA) for more 8 days. After 8 days of contingent punishment, we assessed relapse behaviors on withdrawal day 1 (WD3) and 15 (WD15) from SA. Both female and male rats escalated their METH intake during the first 22 days of SA training. There were significant effects of sex and training days, but their interaction was not significant, indicating that both female and male rats increased their METH intake. Interestingly, male rats took more METH than female rats during the first 10 days and last 7 days of training. Analysis of METH intake for all 22 days revealed only a trend towards significance ($p=0.0601$), with for male rats exhibiting somewhat higher total METH intake then females. Contingent punishment significantly reduced METH taking in

some of the female and male rats (shock-sensitive, SS; non-compulsive) but not in others (shock-resistant, SR; non-compulsive). Moreover, SR rats displayed a significant effect of training day (statistics), but a trend towards significance for sex (p=0.0772) and its interaction (p=0.0806). Furthermore, 72% (13/18) of males while 53% (9/17) of females showed compulsive METH intake in the presence of adverse consequences. Non-compulsive female rats were more prone to relapse during forced abstinence in comparison to non-compulsive male rats. In contrast, both compulsive female and male rats showed similar METH craving behavior during abstinence. These observations indicate that non-compulsive females that take less METH than males might be more prone to relapse to METH taking behaviors. **Acknowledgement:** This work is supported by DHHS/ NIH/ NIDA-IRP.

Disclosures: A. Daiwile: None. **J. Subramaniam:** None. **B. Ladenheim:** None. **M.T. McCoy:** None. **M. Job:** None. **J.L. Cadet:** None.

Poster

PSTR430. Amphetamines, Neural Plasticity, and Behavior

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Program #/Poster #: PSTR430.03/QQ20

Topic: G.09. 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: Modeling methamphetamine use disorder and relapse in animals: short- and long-term, transcriptional consequences in the brain

Authors: *M. MCCOY, K. ELHADI, A. P. DAIWILE, J. L. CADET;
DHHS/NIH/NIDA/IRP, Baltimore, MD

Abstract: Methamphetamine (METH) use disorder (MUD) is a chronic neuropsychiatric disorder characterized by binge drug taking episodes, intervals of abstinence, and relapses to the use of the drug even while in treatment. MUD has been modeled in rodents in order to identify the molecular bases for persistent drug taking behaviors. These experiments promise to shed some light on potential neurobiological substrates of METH addiction, with the hope of developing more target-driven pharmacological interventions. In present study, we trained male Sprague-Dawley rats to self-administered METH (0.1 mg/kg/injection, i.v.) while control rats received saline infusions on an FR-1 schedule for 8 days [15-hours (hrs) sessions/day]. After the last METH SA session, rats were euthanized at 2 hrs, 24 hrs, or 1 month after cessation of drug exposure. Dorsal striata were dissected and used in microarray analysis. Rats escalated their METH intake over time. The microarray data were analyzed using **D**atabase for **A**nnotation, **V**isualization and **I**ntegrated **D**iscovery (DAVID); Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways; and Sankey analyses. At the 2-hr time point, calcium, cAMP, and Ras

signaling pathways were significantly enriched. At the 24-hr time point, there was significant enrichment of genes involved in infectious diseases and immune responses. Interestingly, most genes showed decreased mRNA expression at the 1-month withdrawal time point. At that time, differentially expressed genes belong to cell differentiation and development processes. Cluster analysis revealed that genes upregulated at early time points tended to be down-regulated later on and vice versa. These observations suggest potential compensatory mechanisms at the later time point. Elucidation of the molecular machinery that form the substrates of cue-induced relapses that tend to be more prominent at later time points will provide better molecular targets for both pharmaceutical interventions for individuals who suffer from MUD.**Acknowledgement:** This work is supported Department of Health and Human Services/ National Institutes of Health/ National Institute on Drug Abuse/ Intramural Research Program.

Disclosures: **M. McCoy:** None. **K. Elhadi:** None. **A.P. Daiwile:** None. **J.L. Cadet:** None.

Poster

PSTR430. Amphetamines, Neural Plasticity, and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR430.04/QQ21

Topic: G.09. Drugs of Abuse and Addiction

Support: American Foundation for Pharmaceutical Education
University of Utah Health Sciences
University of Utah College of Pharmacy

Title: The effects of bupropion on Arc mRNA expression in mice with methamphetamine-induced neurotoxicity

Authors: J. EARL[1,2], A. S. GIBSON[1,3], *K. KEEFE[1];
[1]Univ. of Utah, Salt Lake City, UT; [2]The Comparative Hlth. Outcomes, Policy, and Econ. (CHOICE) Inst., Univ. of Washington, Seattle, WA; [3]Neurosci., Westminster Col., Salt Lake City, UT

Abstract: Studies in humans and in animal models suggest that exposure to methamphetamine (METH) leads to decreased striatal dopamine. Such toxicity is associated with decreased amplitude of phasic dopamine signals and alterations in gene expression in striatal efferent neurons, such as Arc. These long-term effects of METH may negatively affect striatally based learning and memory, contributing to a low probability of behavioral recovery. Bupropion (Bup), a dopamine transporter inhibitor, is theorized to increase phasic dopamine signaling and, consequently, restore Arc expression in striatum. The purpose of this study was to provide a preliminary test this hypothesis by determining whether acute treatment with Bup restores Arc expression in striata of mice with METH-induced toxicity. Adult male C57BL/6J mice (n=4/group) were pretreated with *d,l*-METH•HCl (4x10 mg/kg free base) or saline (Sal). Approximately 3 weeks later mice were randomized to receive and were acutely treated with Sal

or Bup•HCl (50 mg/kg free base) and then placed in a novel environment for 30 min. Mice were then sacrificed, and brains harvested, sectioned, and processed for Arc and preproenkephalin (ppe) mRNAs using RNAScope™. Digitized images were captured and analyzed to determine the numbers of Arc+/ppe+ (*i.e.*, D2-MSNs) and Arc+/ppe-negative (*i.e.*, D1-MSNs) cells in a 0.5x0.5 mm field. The numbers of cells were compared across groups via ANOVA. Statistical significance was set at $p \leq 0.05$. All RNAscope assays, imaging, and data analyses were conducted with the experimenter blinded to the treatment group of the animals. Acute treatment with Bup increased the number of Arc+ D1-MSNs. There was a significant main effect of treatment group, as well as a significant group x cellular phenotype interaction. *Post-hoc* analysis revealed significantly more Arc+ D1-MSNs in both the Sal-pretreated and METH-pretreated mice treated with Bup. Although the number of Arc+ D1-MSNs was significantly less in the METH-pretreated mice treated with Bup vs. the Sal-pretreated mice given Bup, the number of Arc+ D1-MSNs was similar to those in the Sal-pretreated mice acutely treated with Sal. Post hoc analysis revealed no effects of treatment group on the number of Arc+ D2-MSNs. These preliminary data suggest that acute treatment with bupropion restores Arc mRNA expression in D1-MSNs of mice with METH-induced neurotoxicity. Given the critical role of Arc in brain plasticity, the findings suggest that bupropion may be an option for improving striatally-based learning and memory processes necessary for optimal response to behavioral treatments for METH-use disorders.

Disclosures: J. Earl: None. **A.S. Gibson:** None. **K. Keefe:** None.

Poster

PSTR430. Amphetamines, Neural Plasticity, and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR430.05/QQ22

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA038058-07A1S1
UAB Center for Addiction and Pain Prevention and Intervention Pilot Award

Title: Mechanistic insight into microbial regulation of psychostimulant abuse

Authors: S. J. MABRY[1], X. CAO[2], S. PATEL[1], T. ROMANAZZI[3], Y. ZHU[1], D. P. SALEEBY[1], C. ROWE[1], A. ELAM[1], H.-T. LEE[1], H. WU[2], A. GALLI[1], ***A. M. CARTER**[1]; [1]Univ. of Alabama, Birmingham, Birmingham, AL; [2]Oregon Hlth. and Sci. Univ., Portland, OR; [3]Univ. of Insubria, Varese, Italy

Abstract: Amphetamines (AMPHs) are highly effective psychostimulants commonly used for the treatment of neuropsychiatric disorders and are abused with devastating outcomes. The stimulant properties and abuse potential of AMPHs have been associated with their ability to increase extracellular dopamine (DA) levels. This increase is mediated, at least in part, by the

reversal of DA transporter (DAT) function, which causes non-vesicular DA release, here defined as DA efflux. Recent studies suggest that imbalances in the gut microbiome (dysbiosis) participate in the pathogenesis of substance use disorders. Microbial products such as short-chain fatty acids (SCFAs), are suspected to play a fundamental role in this process. Among SCFAs, butyrate is known to cross the blood-brain barrier and directly act on neurons and glial cells. *Fusobacterium nucleatum* (*F. nucleatum*) is a bacterial species that secretes butyrate and whose abundance is increased by AMPH abuse in both rodents and humans. It is important to note that butyrate is a potent inhibitor of histone deacetylases (HDACs) and that inhibition of HDACs robustly increases expression of both DAT mRNA and protein levels in cultured cells. Changes in DAT expression regulates both AMPH-induced DA efflux and psychomotor actions. Here, we report that colonization of the intestinal tract of gnotobiotic *Drosophila* with *F. nucleatum* significantly enhances AMPH-induced DA efflux and associated behaviors such as locomotion, courting, and preference. This potentiation of AMPH actions by *F. nucleatum* was paralleled by oral administration of butyrate. In contrast, acetate and propionate, other SCFAs secreted by *F. nucleatum*, failed to enhance both AMPH-induced DA efflux and locomotion. Further, inhibition of HDACs using Trichostatin A, as well as genetic knockdown of HDAC1 in DA neurons *via* RNAi, both increased AMPH-induced DA efflux and locomotion. Importantly, both *F. nucleatum* colonization, as well as direct butyrate administration, also lead to a significant increase in DAT expression. These data provide evidence that *F. nucleatum* modulates AMPH-induced behaviors through secretion of butyrate, inhibition of HDACs, elevation of DAT expression, and increased DA efflux. These findings suggest modulation of the gut microbiome as a therapeutic approach for the treatment of substance use disorders.

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Poster

PSTR430. Amphetamines, Neural Plasticity, and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR430.06/QQ23

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant P20GM130461

Title: Activation of Melanin Concentrating Hormone Neurons in the Lateral Hypothalamus alter the expression of methamphetamine intravenous self-administration

Authors: *M. SUAREZ, H. SCHNEIDER, I. R. K. KUEBLER, K. T. WAKABAYASHI; Psychology, Univ. of Nebraska-Lincoln, Lincoln, NE

Abstract: Sensitivity to the reinforcing effects of methamphetamine (METH) can influence the motivation to work for the drug, or its associated cues; this in turn can contribute to the

development of METH use disorder (MUD). Several brain areas play a role in regulating METH associated behaviors. The lateral hypothalamus (LH) is a region that interfaces with mesolimbic neural circuitry associated with reward and MUD. Within the LH, melanin-concentrating hormone (MCH) neurons, mainly studied in the context of sleep wakefulness and energy homeostasis, have been shown to impact METH psychomotor sensitization. These and other findings suggest that LH MCH neurons regulate METH seeking and taking behavior, but relatively little is known about how the activity of these neurons influence METH self-administration behavior. In this study, male and female Wistar rats were trained to intravenously self-administer METH (0.05 mg/kg/inf) via nose pokes for 10 days. After this, a dose-response curve was generated by allowing rats to self-administer one of five doses (0.0625, 0.0125, 0.025, 0.05, or 0.1 mg/kg/inf, presented in a Latin-Square design) every other day. Using a combinatorial viral approach, we targeted activating designer receptors exclusively activated by designer drugs (DREADDs) to MCH-positive LH neurons. On test days, 30 minutes before self-administration sessions, rats received a saline vehicle control or clozapine-n-oxide (CNO, 0.3 or 1 mg/kg) in order to activate MCH neurons. Male rats readily self-administered METH and a dose response curve was generated where the lowest dose resulted in the most nose pokes, and the highest dose resulted in the fewest. Stable responding to the training dose was seen in between dose response days. In male rats, CNO administration of 0.3 mg/kg selectively increased infusions of METH, with the greatest effects on the two lowest doses. Conversely, CNO administration of 1 mg/kg reduced METH infusions, flattening the dose response function. Results for female rats and associated sex differences will be discussed at the conference. These finding suggest a nuanced behavioral interaction between LH MCH neuron activity and the reinforcing effects of self-administered METH, which may have further implications for MUD in humans.

Disclosures: M. Suarez: None. **H. Schneider:** None. **I.R.K. Kuebler:** None. **K.T. Wakabayashi:** None.

Poster

PSTR430. Amphetamines, Neural Plasticity, and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA042110

Title: Reduced polysubstance self-administration and stress-induced reinstatement following systemic or central amygdala hypocretin-receptor blockade in rats

Authors: *T. A. ZARIN, B. E. SCHMEICHEL;
Biomed. Sci., East Tennessee State Univ., Johnson City, TN

Abstract: The hypocretin/orexin (HCRT) system is associated with compulsive stimulant and opioid use, involving both HCRT-receptor 1 (-R1) and HCRT-receptor 2 (-R2). Few studies, however, have examined the role of combined HCRT-R1/2 on compulsive drug taking behavior, and fewer have examined the role of HCRT in drug-taking behavior involving a combined methamphetamine and fentanyl polysubstance. In this study, we examined the effects of systemic and intracranial HCRT-R1/2 antagonism on compulsive polysubstance intravenous self-administration and stress-induced reinstatement, as modeled by escalated intake in rats allowed extended access to polysubstance. Male and female adult Wistar rats (N=40) were allowed either short (1h; ShA; n=8 male) or long (6h; LgA; n=16 male, n=16 female) access to polysubstance intravenous self-administration (fixed ratio 1) for 12 sessions. Rats in the LgA group significantly escalated their drug-taking over the 12 sessions ($p<.01$), and were taking significantly higher amounts of polysubstance during the first hour compared to the ShA group ($p<.01$). After escalation, a subset of LgA males and females were systemically administered a HCRT-R1/2 antagonist (suvorexant; SUV, 0 and 30mg/kg) 30 min prior to polysubstance self-administration testing. Systemic SUV administration elicited significant attenuation of polysubstance-taking in both the first hour ($p<.01$) and full 6h ($p<.05$) session for male and female LgA rats. Brains from drug-naïve, ShA and LgA (n=8/group) male rats were collected 1 day after the last self-administration session, and assessed for HCRT-1, HCRT-R1 and -R2 optical density using immunohistochemical assays. LgA rats had a significantly higher density of HCRT-1 peptide in the central amygdala (CeA) when compared to naïve rats ($p<.05$), and higher HCRT-R1 compared to both ShA and naïve rats ($p<.01$). There was no change in HCRT-R2 optical density across groups. A final subset rats extinguished their drug-seeking behavior via substitution of saline for polysubstance during self-administration. Once lever pressing was extinguished, the rats were tested for yohimbine-induced (2mg/kg) reinstatement of drug-seeking behavior. Stress-induced reinstatement was significantly attenuated in both males and females with systemic SUV administration ($p<.01$), and in females following intracerebral injections of SUV into the CeA (9 µg/hem; $p<.01$). Combined, these results suggest HCRT neurotransmission at both HCRT-R1 and -R2, particularly within the CeA, likely contributes to polysubstance-taking and stress-induced polysubstance-seeking behavior.

Disclosures: T.A. Zarin: None. **B.E. Schmeichel:** None.

Poster

PSTR430. Amphetamines, Neural Plasticity, and Behavior

Location: WCC Halls A-C

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Topic: G.09. Drugs of Abuse and Addiction

Support: AMC21 Scholarship (SJM) NIH 5T32NS061788-12
(SJM) NIMH F31 MH114316-01
NIH TR32GM007628-38/39 (JIA)
NIH grant NIH DA035263 (AG and HM)

Title: Syntaxin 1 Ser^{14} phosphorylation is required for nonvesicular dopamine release via the dopamine transporter

Authors: S. J. MABRY[1], M. H. CHENG[2], ***Y. ZHU**[4], J. AGUILAR[5], S. PATEL[4], D. ZANELLA[6], D. SALEEBY[4], T. ROMANAZZI[8], H. J. MATTHIES[9], A. CARTER[7], I. BAHAR[3], A. GALLI[10];
[1]Univ. of Alabama Birmingham, Univ. of Alabama Birmingham, Birmingham, AL; [3]Univ. of Pittsburgh, [2]Univ. of Pittsburgh, Pittsburgh, PA; [4]Univ. of Alabama at Birmingham, Birmingham, AL; [5]Vandebilt Univ., Vandebilt Univ., Nashville, TN; [6]Private Residence, [7]Univ. of Alabama, Birmingham, Univ. of Alabama, Birmingham, Birmingham, AL; [8]Univ. of Insubria, Varese, Italy; [9]Univ. of Alabama at Birmingham, Vanderbilt Univ., birmingham, AL; [10]UAB, UAB, Brentwood, TN

Abstract: Amphetamine (AMPH) is a commonly abused psychostimulant. The stimulant properties of AMPH are associated with its ability to increase dopamine (DA) neurotransmission via nonvesicular DA release, which is mediated by reversal of DA transporter (DAT) function. Syntaxin 1 (Stx1) is a SNARE protein that is phosphorylated at Ser^{14} by casein kinase II. We show that Stx1 phosphorylation is critical for AMPH-induced nonvesicular DA release and regulates the expression of AMPH-induced preference and sexual motivation in *Drosophila melanogaster*. Our molecular dynamics simulations of the DAT/Stx1 complex demonstrate that phosphorylation of these proteins is pivotal for DAT to dwell in a DA releasing state. This state is characterized by the breakdown of two key salt bridges within the DAT intracellular gate, K66-D345 and E428-R445, causing the opening and hydration of the DAT intracellular vestibule, allowing DA to bind from the cytosol, a mechanism that we hypothesize underlies nonvesicular DA release.

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Poster

PSTR430. Amphetamines, Neural Plasticity, and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR430.09/QQ26

Topic: G.09. Drugs of Abuse and Addiction

Title: A locomotor activity sign of amphetamine acute withdrawal is attenuated by kappa opioid receptor antagonist in rats

Authors: *W. WHITE, I. WHITE;
Morehead State Univ., Morehead, KY

Abstract: When rats are given 2.0 mg/kg amphetamine near light onset of a 12-12 hour light-dark cycle, hyperactivity, indicative of an acute state, occurs during the first four hours following

treatment, and reduced activity, indicative of an acute withdrawal, occurs from around 13 to 24 hours post-treatment. Recently, kappa opioid antagonists, such as nor-BNI, have been shown to attenuate withdrawal-related signs of stress, anxiety, and depression produced by chronically administered drugs of abuse. nor-BNI is a long-acting selective kappa opioid receptor antagonist. It is often administered 24-hours prior to a target manipulation, and the effects of a single nor-BNI treatment can persist for weeks. This study examined the capacity of nor-BNI to block acute withdrawal from amphetamine. Adult male Wistar rats were given a series of six one-week tests. On Day 1 of a test, animals were given a control treatment, and on Day 4, they were given a drug treatment. Distance moved in an open field was monitored for 24 hours following each treatment. During Tests 1-3, Day 4 drug treatments were: 2.0 mg/kg amphetamine alone ; 2.0 mg/kg amphetamine followed 15 minutes later by the non-selective opioid receptor antagonist naloxone (0.1 mg/kg); and 2.0 mg/kg amphetamine preceded 24 hours earlier by nor-BNI (7.5 mg/kg). During Tests 4-6, Day 4 drug treatment was 2.0 mg/kg amphetamine, so that the effect of nor-BNI on acute withdrawal could be assessed 1 week (Test 4), 2 weeks (Test 5), and 3 weeks (Test 6) following nor-BNI administration. Amphetamine alone (Test 1) produced hyperactivity 1-4 hours following treatment and reduced activity 13-24 hours following treatment. Neither naloxone (Test 2) nor nor-BNI (Test 3) appeared to alter acute amphetamine hyperactivity, but they both prevented the reduction in activity from hours 13-24, that is, they prevented acute withdrawal as assessed with a locomotor activity measure. Acute withdrawal to amphetamine also did not occur 1 week after nor-BNI (Test 4), but it began to reemerge 2 weeks following nor-BNI (Test 5). Withdrawal from both intermittently and chronically used drug appears to involve kappa opioid receptor activation. Careful assessment of determinants of acute withdrawal may disclose factors that promote the transition from intermittent recreational drug use to chronic drug abuse.

Disclosures: W. White: None. **I. White:** None.

Poster

PSTR430. Amphetamines, Neural Plasticity, and Behavior

Location: WCC Halls A-C

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Program #/Poster #: PSTR430.10/QQ27

Topic: G.09. Drugs of Abuse and Addiction

Support: 1-437-5169

Title: A comparison of the neurobehavioral responses of rats exposed to original and counterfeit Captagon

Authors: *Y. ALTHOBAITI, A. ALSHANBARI, D. ALSHOAIBI, E. KHAN, H. ALGHAMDI, S. ALJUAID, A. ALZILIFI, N. ALSULIMANI, O. ALRIZQI, W. ALSANIE, A. GABER;
Taif Univ., Taif, Saudi Arabia

Abstract: A common drug of abuse in the Middle East is captagon. Captagon contains the active ingredient fenethylline, which has two major active metabolites: amphetamine and theophylline. Due to the lake and difficulty of obtaining the original Captagon (FEN), counterfeit Captagon (CC) has emerged. In addition to theophylline and amphetamine, CC contains paracetamol, caffeine, diphenhydramine, and lidocaine. The neurobehavioral effects of FEN and CC have never been compared previously. Therefore, the aim of this study was to investigate the neurobehavioral reactions of rats that were exposed to different doses of FEN and CC. A total of 36 male Sprague Dawley rats were randomly assigned to receive the vehicle, FEN (50 or 100 mg/kg), or CC (50 or 100 mg/kg). Body temperature was monitored, and locomotor activity was assessed following the treatment. Prefrontal cortex samples were extracted to assess the gene expression of antioxidant enzymes. Interestingly, CC as compared to the FEN caused more increases in body temperature posing more risks of fatal hyperthermia. Moreover, this new Captagon induced a significant increase in locomotor activity as compared to the original one. These behavioral changes were linked to increases in oxidative stress in the CC group due to modified gene expression of the antioxidant enzymes in the prefrontal cortex. These findings are the first to demonstrate the potential health harm caused by the new Captagon as compared to the original one. More efforts should be made to educate our society about the danger of these new drugs and to control their use.

Disclosures: Y. Althobaiti: None. **A. Alshanbari:** None. **D. Alshoaibi:** None. **E. Khan:** None. **H. Alghamdi:** None. **S. Aljuaid:** None. **A. Alzilifi:** None. **N. alsulimani:** None. **O. Alrizqi:** None. **W. Alsanie:** None. **A. Gaber:** None.

Poster

PSTR430. Amphetamines, Neural Plasticity, and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR430.11/QQ28

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA009621

Title: Cell-type and pathway specific synaptic adaptations in the rat nucleus accumbens core after incubation of methamphetamine craving

Authors: E.-K. HWANG, M. M. BEUTLER, ***M. E. WOLF**;
Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Use of methamphetamine (meth) is highly prevalent and the high rate of relapse even after prolonged abstinence is a major problem in treating meth use disorder. In a rat model of drug craving and relapse, cue-induced drug seeking progressively intensifies after withdrawal from drug self-administration (SA) (incubation of craving), which is associated with long-term synaptic adaptations of glutamatergic transmission in nucleus accumbens core (NAcc) medium spiny neurons (MSN). Our lab previously demonstrated that incubation of meth craving is

associated with synaptic calcium-permeable AMPA receptor (CP-AMPAR) upregulation in the NAcc and that CP-AMPAR activation is required for expression of incubated meth craving (Scheyer et al., 2016). However, it is not known whether meth incubation is associated with cell-type specific upregulation of CP-AMPAR in MSN that express either the dopamine D1 or D2 receptor and which specific glutamatergic inputs are involved in CP-AMPAR-mediated synaptic alterations in NAcc. Here, we examined CP-AMPAR plasticity during meth incubation in a cell-type and pathway-specific manner in the NAcc. Male and female rats self-administered saline or meth under extended access conditions (6 h/day for 10 days; 0.1 mg/kg/infusion; infusions paired with light cue). We measured cue-induced meth seeking after 1 and 21 days of abstinence. On withdrawal day (WD) 25-50, whole-cell patch clamp recordings were performed to characterize AMPAR-mediated synaptic transmission by measuring the rectification index and NASPM (CP-AMPAR selective antagonist) sensitivity. To enable identification of MSN subtypes, we use D1-Cre or A2a-Cre rats (the A2aR colocalizes with the D2R) crossed with ZsGreen or TdTomato reporter rats. During seeking tests, significantly higher cue-induced seeking was observed on WD21 than on WD1. We found upregulation of CP-AMPAR in D1+ but not D1- or A2a+ MSN after withdrawal from meth SA compared to saline controls. Furthermore, combining electrophysiology and optogenetics, we examined input-specific plasticity in the NAcc synapses projecting from the medial prefrontal cortex (mPFC), basolateral amygdala (BLA) and paraventricular thalamus (PVT). We found potentiated glutamatergic synaptic transmission through upregulation of CP-AMPAR in mPFC and PVT to NAcc pathways, but not the BLA to NAcc pathway. Experiments combining pathway and cell-type specific approaches are in progress. Our results are the first to demonstrate plasticity of specific excitatory pathways to NAc MSN after incubation of meth craving.

Disclosures: E. Hwang: None. **M.M. Beutler:** None. **M.E. Wolf:** None.

Poster

PSTR430. Amphetamines, Neural Plasticity, and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR430.12/RR1

Topic: G.09. Drugs of Abuse and Addiction

Title: Role of histone deacetylase 5 in nucleus accumbens in incubation of methamphetamine craving

Authors: *M. BURKE[1], A. OLANIRAN[2], X. LI[1];
[2]Psychology, [1]Univ. of Maryland, College Park, MD

Abstract: Methamphetamine seeking progressively increases after abstinence in both rats and humans. We previously showed that overexpressing histone deacetylase 5 (HDAC5) in rat dorsal striatum potentiates this incubation of methamphetamine craving, while knocking down HDAC5 suppresses it. Together these results demonstrated a critical role of HDAC5 in rat dorsal striatum in incubation of methamphetamine craving. However, whether the role of HDAC5 in this

incubation generalizes to nucleus accumbens (NAc) is unknown. Anatomically, NAc is further divided into two subregions, core and shell. Based on previous literature showing that the NAc core and shell play distinct roles in drug relapse behaviors, here we aimed to examine the role of HDAC5 in incubation of methamphetamine craving in NAc core and shell, respectively. We first injected adeno-associated viruses (AAVs) expressing either a nuclear-localized HDAC5 (AAV-HDAC5) or GFP (control, AAV-GFP) bilaterally into NAc shell. One week later, we trained both groups of rats to self-administer methamphetamine for 10 days (0.1 mg/kg/infusion, 9 h/d). After the training, we tested all rats for methamphetamine seeking on both abstinence days 2 and 35. We found that overexpressing HDAC5 in NAc shell had no effect on methamphetamine seeking on either abstinence day 2 or 35, suggesting that HDAC5 in NAc shell does not play a role in incubation of methamphetamine craving. Our ongoing study is examining whether overexpressing HDAC5 in NAc core impacts incubation of methamphetamine craving. Together, these results may reveal a sub-region-specific role of HDAC5 in NAc in mediating methamphetamine relapse.

Disclosures: **M. Burke:** None. **A. Olaniran:** None. **X. Li:** None.

Poster

PSTR430. Amphetamines, Neural Plasticity, and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR430.13/RR2

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01 DA033641

Title: Deep brain stimulation of nucleus accumbens shell does not alter methamphetamine seeking in male rats

Authors: *T. J. SACKO, S. E. SWINFORD-JACKSON, R. C. PIERCE;
Brain Hlth. Inst. and Dept. of Psychiatry, Rutgers Robert Wood Johnson Med. Sch., Piscataway, NJ

Abstract: Methamphetamine use disorder is a chronic, relapsing condition for which there are currently no available FDA-approved treatments. Deep brain stimulation (DBS) is a well-established surgical intervention approved for Parkinson's Disease and other neurological conditions which is also under exploration as a possible treatment for neuropsychiatric disorders including depression and substance use disorders. Relapse, a major obstacle to recovery, is often precipitated by craving which can be modeled as drug seeking in rodents. DBS of the nucleus accumbens shell reduces cocaine-seeking behavior in rats; however, its effectiveness for methamphetamine is not well understood. Here, we examined the effect of deep brain stimulation of the nucleus accumbens shell on methamphetamine priming-induced reinstatement of drug-seeking behavior in male Sprague Dawley rats. Rats were allowed to self-administer intravenous methamphetamine (0.1 mg/kg/infusion) for 2-hours daily over 21 days, followed by

an extinction phase where lever pressing was extinguished by replacing methamphetamine with saline. Methamphetamine seeking was reinstated by a priming injection of methamphetamine (1.0 mg/kg, i.p.) administered 15 minutes prior to the session. Immediately after the rat was placed in the operant chamber, DBS was administered bilaterally to the nucleus accumbens shell through bipolar electrodes. Biphasic symmetrical pulses were delivered at a frequency of 160 Hz and a current intensity of 150 µA throughout the entire 2-hour reinstatement session. Rats received sham (0 µA) and stimulation (150 µA) in a counterbalanced within-subjects design. DBS of the nucleus accumbens shell had no significant effect on methamphetamine seeking behavior in male rats, contrary to its effect on cocaine reinstatement. Future experiments could explore the effect of DBS delivered using different parameters or in other limbic regions, and should investigate the effect of DBS on methamphetamine seeking in female rats.

Disclosures: T.J. Sacko: None. **S.E. Swinford-Jackson:** None. **R.C. Pierce:** None.

Poster

PSTR430. Amphetamines, Neural Plasticity, and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR430.14/RR3

Topic: G.09. Drugs of Abuse and Addiction

Support: MEXT KAKENHI 22K10612

Title: Role of TRUSS in the development of methamphetamine-induced rewarding effect

Authors: *K. MIZUO, T. YAGMAGUCHI, S. HATTORI, S. WATANABE;
Sapporo Med. Univ., Sapporo, Japan

Abstract: Methamphetamine is one of the most abused drugs in Japan and produces a strong rewarding effect. However, little is known about the mechanisms underlying methamphetamine-induced rewarding effect. Our recent findings suggested that tumor necrosis factor receptor-associated ubiquitous scaffolding and signaling protein (TRUSS) may play role in the development of methamphetamine-induced rewarding effect. In the present study, we investigated the expression of TRUSS in methamphetamine-induced rewarding effect. The rewarding effect was evaluated by conditioned place preference. Methamphetamine (1mg/kg, s.c.) produce a significant rewarding effect. The mice were killed by decapitation and the limbic forebrain (containing nucleus accumbens) was dissected. The expression of TRUSS in limbic forebrain was significantly decreased in methamphetamine-induced rewarding effect. We next investigated the role of TRUSS in the development of methamphetamine-induced rewarding effect. Methamphetamine-induced rewarding effect was significantly enhanced by i.c.v. treatment of TRUSS antibody. In conclusion, our findings suggest that the TRUSS may be a negative regulator for methamphetamine-induced rewarding effect.

Disclosures: K. Mizuo: None. **T. Yagmaguchi:** None. **S. Hattori:** None. **S. Watanabe:** None.

Poster

PSTR430. Amphetamines, Neural Plasticity, and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR430.15/RR4

Topic: G.09. Drugs of Abuse and Addiction

Title: Sex differences in the effect of exercise on the cognitive consequences of methamphetamine abuse

Authors: *I. DE LEON, *I. DE LEON, K. DE LEON, J. PELLETIER, N. CABRERA, S. GALEANO, A. FRICKS-GLEASON;
Psychology and Neurosci., Regis Univ., Denver, CO

Abstract: Methamphetamine (METH) is a widely used psychostimulant drug, and its use in the United States has reached a near-epidemic in the past 15 years, due to the ease with which METH can be manufactured, as well its highly addictive properties. METH use costs the government billions per year through crime, foster care, lost workplace productivity, and other social problems, in addition to causing destructive effects in the lives of users. In humans, METH abuse has been shown to result in long-lasting brain injury as well as significant cognitive impairments. METH interacts with the catecholamine nerve terminals in the brain, inducing non-exocytotic transmitter release, which results in the initial euphoria after taking the drug but then leads to long-lasting brain injury for the user. The neurotoxic effects of the drug are responsible for inducing the cognitive consequences associated with abuse, which include impairments in memory, attention, executive functioning, and decision making skills. The memory impairments caused by METH are seen as the most prominent and persistent cognitive problems, because they interfere with the user's ability to adhere to and benefit from addiction treatment. Therefore, it is of utmost importance to find ways to attenuate these cognitive deficits and thereby improve treatment outcomes for METH users. The impacts of exercise on the rodent brain, such as induction of synaptic plasticity, increased production of neurotrophins, and enhanced neurogenesis, have been extensively characterized, and the beneficial effects of exercise on cognition are well-documented. Here we investigated whether exercise can improve performance on cognitive tasks known to exhibit METH-induced deficits. The studies described herein focused on two well-validated tests of memory in an animal model: object recognition and odor recognition. Notably, work on this question has historically left out a critical portion of the population - females. Women are just as likely as men to develop substance use disorder, but women often use and respond to drugs differently. Research in both humans and animals suggests that women may be more vulnerable to the reinforcing effects of stimulants, potentially making them more susceptible to craving and relapse. Here we demonstrate critical sex differences in the ability for exercise to attenuate METH-induced cognitive deficits, as evidenced by variability in scores on object-in-place and odor recognition tasks.

Disclosures: I. De Leon: None. **I. De Leon:** None. **K. De Leon:** None. **J. Pelletier:** None. **N. Cabrera:** None. **S. Galeano:** None. **A. Fricks-Gleason:** None.

Poster

PSTR430. Amphetamines, Neural Plasticity, and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR430.16/RR5

Topic: G.09. Drugs of Abuse and Addiction

Title: Sex differences in the ability of exercise to attenuate methamphetamine-induced monoaminergic neurotoxicity

Authors: *M. GOLDSCHMIDT[1], J. PELLETIER[2], I. DE LEON[3], K. A. DE LEON[3], L. J. HERRERA[3], A. A. FINEBERG[3], E. SAURINI[3], Y. K. VILLAGOMEZ[3], A. FRICKS-GLEASON[3];
[1]Regis Univ. Neurosci. Program, Denver, CO; [2]Neurosci., Regis Univ. Neurosci. Program, Westminster, CO; [3]Regis Univ., Denver, CO

Abstract: Methamphetamine (METH) use continues to be a major public health concern. Upwards of 14.7 million people in the U.S. report having tried METH. The use of METH is highly problematic, not only due to the acute effects of the drug which can include psychosis and aggressive behavior, but also due to the long-term consequences including neurotoxicity, cognitive deficits, and addiction. METH-induced monoaminergic neurotoxicity has been modeled in numerous species. One well-known rodent model of METH use utilizes binge administration, where repeated doses of METH are given in a single day. This dosing regimen has been shown to cause long-lasting damage to monoaminergic nerve terminals in the striatum and prefrontal cortex similar to that seen in human METH users. In fact, individuals who use METH are more likely to develop Parkinson's disease, suggesting enduring and possibly progressive dopamine loss as a consequence of METH use. Exercise is well known for its beneficial physiological effects and cognition-enhancing properties and has long been investigated in the context of neurodegenerative disease; only recently has exercise gained traction in the treatment of drug use and addiction. Here we demonstrate a critical sex difference in the ability of exercise to attenuate METH-induced neurotoxicity. Previously, we've shown that 3 weeks of voluntary running after a METH binge protects against METH-induced dopaminergic neurotoxicity. Critically, delaying the start of exercise for 7 or 30 days also results in attenuated neurotoxicity, suggesting that post-METH exercise isn't simply disrupting the mechanisms that lead to neurotoxicity, but is reversing the neurotoxic effects post-hoc. While METH-induced neurotoxicity has been modeled in many species, these studies have largely excluded female subjects. The goal of this project was to replicate our previous work in females. Female Sprague Dawley rats were dosed with a neurotoxic regimen of (+)-METH-HCl (4 x 3 mg/kg, s.c. at 2-hr intervals) or saline (4 x 1 ml/kg, s.c. at 2-hr intervals). Beginning 1, 7, or 30 days after injections, animals were then subdivided into one of two exercise conditions, voluntary exercise (rats were given continuous access to a running wheel for 3 weeks) or sedentary control (rats were housed with a locked wheel for the same duration). Our results demonstrate critical sex differences in the ability for exercise to attenuate METH-induced neurotoxicity, as evidenced by variability in degree of damage to dopaminergic nerve terminals.

These results highlight the necessity of including sex as a biological variable in methamphetamine neurotoxicity studies going forward.

Disclosures: M. Goldschmidt: None. **J. Pelletier:** None. **I. De Leon:** None. **K.A. De Leon:** None. **L.J. Herrera:** None. **A.A. Fineberg:** None. **E. Saurini:** None. **Y.K. Villagomez:** None. **A. Fricks-Gleason:** None.

Poster

PSTR430. Amphetamines, Neural Plasticity, and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR430.17/RR6

Topic: G.09. Drugs of Abuse and Addiction

Title: The role of neurotrophic factors in the ability of exercise to facilitate extinction of methamphetamine conditioned place preference and attenuation of drug-primed reinstatement.

Authors: *G. AIMALE, H. SIMPSON, E. SAURINI, S. GALEANO, A. FRICKS-GLEASON; Psychology and Neurosci., Regis Univ., Denver, CO

Abstract: Over 60 million people worldwide report using amphetamine-type stimulants, especially methamphetamine (METH), and use is endemic in the western United States. METH use is particularly problematic due to the well-documented long-term consequences on the structure and function of the brain. Exposure to multiple high doses of METH produces damage to central monamine systems. Long-lasting decreases in markers of dopamine (DA) innervation of the striatum have been reported in both human METH users and rodent models of binge METH use. Importantly, this METH-induced damage is accompanied by impairments in cognition in both humans and rodent models. These cognitive deficits include impairments in memory, decision making, and executive function and significantly impact the ability of METH users to engage in and ultimately benefit from treatment. Reported relapse rates for METH users are as high as 61% during the first year post-treatment and 25% during the 2-5 years post-treatment. Conditioned place preference (CPP) is a widely used paradigm used to study the impact of cue and stress triggers on relapse to drug seeking behavior. While exercise has been reported to attenuate the rewarding effects of drugs of abuse, no studies to date have directly investigated the effects of exercise on extinction and reinstatement of methamphetamine-cue memory. Here we show that voluntary exercise led to facilitated extinction of methamphetamine-cue memory and attenuated reinstatement to a priming injection of the drug. We also investigated the role that neurotrophic factors, such as BDNF, might play in the ability for exercise to impact methamphetamine-cue memory. Together these results demonstrate the potential for exercise to serve as a non-pharmacological treatment for METH addiction by protecting against risk of relapse.

Disclosures: G. Aimale: None. **H. Simpson:** None. **E. Saurini:** None. **S. Galeano:** None. **A. Fricks-Gleason:** None.

Poster

PSTR431. Opioids: Reinforcement, Seeking, and Reinstatement

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR431.01/RR7

Topic: G.09. Drugs of Abuse and Addiction

Support: 1ZIADA000434-22

Title: Modeling opioid maintenance treatment in Oprm1-Cre knock-in rats: effect of chronic buprenorphine on incubation of heroin seeking

Authors: *K. E. CALDWELL, I. FREDRIKSSON, A. BATISTA, Y. GERA, R. MADANGOPAL, Y. SHAHAM, J. M. BOSSERT;
Natl. Inst. of Drug Abuse, Baltimore, MD

Abstract: Background: We recently modeled opioid maintenance treatment in rats and found that chronic buprenorphine decreased several relapse-related behaviors, including incubation of heroin seeking during abstinence. We also recently introduced a novel CRISPR-based *Oprm1-Cre* knock-in rat that allow us to visualize and manipulate μ-opioid receptor (MOR)-expressing cells in the brain. Here, we used the *Oprm1-Cre* rats to identify brain MOR-expressing cells involved in the inhibitory effect of buprenorphine on incubation of heroin seeking. Methods: We trained male and female *Oprm1-Cre* rats and their wildtype littermates to self-administer heroin in Context A for 12 days (6-h/day). One day after the last training session, we tested the rats under extinction conditions in Context B. Two weeks later, we implanted osmotic minipumps containing buprenorphine (0 or 6 mg/kg/day). One week later, we tested the rats for incubation of heroin seeking in context B and then anesthetized, perfused, and extracted their brains for immunohistochemistry. Results: There were no genotype or sex differences in heroin self-administration and non-incubated heroin seeking on abstinence day 1 prior to buprenorphine treatment. In both genotypes and sexes, chronic buprenorphine decreased incubated heroin seeking on abstinence day 21. We currently perform double immunohistochemistry of Fos (a neuronal activity marker) and MOR protein to determine which brain areas are activated during incubation of heroin seeking and subsequently inhibited by chronic buprenorphine. Conclusions: Our study using the new *Oprm1-Cre* rats confirms previous results on incubation of heroin seeking after homecage abstinence. We will present the immunohistochemistry results at the meeting.

Disclosures: K.E. Caldwell: None. **I. Fredriksson:** None. **A. Batista:** None. **Y. Gera:** None. **R. Madangopal:** None. **Y. Shaham:** None. **J.M. Bossert:** None.

Poster

PSTR431. Opioids: Reinforcement, Seeking, and Reinstatement

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR431.02/RR8

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant 1ZIADA000434-22

Title: Role of the posterior claustrum in incubation of opioid seeking after electric-barrier induced voluntary abstinence

Authors: *A. BATISTA, K. E. CALDWELL, S. CLAYPOOL, J. BOSSERT, Y. SHAHAM, I. FREDRIKSSON;
Natl. Inst. of Drug Abuse, Baltimore, MD

Abstract: Background: We recently found a critical role of ventral subiculum (vSub) in incubation of oxycodone seeking after electric barrier-induced abstinence, a procedure mimicking human voluntary abstinence due to adverse consequences of drug seeking. Here, we used this model to further study the role of vSub afferent projections in incubation of oxycodone seeking. **Methods:** We trained male and female Sprague-Dawley rats to self-administer oxycodone for 14 days. Next, we exposed the rats for 13 days to an electric barrier of increasing shock intensity near the drug-paired lever, which produced voluntary abstinence. We tested the rats for relapse to oxycodone seeking without shock and drug on abstinence day 1 and 15. First, we determined projection-specific activation of vSub afferents during incubated oxycodone seeking with Fos plus the retrograde tracer cholera toxin B. Oxycodone relapse was associated with modest (anterior claustrum) and strong (posterior claustrum) Fos induction in claustrum neurons projecting to vSub. We then determined the causal role of posterior claustrum in incubation of oxycodone seeking by inactivating this region with the GABAa and GABAb receptor agonists muscimol-baclofen. **Results:** Incubation of oxycodone seeking after electric barrier-induced abstinence was associated with increased Fos expression in anterior and posterior claustrum neurons projecting to vSub. Muscimol-baclofen inactivation of the posterior claustrum decreased incubated oxycodone seeking on day 15 after electric barrier stress-induced abstinence but not non-incubated oxycodone seeking on day 1. **Conclusion:** Results indicate that the posterior claustrum is critical to incubation of opioid seeking after voluntary abstinence induced by adverse consequences of drug seeking.

Disclosures: A. Batista: None. **K.E. Caldwell:** None. **S. Claypool:** None. **J. Bossert:** None. **Y. Shaham:** None. **I. Fredriksson:** None.

Poster

PSTR431. Opioids: Reinforcement, Seeking, and Reinstatement

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR431.03/RR9

Topic: G.09. Drugs of Abuse and Addiction

Support: 1ZIADA000434-22
FI2GM142476

Title: Avoiding naloxone-induced precipitated withdrawal: A procedure for studying opioid negative reinforcement in rats

Authors: *J. CHOW[1], K. M. PITTS[1], J. M. CHABOT[1], R. ITO[2], Y. SHAHAM[1];
[1]NIDA IRP, Baltimore, MD; [2]Univ. of Toronto, Toronto, ON, Canada

Abstract: Background: Operant negative reinforcement (withdrawal avoidance) plays a key role in human opioid addiction. Preclinical rat model of this human condition do not exist. Here we describe a rat model of operant negative reinforcement inspired by the primate model of Downs & Woods (Pharmacol Rev. 1975). **Methods:** We trained rats (n=30,15 females) to lever-press to escape and then avoid mild-footshocks (0.15-0.31, mA) for 35 days (30 trials/d). Next, we catheterized them and implanted minipumps containing methadone (10 mg/kg/day) or saline. We then paired (4 times), in a single session, a light cue (20-s) with naloxone infusions (20 µg/kg, i.v) that precipitated opioid withdrawal symptoms. Next, we trained the rats on an operant procedure for naloxone escape for 10 days (30 trials/d). Each trial started with the onset of the opioid-withdrawal cue. After 20-s, a lever extended, and an infusion of a low naloxone dose (1 µg/kg) began; a lever-press during an 11-s window would terminate the withdrawal-paired cue and the infusion. **Results:** The rats learned to lever-press to escape or avoid mild-footshocks. Methadone-dependent rats, but not drug-naïve rats, lever-pressed to escape naloxone infusions. **Conclusion:** We introduce an operant negative reinforcement procedure in which opioid-dependent rats lever-press to escape infusions of naloxone that induce opioid withdrawal. Pending independent replications, the procedure can be used to study mechanisms of operant negative reinforcement in opioid-dependent rats.

Disclosures: J. Chow: None. **K.M. Pitts:** None. **J.M. Chabot:** None. **R. Ito:** None. **Y. Shaham:** None.

Poster

PSTR431. Opioids: Reinforcement, Seeking, and Reinstatement

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR431.04/RR10

Topic: G.09. Drugs of Abuse and Addiction

Support: 1ZIADA000434-22
FI2GM142476

Title: Effect of peer-sex on operant responding for social interaction: Role of estrous cycle and striatal dopamine

Authors: *K. M. PITTS, A. SCHOENBAUM, K. M. COSTA, G. SCHOENBAUM, Y. SHAHAM, J. CHOW;
Natl. Inst. of Drug Abuse, BALTIMORE, MD

Abstract: Background: Preclinical models that simulate human social interaction demonstrate that rats will choose a social peer over addictive drugs, even after extensive drug self-administration experience. Investigation into how operant social interaction functions as a reward indicates that it supports lever-pressing like other self-administered rewards. However, these studies were conducted with peers of the same sex. Here we examined if peer-sex influences operant social interaction and the role the estrous cycle and striatal dopamine on same- vs. opposite-sex social interaction.
Methods: We trained rats (n=13; 7 females) to lever-press for access (15 s) to a same- or opposite-sex peer for 16 d (8 d/sex) and tracked females' estrous cycle. Next, we transfected GRAB-DA2m and implanted optic fibers into nucleus accumbens core and dorsal medial striatum. We then retrained the rats for social interaction for 16 d (8 d/sex) and recorded striatal dopamine during operant responding for a peer for 8 d (4 d/sex). Finally, we recorded striatal dopamine during assessment of economic demand for a peer (10 d/sex).
Results: Male rats responded more when a female peer was present; there were no differences in responding in female rats. Estrous cycle had no effect on operant social interaction. Striatal dopamine signals during operant social interaction were dependent on the peer's sex.
Conclusions: Results indicate that, unexpectedly, estrous cycle fluctuations did not influence opposite-sex operant social interaction. Additionally, striatal dopamine activity was dependent on the peer's sex.

Disclosures: K.M. Pitts: None. **A. Schoenbaum:** None. **K.M. Costa:** None. **G. Schoenbaum:** None. **Y. Shaham:** None. **J. Chow:** None.

Poster

PSTR431. Opioids: Reinforcement, Seeking, and Reinstatement

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR431.05/RR11

Topic: G.09. Drugs of Abuse and Addiction

Support: 1ZIADA000434-22

Title: An operant procedure to study choice between rewarding physical activity and heroin in male and female rats

Authors: *S. CLAYPOOL, H. BONBREST, K. CALDWELL, R. MADANGOPAL, J. J. CHOW, H. P. JEDEMA, Y. SHAHAM;
Natl. Inst. of Drug Abuse, Baltimore, MD

Abstract: Background and rationale: We and others have previously reported that in a discrete choice procedure, rats of both sexes strongly prefer palatable food or social interaction over

opioid drugs. Here, we adapted this procedure to test whether rats will prefer rewarding physical activity (wheel running) over the opioid drug heroin. Methods: We gave rats (n=6, 3 females) free access to a running wheel for 30-60 min/d for 6 d. Next, we paired in a single 30-min session a tone-light cue with unlocking the wheel, signaling running availability (15 1-min trials/d;1-min intertrial-interval). Next, we trained them to lever-press to unlock the running wheel for 30-s to 1-min for 1-h/d for 14 d; fixed-ratio-1 schedule. During this phase, we also trained the rats to lever-press for heroin (0.05-0.1 mg/kg infusion for 11 d; fixed-ratio-1 schedule). Next, we tested the rats for 8 d in a discrete choice procedure where they chose between lever-pressing for access to the wheel running for 30-s versus lever-pressing for heroin infusions (15 trials/d; 2-min intertrial interval, 4-min to make a choice, 2-min between trials, 6-min total). Results: Our preliminary results indicate that rats would reliably lever-press to gain access to a running wheel and that, under our current experimental conditions, some rats preferred running wheel while others preferred heroin, resulting in ~50% preference for wheel running at the group level. Conclusions: We introduce an operant procedure to study choice between rewarding physical activity versus an addictive drug that can be used to study mechanisms of choice between the two rewards.

Disclosures: S. Claypool: None. **H. Bonbrest:** None. **K. Caldwell:** None. **R. Madangopal:** None. **J.J. Chow:** None. **H.P. Jedema:** None. **Y. Shaham:** None.

Poster

PSTR431. Opioids: Reinforcement, Seeking, and Reinstatement

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR431.06/RR12

Topic: G.09. Drugs of Abuse and Addiction

Support: This work was supported by startup funds from OSU-CHS (C.T.W.), startup funds from MURC (S.M.), and NCAST funds to NCWR.

Title: Fentanyl seeking is attenuated by iboga alkaloid congeners

Authors: *J. D. LAYNE[1,2], M. J. HOCHSTETLER[1,2], B. M. CURRY[1,2], M. MAULIK[3], A. F. CARMEN[3,4], C. SMITH[3], K. LOOSCHEN[3], H. R. ARIAS[1], S. MITRA[3], C. T. WERNER[1,2]; [1]Pharmacol. and Physiol., [2]Natl. Ctr. for Wellness and Recovery, Oklahoma State Univ. Ctr. for Hlth. Sci., Tulsa, OK; [3]Biomed. Sci., Joan C Edwards Sch. of Med., Huntington, WV; [4]Jacobs Sch. of Med., The State Univ. of New York at Buffalo, Buffalo, NY

Abstract: Background. The prevalence of opioid use disorder (OUD) has reached epidemic proportions with a record-breaking number of overdose deaths. Over 70% of the overdose deaths are caused by synthetic opioids, including fentanyl. Fentanyl is commonly administered intravenously or by inhalation (smoking/vaping), which results in rapid drug bioavailability in the brain. There is an urgent need to identify novel pharmacotherapies to treat OUD. There is increasing evidence to support the use of ibogaine analogs, also known as iboga alkaloids, to

treat OUD and other psychiatric illnesses. In preclinical models, iboga alkaloids, such as 18-methoxcoronaridine (18-MC), have been shown to decrease opioid self-administration and seeking and induce antidepressant and anxiolytic effects. To our knowledge, there is no published research examining the effects of catharanthine (Cath) on drug seeking behaviors. Here we used a preclinical fentanyl vapor self-administration model to study the anti-addictive effects of two iboga alkaloid congeners, 18-MC and Cath.
Methods. Male and female C57BL/6 mice were trained to self-administer vaporized fentanyl (5 mg/mL) or vehicle in air-tight operant chambers for 10 1-hour sessions. Mice learned to self-administer vapor reward with 3-second vapor deliveries for the first 3 sessions of training, which was then reduced to 1.5-second vapor deliveries for the remaining 7 sessions. After training, mice were returned to their home cages for a forced abstinence period. Cue-induced drug seeking tests were conducted on abstinence day (AD) 20 and AD25. Cue-induced drug seeking tests were conducted using a crossover design where half of subjects were injected (i.p.) with iboga treatment (18-MC or Cath), while the other half received vehicle (ddH2O), on AD20 1 hour before seeking tests. On AD25, subjects received the opposite treatment compared to AD20. To examine the molecular mechanisms of Cath, a separate cohort of male and female mice were euthanized 1 hour after Cath injection (i.p.), and brain tissue was extracted and processed for molecular analyses.
Results. We found that both 18-MC and Cath significantly reduced fentanyl seeking during prolonged abstinence in both males and females with no effect on mice that had previously self-administered vehicle vapor. Molecular analysis provides a potential mechanism for this behavioral effect.
Conclusion. In this study, we report that both 18-MC and Cath decrease fentanyl seeking during prolonged abstinence in both males and females. These results add evidence to the existing literature that iboga alkaloids may be a promising class of compounds for novel pharmacotherapeutic treatment of OUD.

Disclosures: J.D. Layne: None. **M.J. Hochstetler:** None. **B.M. Curry:** None. **M. Maulik:** None. **A.F. Carmen:** None. **C. Smith:** None. **K. Looschen:** None. **H.R. Arias:** None. **S. Mitra:** None. **C.T. Werner:** None.

Poster

PSTR431. Opioids: Reinforcement, Seeking, and Reinstatement

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR431.07/RR13

Topic: G.09. Drugs of Abuse and Addiction

Support: This work was supported by OSU-CHS startup funds to C.T.W. and NCAST funds to NCWR

Title: Examining how prelimbic cortical neuronal ensembles encode behaviors during fentanyl vapor self-administration

Authors: *M. J. HOCHSTETLER[1,3], J. D. LAYNE[1,3], B. M. CURRY[1,3], C. T. WERNER[3,2]; [1]Pharmacol. and physiology, Oklahoma State Univ., tulsa, OK; [2]Pharmacol. and physiology, Oklahoma State Univ., Tulsa, OK; [3]Natl. Ctr. for Wellness and Recovery, Tulsa, OK

Abstract: The prevalence of opioid use disorder (OUD) and overdose deaths have reached epidemic proportions that constitute a global crisis. Comparing January 2021 to January 2020 synthetic opioids, including fentanyl, were responsible for a 31% increase in non-fatal opioid overdoses across the United States. Fentanyl, which is often used clinically for anesthesia and analgesia, is commonly administered intravenously or by inhalation (smoking/vaping), which results in rapid drug bioavailability in the brain. Technical challenges have contributed greatly to our lack of understanding of the neurobiology of OUD, including limitations of behavioral models, difficulty tracking individual neurons longitudinally in freely behaving animals, and inadequate behavioral analysis tools. Intravenous drug self-administration is considered the "gold standard" model to investigate the neurobiology of OUD preclinically, but it remains difficult to perform *in vivo* electrophysiology or calcium imaging during drug self-administration due to the tangling of drug catheter and recording cable. This technical challenge was overcome with the development of a noninvasive mouse model of opioid self-administration using vaporized fentanyl that recapitulates key features of OUD. Imaging freely behaving animals is difficult, and conventional single-unit recordings can neither distinguish neuron subtypes nor track individual neurons longitudinally. In contrast, *in vivo* imaging using miniaturized fluorescence microscope (miniscope) systems allows for examining spatially and temporally coordinated activity in hundreds of individual neurons longitudinally in freely behaving animals. Complex behavioral analysis is infrequently incorporated in preclinical models, which likely contributes to limited translational impact. Recent computational advances in convolutional neural networks, pose estimation, and machine learning analysis has overcome these challenges to provide tools for computational neuroethology. Here, we implemented a multi-faceted approach combining custom operant vapor chambers and single-photon miniscope to examine how prelimbic cortical neuronal ensembles encode behaviors during fentanyl vapor self-administration . Following data collection, we utilized deep behavior learning to perform a fine-grain analysis of behavior, which we overlayed with calcium transient data. We are leveraging these cutting-edge imaging technologies and behavioral analysis tools to gain a deeper insight into how neuronal ensembles in the prefrontal cortex encode opioid-related behaviors during fentanyl vapor self-administration and relapse.

Disclosures: M.J. Hochstetler: None. **J.D. Layne:** None. **B.M. Curry:** None. **C.T. Werner:** None.

Poster

PSTR431. Opioids: Reinforcement, Seeking, and Reinstatement

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR431.08/RR14

Topic: G.09. Drugs of Abuse and Addiction

Support: DA726129
DA041781
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WM Keck Fellowship

Title: Estradiol protects against pain-facilitated fentanyl use via suppression of opioid-evoked dopamine activity

Authors: *J. HIGGINBOTHAM[1], J. ABT[2], R. TEICH[2], T. LINTZ[2], J. DEARMAN[2], J. MORON-CONCEPCION[3];
[2]Anesthesiol., [1]Washington Univ. Sch. of Med., St. Louis, MO; [3]Washington Univ., Saint Louis, MO

Abstract: Opioid analgesics are commonly used for pain management despite their potential for abuse. Evidence suggests that the risk for opioid misuse under conditions of pain may vary based on gender/sex, but the biological basis of this relationship is unclear. Using a rat model of fentanyl self-administration (SA) with wireless *in vivo* fiber photometry, we showed that males with inflammatory pain exhibit increased rates of fentanyl intake that are associated with, and dependent on, maladaptations within mesolimbic reward circuitry. A cre-dependent calcium indicator (jGCaMP7c) was targeted to the ventral tegmental area (VTA) of male and female TH-Cre+ rats (2-3 mo.) and optic fibers were implanted in the VTA or nucleus accumbens (NAc) to monitor VTA dopamine neuron at the soma (VTA^{DA}) or terminal (VTA^{DA}->NAc), respectively. Rats were implanted with IV catheters, received hind-paw injections of Complete Freund's Adjuvant (CFA) to produce inflammatory pain, and were trained for fentanyl SA (2-5 µg/kg/infusion) during five 2-hr sessions/week for 3 weeks. Pain did not affect the acquisition of fentanyl SA during week 1, but males with pain had greater intake than any other group by week 3. This pain and sex-specific behavior was associated enhanced fentanyl-evoked VTA^{DA} and VTA^{DA}->NAc activity that developed in paralleling magnitude with fentanyl SA. Chemogenetic inhibition of VTA^{DA} cells during week 3 prevented males with pain from increases in fentanyl use. Ovariectomized (OVX) females developed a male-like phenotype with increased fentanyl SA and associated VTA^{DA} activity. Estradiol (E2) treatment failed to reverse the effects of OVX. Instead, we demonstrate the therapeutic potential of E2 treatment in males and its ability to reverse the effects of pain on VTA^{DA} function and fentanyl use via VTA estrogen receptors. These findings are the first to implicate a role for gonadal hormones in pain-facilitated opioid use and provide novel therapeutic targets within the mesolimbic DA reward circuitry that may contribute to pain-related motivational impairments and risk for opioid abuse.

Disclosures: J. Higginbotham: None. **J. Abt:** None. **R. Teich:** None. **T. Lintz:** None. **J. Dearman:** None. **J. Moron-Concepcion:** None.

Poster

PSTR431. Opioids: Reinforcement, Seeking, and Reinstatement

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR431.09/RR15

Topic: G.09. Drugs of Abuse and Addiction

Support: Intramural Research Program at NIDA

Title: Effect of suvorexant on economic choice between remifentanil and food in squirrel monkeys.

Authors: *J. HECKER, N. BOOKWALA, H. JEDEMA, C. BRADBERRY;
NIH, NIDA IRP, Baltimore, MD

Abstract: Opioid use disorder (OUD) is clinically treated through opioid maintenance therapy using mu-opioid agonists and partial agonists such as methadone and buprenorphine. These compounds may cause unfavorable side effects such as respiratory depression, constipation, and a continuing state of dependence. Because of this, research into non-conventional therapeutics that don't involve the mu-opioid receptor is needed. One area of interest includes the orexin receptor sites, as they have been linked to arousal and motivation. Suvorexant is a dual orexin antagonist clinically used for the treatment of insomnia, although recent studies have shown potential for the attenuation of opioid reward. To evaluate the effects of suvorexant on opioid reward, we are utilizing a nonhuman primate (squirrel monkey) model, in which opioid reward is evaluated using an established economic choice paradigm that tracks subject choice allocation between differing quantities of remifentanil (an ultrashort-acting opioid) and a food reward. Additionally, actigraphy monitors (Axivity AX3) were placed on each subject as a non-invasive way to record activity data. Based on these raw activity scores, total sleep time (TST) and sleep onset latency (SOL) for each night were determined using an algorithm in Matlab. These sleep parameters provide supplementary information to determine if suvorexant is altering sleep, an expected behavioral effect. Suvorexant was administered orally at 0.3-3.0 mg/kg with no significant differences in choice valuation, TST, or SOL compared to contemporaneous individual baselines. At these doses, plasma level concentrations of suvorexant were much lower than expected, suggesting inadequate delivery to their bloodstream. Ongoing studies using slow, intravenous (IV) delivery of suvorexant will continue to evaluate the effects on opioid reward, sleep parameters, and achieved plasma levels.

Disclosures: J. Hecker: None. **N. Bookwala:** None. **H. Jedema:** None. **C. Bradberry:** None.

Poster

PSTR431. Opioids: Reinforcement, Seeking, and Reinstatement

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR431.10/RR16

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant UL1TR002544
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Program in Pharmacology and Drug Development, Tufts Univ. Grad Sch Biomed Sciences

Title: An Intranasal GDNF Gene Therapy for Opioid Relapse Reduction: Effects on Central Dopamine Exocytosis

Authors: Y. R. ABUHASAN[1], L. LI[1], J. S. YANG[1], S. AGRAWAL[1], J. EGHOLM[1], K. E. BUDGE[2], O. SESENOGLU-LAIRD[3], M. J. COOPER[3], R. C. MOHEN[3], B. L. WASZCZAK[4], E. M. BYRNES[2], ***E. N. POTHOS**[1];
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Abstract: The ongoing opioid epidemic has underlined the need for treatment of opioid use disorder (OUD) with alternatives to opioid medications. Such interventions could impact central dopamine systems regulating opioid reward and the aversive aspects of opioid withdrawal, abstinence, and craving. We and others have shown that glial cell derived neurotrophic factor (GDNF) has protective and restorative effects on midbrain dopamine neurons and synaptically facilitates dopamine release. At the same time, chronic use of opioids induces deficits in mesolimbic dopamine release, which can drive relapse. Our current studies test whether intranasal administration of GDNF plasmid DNA nanoparticles (pGDNF NPs) can correct central dopamine deficits in a rat model of oxycodone (OXY) intravenous self-administration (IVSA); whether any observed effects are different in male and female animals; and whether changes in dopamine exocytosis in real time correlate with behavioral parameters of oxycodone IVSA that assess motivation to work for receiving oxycodone injections. We assessed *ex vivo* dopamine release kinetics in dorsal striatum (DS), nucleus accumbens (Nacc), and medial prefrontal cortex (PFC) of male and female rats trained identically in OXY IVSA (0.1 mg/kg/infusion; 6 h/day; 13 training days). Rats were then administered either intranasal saline vehicle or pGDNF NPs (90 μg DNA). This was followed by 28 days of abstinence from OXY prior to the *ex vivo* dopamine release studies. Amperometric electrodes with 5 μm carbon fibers (Amoco) and a positive 700 mV voltage (vs Ag-AgCl ground) were employed to measure dopamine. The electrodes were placed in acute DS, Nacc or PFC 300 μm coronal slices. A bipolar stimulating electrode was placed 100-200 μm away from the carbon fiber electrode and a current stimulus of +500 μA was applied 3 times per site every 5 min for 2 msec. The output was digitized at 50 kHz to record dopamine exocytosis in real time and low-pass filtered at 1 kHz. The number of dopamine molecules per stimulation was determined by Faraday's equation, and, so far, estimated to increase up to 2-fold in the Nacc of pGDNF NPs-treated rats. We are running further correlation analyses between behavioral (IVSA) and dopamine release parameters to detect any differences between subpopulations of pGDNF NP and vehicle-treated groups, such as males versus females and high- versus low-responders in the OXY progressive ratio session. These results will determine if correction of dopamine deficiencies in the brain's reward centers may serve as a mechanism for the efficacy of intranasal pGDNF NPs in reducing relapse behavior in our OXY IVSA studies.

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Poster

PSTR431. Opioids: Reinforcement, Seeking, and Reinstatement

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant UL1TR002544
NIH Grant UG3DA050942

Title: An intranasal GDNF gene therapy for opioid relapse reduction: Neural modifications associated with reduced opioid reinstatement following intranasal administration of GDNF plasmid DNA nanoparticles

Authors: K. E. BUDGE[1], J.-L. LOPES[1], K. R. GILDAWIE[1], F. M. VASSOLER[1], O. SESENOGLU-LAIRD[2], R. C. MOEN[2], M. J. COOPER[2], J. D. ELSWORTH[3], E. N. POTHOS[4], B. L. WASZCZAK[5], *E. BYRNES[1];
[1]Tufts Univ. Cummings Sch. Vet Med., North Grafton, MA; [2]Copernicus Therapeutics, Inc, Cleveland, OH; [3]Virscio, Inc, New Haven, CT; [4]Dept of Immunol., Progs. in Pharmacol & Exp Therapeut., Pharmacol & Drug Develop. & Neurosci., Tufts Univ. Sch. Med., Boston, MA; [5]Dept Pharmaceut Sci., Northeastern Univ., Boston, MA

Abstract: Treatments for opioid use disorder (OUD) include the opioids methadone and buprenorphine, which moderate withdrawal symptoms and reduce craving. However, both of these treatments have abuse potential. Thus, new non-opioid based treatments that can effectively reduce craving and decrease the risk of relapse are still needed. We are testing the hypothesis that an intranasal glial cell line-derived neurotrophic factor (GDNF) gene therapy may be able reduce craving and relapse due to effects on the brain dopamine system. Adult male and female Sprague Dawley rats were implanted with jugular catheters and trained to lever press for oxycodone (OXY) (0.1 mg/kg/infusion; 6 h/day) for 12 days. On Day 13 all animals were tested for OXY motivated responding using a progressive ratio (PR) schedule. The next day rats were administered either intranasal saline vehicle or GDNF plasmid DNA nanoparticles (pGDNF NPs) (90 μg DNA; Copernicus Therapeutics, Inc.), with groups counterbalanced based on total drug intake and PR breakpoint. All animals then underwent forced abstinence for 30 days and were then tested for cue-induced reinstatement (90 min). Consistent with our

hypothesis, intranasal administration of pGDNF NPs significantly decreased cued reinstatement in abstinent oxycodone self-administering males and females when examined 30 days after intranasal delivery. Having shown potential for reducing relapse behavior, our current studies are addressing several follow-up questions: 1) Do levels of GDNF mRNA and protein remain elevated several weeks after intranasal infusion in rats? 2) Are the effects of intranasal GDNF NPs on cued reinstatement in rats associated with changes in Fos activity in tyrosine hydroxylase (TH) expressing dopamine neurons? 3) Do intranasal infusions of pGDNF DNA NPs increase GDNF mRNA and protein in the brains of non-human primates (NHPs)? For the rat studies, all animals were immediately euthanized at the end of reinstatement; half were used to quantify Fos positive TH neurons using immunohistochemistry with florescence while half were used to measure GDNF mRNA using qPCR and GDNF protein using ELISAs. For the NHP study, African Green Monkeys were anesthetized and intranasally administered different doses of pGDNF NPs. Two weeks later levels of GDNF mRNA and protein were assessed across a number of brain regions (prefrontal cortex, striatum, VTA, and cerebellum). These studies will begin to identify the neural mechanisms underlying decreased reinstatement following intranasal pGDNF NPs in rats and determine the potential efficacy of intranasal delivery of pGDNF NPs to upregulate GDNF mRNA and protein in the primate brain.

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Poster

PSTR431. Opioids: Reinforcement, Seeking, and Reinstatement

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR431.12/RR18

Topic: G.09. Drugs of Abuse and Addiction

Support: 1K99DA056573
1K08DA055157

Title: Gating of opioid withdrawal aversion by a unique class of neurons in the nucleus accumbens

Authors: *J. TUCCIARONE[1,2], M. POMRENZE[2], J. BAEK[2], Z. ZHANG[2], A. SHANK[2], P. A. NEUMANN[2], N. ESHEL[2], R. MALENKA[2];
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Abstract: Lethal overdose from opioids have increased dramatically over the past decade, critically contributing to the "opioid crisis". The aversion of opioid withdrawal and its associated environmental cues act as potent drivers of relapse and increase the risk of future overdose. More detailed understandings of circuit mechanisms underlying opioid withdrawal aversion are needed to develop new therapeutic strategies to target opioid dependence and relapse. The nucleus accumbens (NAc) has been long known to be involved in drug reward and cued reinstatement. We identified neurons in the NAc that express mu opioid receptor (MOR) and found two major populations expressing D1 dopamine receptors, one marked by *Pdyn* and another with *Tshz1*, a cell-type recently found to mediate aversion learning in the dorsal striatum. Photometry recordings with GCaMP6f revealed distinct activity dynamics, such that acute morphine intoxication reduced activity in both cell-types yet naloxone precipitated withdrawal triggered a large rebound in activity selectively in *Tshz1* cells. Reducing activity of *Tshz1* neurons with hM4Di disrupted morphine withdrawal conditioned place aversion (CPA), while inhibition of *Pdyn* neurons had no effect on withdrawal CPA but reduced morphine conditioned place (CPP). Acute stimulation of *Tshz1* neurons with ChR2 led to avoidance of the stimulation-paired chamber in a real time place preference test, whereas stimulation of *Pdyn* neurons led to a preference. We hypothesized that these behavioral effects are mediated through cell-type specific modulation of dopamine (DA) release in the NAc. To test this idea, we stimulated *Tshz1* or *Pdyn* cells with ChRmine while performing photometry recordings with GRAB DA. Stimulation of *Tshz1* neurons led to a rapid suppression of DA release in the NAc, whereas stimulation of *Pdyn* neurons increased DA release. DA levels rose substantially after morphine administration but were significantly suppressed below baseline after naloxone injection, indicating a hypodopaminergic state during withdrawal. Ongoing studies are examining the discrete mechanism by which *Tshz1* neurons modulate DA release in the NAc, whether *Tshz1* neuron activity mediates withdrawal-induced decreases in DA, and the role of MOR in *Tshz1* neurons in these effects. Together, our data demonstrate a unique population of NAc neurons that mediate the aversive nature of acute opioid withdrawal through strong modulation of mesolimbic DA release.

Disclosures: **J. Tucciarone:** F. Consulting Fees (e.g., advisory boards); Headlamp Health. **M. Pomrenze:** None. **J. Baek:** None. **Z. Zhang:** None. **A. Shank:** None. **P.A. Neumann:** None. **N. Eshel:** F. Consulting Fees (e.g., advisory boards); Boehringer Ingelheim. **R. Malenka:** F. Consulting Fees (e.g., advisory boards); Maplight Therapeutics, MindMed, Bright Minds Biosciences, AZ Therapies, Cyclerion.

Poster

PSTR431. Opioids: Reinforcement, Seeking, and Reinstatement

Location: WCC Halls A-C

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Program #/Poster #: PSTR431.13/RR19

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant F31DA056194

Title: Opioid withdrawal increases excitability and synaptic output of ventral pallidal glutamatergic neurons

Authors: *J. TOOLEY[1], M. CREED[2];
[2]Anesthesiol., [1]Washington Univ. in St. Louis, St. Louis, MO

Abstract: Withdrawal from opioids is associated with a highly aversive affective state, and avoidance of this state is a main reason people with opioid use disorder continue to use or relapse to opioids. Activity in the ventral pallidum (VP) is necessary for opioid relapse after withdrawal. Recent studies from our group and others have shown that glutamatergic VP neurons (VP_{Glu}) constitute a unique subpopulation of VP neurons, and that VP_{Glu} activity constrains reward seeking, particularly in decision-making tasks where negative consequences are associated with choosing a reward. However, how self-administration and withdrawal from prescription opioids alters the function of VP_{Glu} neurons and their activity in conflicted decision-making tasks is completely unknown. With fluorescent in situ hybridization, we discovered that VP_{Glu} neurons are enriched in mu opioid receptors. Patch-clamp electrophysiology experiments revealed that application of mu opioid agonists decreased excitability of VP_{Glu}. Thus, we predicted that self-administration of oxycodone, the mu opioid receptor agonist, would potently modulate function of these neurons. Indeed, protracted abstinence from oxycodone increased the intrinsic excitability of VP_{Glu} neurons and their synaptic output. Using in vivo calcium imaging and Pavlovian behavior tasks, we investigated the response of VP_{Glu} neurons to positive and negative stimuli throughout oxycodone self-administration to determine whether the response of these neurons is altered throughout opioid exposure. Given our previous work showing that activation of VP_{Glu} neurons is highly aversive, we hypothesize that these opioid-induced adaptations contribute to the aversive state of drug withdrawal that drives opioid relapse through negative reinforcement.

Disclosures: J. Tooley: None. **M. Creed:** None.

Poster

PSTR431. Opioids: Reinforcement, Seeking, and Reinstatement

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR431.14/Web Only

Topic: G.09. Drugs of Abuse and Addiction

Title: Mitragynine-associated drug intoxication increases with fentanyl use

Authors: *A. SURIAGA;
Epidemiology, Harvard Univ., West Palm Beach, FL

Abstract: The neurobiology of mind-altering substances, particularly Mitragynine, and how it disrupts the chemical processes that maintain homeostasis and essential functions such as breathing within the brain is complex. Mitragynine is a natural indole alkaloid of Mitragyna speciosa that grows in Thailand, Indonesia, and the Philippines; used primarily for pain relief and

to improve work endurance. People in Western countries use Mitragynine for its analgesic effect, self-treatment for opioid withdrawal, and to reduce cannabis and opioid use. Mitragynine users among U.S. adults 12 years and older account for > 2,00,000 in the past year. Consuming Mitragynine in higher doses stimulates the brain while producing a sedative/hypnotic effect in lower doses. Polysubstance use potentiates the dose-dependent adverse effects of Mitragynine. While Mitragynine depresses the respiratory center lesser than Morphine, its mechanism of action remains unclear. In this study, we report the risk of Mitragynine-Associated Drug Intoxication (MADI) when co-using an opioid. We used de-identified data from the Florida Department of Law Enforcement in 2021. We used descriptive statistics to describe decedents with MADI and logistic regressions to examine the odds of dying from MADI with opioids (n=10). We analyzed all data using Stata 17. Results: 305 people died from Mitragynine use in Florida in 2021, up 24% from 2020. The average age was 37.79 (SD=9.38). More males died than females (81.64% vs. 18.36%), 24-44 years old were mostly affected (>75%), and white at 94.43%. 85% (n=260) died from drug intoxication, suicide (n=16), natural cause (n=11), and the rest from homicide, suicide, and accidents. More than 90% of decedents had used multiple drugs other than Mitragynine (n=281). Fentanyl was the most frequent drug used among Mitragynine users (n=221); then fentanyl analogs (n=138), Morphine (n=33), Ethanol (n=79), Cannabinoids (n=57), Methamphetamine (n=43), and Amphetamine (n=40). No statistically significant relationship between gender, ethnicity, age, population density, and MADI (p>.05). The odds of dying from MADI increased by six-fold among Fentanyl users versus non-Fentanyl users (OR=6.48, 95% CI, 3.87, 11.02), *p*= .001. Adjusting for age, gender, ethnicity, and population density, the odds of dying from MADI went up by 6.53 times the odds versus those who did not use Fentanyl. Controlling for neurocognitive, cardiovascular, and respiratory conditions, the odds of dying from MADI remain stable (OR=6.50, 95% CI, 3.70, 11.43), *p*=.001. MADI is a severe public health problem. More research is needed to curb preventable deaths from this psychoactive substance.

Disclosures: A. Suriaga: None.

Poster

PSTR432. Human Perception, Imagery, and Imagination

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR432.01/RR20

Topic: H.02. Perception and Imagery

Title: Identification of dot clusters in a Gaussian-distributed field: the range of human perception and a comparison with spectral graph clustering.

Authors: *A. D. CATE;
Psychology, Roanoke Col., Salem, VA

Abstract: This study characterized human biases in the perception of clusters from random dot fields. It also compared human-chosen clusters with those identified by a state-of-the-art

machine learning algorithm. This study introduced a novel cluster identification task that was ensured to reveal participants' individual differences. Unlike other visual cluster identification displays, which present several potential clusters with different features for participants to choose, this study presented random dot fields with Gaussian-distributed density. These were densest in the center of the display (the peak of the distribution) and became gradually less dense radially toward the periphery; there was no salient discontinuity in dot spacing. 24 18-27 year-old participants viewed 180 displays, and were instructed to select the dots that appeared to form the most noticeable cluster on each display using a mouse interface, with no time limit. Their clusters were analyzed for number of dots; convex hull area, perimeter, and compactness; dot density within the hull; and mean nearest neighbor distance. A spectral clustering algorithm using a density-aware kernel (Zhang, 2011) that assigns higher scores in denser regions of a graph and which is capable of identifying concentrically-arranged clusters (the Spectrum R package; John et al., 2020) was applied to the dot displays for comparison with the performance of human participants. The participants' clusters had a range of dot numbers, in a distribution that was not significantly different from normal (Shapiro-Wilk's test $p=0.85$). However, unlike in many perceptual magnitude estimation or production tasks, the standard deviations of the participants' means, did not scale in correlation with the mean values. The degree of overlap between the human and the spectral algorithm-defined clusters was modest, with a mean F1-score of 0.63 (SD 0.035). It was found that the clusters (all produced by Gaussian point processes) showed strong correlations among all of the features measured, provided that they were centered on the Gaussian distribution's center. It was determined that the spectral clustering algorithm may have matched the human participants less well because its matching cluster was frequently not centered. Although different individuals consistently preferred clusters of distinct sizes, cluster perception appears to function differently from magnitude perception processes.

Disclosures: A.D. Cate: None.

Poster

PSTR432. Human Perception, Imagery, and Imagination

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR432.02/SS1

Topic: H.02. Perception and Imagery

Support: NIMH Intramural Research Program (ZIAMH002783)

Title: A functionally time-resolved reconstruction technique for high-resolution fMRI (fTR-MRI)

Authors: *A. MORGAN, I. GEPHART, D. A. HANDWERKER, J. GONZALEZ-CASTILLO, P. A. BANDETTINI;
NIMH, Bethesda, MD

Abstract: Functional MRI (fMRI) with sub-millimeter spatial resolution is a promising technique to probe the human brain's mesoscopic scale [1,2]. However, typical spatial resolutions remain too coarse to sample individual human columnar and laminar structures. Moreover, high-resolution fMRI measurements using echo-planar trajectories (EPI) and blood oxygen-dependent (BOLD) contrast suffer from spatial distortions and T2* blurring due to long readout trains. Recently, time-resolved reconstruction methods have alleviated some of these issues by keeping track of the timing of data acquisition in reference to signal properties [3] or physiological cycles [4]. We utilize this conceptual framework to time resolve data with respect to events in a neuroscience experiment. The current work, which we call functionally time-resolved MRI (fTR-MRI), has high spatial resolution (0.5 mm) and is not affected by phase-encoding distortions yet reconstructs brain responses with reasonable temporal resolution (400 ms).

We acquired data from 3 participants (2 male) on a Siemens MAGNETOM 7T+ with a Nova 32-channel head coil. We collected a multi-echo 2D-GRE sequence (TR=31 ms, TEs=[4.22, 8.38, 12.54, 16.7, 20.86, 25.02] ms, slice thickness=0.8 mm, matrix=360x270, no acceleration or Partial Fourier). Acquisition times were tracked for each k-space line by sending an external trigger to a stimulation computer. The experimental paradigm consisted of a 10 Hz flashing radial checkerboard presented for 2 s (15 s ISI). We reconstructed data via low-rank tensor completion [5] with modes for k-space, receivers, echoes, and experimental response time. The resulting reconstruction depicts brain responses from -2 to 32 seconds after stimulus presentation with 6 echoes.

Primary visual cortex displayed a prominent dip in T2* decay times in middle layers, allowing us to identify infra- and supra-granular layers. Functional responses peaked between 2.5 and 3 s after the short stimulus presentation and superficial layers showed larger peak response and post-stimulus undershoot amplitudes, as reported in rodent studies [6].

The fTR-MRI reconstruction method incorporates experimental designs into the image reconstruction process to capture high spatial and temporal resolution brain responses. These features expand the arsenal of tools available to non-invasively examine mesoscopic responses in the human brain.

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[2] E. Finn, et al., Prog Neurobiol. 2021
[3] F. Wang et al., Magn. Reson. Med. 2019
[4] A.G. Christodoulou et al., Nat. Biomed. Eng. 2018
[5] M.A.O. Vasilescu, University of Toronto Thesis. 2009
[6] P. Tian et al., Proc. Natl. Acad. Sci. 2010

Disclosures: A. Morgan: None. **I. Gephart:** None. **D.A. Handwerker:** None. **J. Gonzalez-Castillo:** None. **P.A. Bandettini:** None.

Poster

PSTR432. Human Perception, Imagery, and Imagination

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR432.03/SS2

Topic: H.02. Perception and Imagery

Support: Israeli Science Foundation Grant 1169/17

Title: Neural correlates of the embodied sense of agency support two-level processing: A pre-registered EEG study

Authors: *A. REGEV KRUGWASSER[1], R. VAN DER GOOT[1], G. MARKUSFELD[1], Y. ZVILICHOVSKY[1], R. SALOMON[2];
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Abstract: The Sense of Agency (SoA) is the subjective experience that 'I am in control of my actions'. It has been suggested that SoA relies on an internal comparator mechanism, whereby predictions about the consequences of an action are compared to the perceived consequences of the performed action. A mismatch between one's predicted and perceived action's consequences leads to a reduced SoA. More recent accounts have distinguished two levels in the formation of SoA - early implicit sensorimotor processes (feeling of agency) and later explicit higher-level processes, incorporating one's thoughts and beliefs (judgment of agency). Even though SoA is fundamental to our interactions with the external world and the construct of the self, its underlying neural mechanism remains elusive. In the current pre-registered electroencephalography (EEG) study, we used time-frequency and Multivariate Pattern Analysis (MVPA) to investigate the electrophysiological characteristics associated with SoA. We used an established virtual reality, embodied SoA paradigm in which visual feedback of a finger movement is modulated to examine the effect of either a match or a mismatch between the predicted and perceived sensory feedback. Participants (N = 30) moved their finger and were presented with an anatomical or a spatial alteration (different finger or angular shift, respectively), then rated their SoA over the observed movement, while their neural activity was recorded using EEG. In accordance with our pre-registered hypothesis, we found that a reduction of SoA is associated with decreased attenuation in the alpha frequency band, in line with the well-established finding of Mu suppression during voluntary motor action. Increased power in the theta frequency band was also associated with SoA reduction. Importantly, we show that trials in which participants reported having SoA vs. not having SoA, can reliably be decoded with ~70% accuracy starting around 200ms after movement onset. Finally, Cross-decoding analyses revealed similar neural patterns between reduced SoA in the anatomical and spatial conditions, at a later processing stage starting around 500ms after movement onset. Together, our results identify and characterize reduced alpha attenuation, as well as increased theta power as cortical signatures of loss of SoA and provide the first neural evidence that supports the hypothesis of a two-level formation of SoA - an early component that could be decoded within condition only (domain-specific), possibly the equivalent of the implicit feeling of agency, and a late domain-general component, possibly the equivalent of the explicit judgment of agency.

Disclosures: A. Regev Krugwasser: None. **R. Van Der Goot:** None. **G. Markusfeld:** None. **Y. Zvilichovsky:** None. **R. Salomon:** None.

Poster

PSTR432. Human Perception, Imagery, and Imagination

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Topic: H.02. Perception and Imagery

Support: This work was supported by the Alchemist Project (20012355, Fully implantable closed loop Brain to X for voice communication) funded by the Ministry of Trade, Industry & Energy (MOTIE, Korea).

Title: Intracranial neural prediction of imagined melodies from relative pitch height decoding

Authors: *J. KWON[1], J. KIM[2,3], C. CHUNG[4];
[1]Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Seoul Nat Univ., Seoul, Korea, Republic of; [3]Clin. Res. Inst., Konkuk Univ. Med. Ctr., Seoul, Korea, Republic of; [4]Seoul Natl. Univ. Hosp., Seoul Natl. Univesity, Seoul, Korea, Republic of

Abstract: Music is our oldest and most fundamental expression. Melody, consisting of a pitch series, is considered an essential component of music, as playing an essential role in distinguishing music. In the perspective of decoding music in general, imagined music decoding remains rudimentary. In the present study of human electrocorticography, we aimed to reconstruct an imagined melody by decoding the relative pitch heights. Furthermore, we tried to elucidate the key areas in imagined pitch decoding. Ten medically intractable epilepsy patients without professional musical backgrounds participated in the present study. They were trained with 5 common children's songs. After training, they performed the task of listening, followed by imagery of certain melody parts. During the music imagery, we observed significantly increased high gamma activities in the temporal cortex, the inferior frontal cortex and the sensorimotor cortex. Neural features of electrodes in those areas activated during music imagery were used in decoding the relative pitch height (Do-Re-Mi-Fa-Sol-La) by a random forest algorithm. To reconstruct the imagined melody, decoding was performed in order within one melody chunk. Pearson's correlation coefficient was employed to evaluate the performance of reconstructing an imagined melody. We could reconstruct imagined melodies by decoding single-pitch heights, with a mean Pearson's correlation coefficient of 0.42 and cosine similarity of 0.86 with the original music. The performance of reconstructing imagined melodies was correlated with familiarity scores for 5 songs for subjects, with a correlation coefficient of 0.53. Notably, the left primary motor cortex (M1) and the right homotopic Broca's area significantly contributed to imagined pitch decoding. A previous EEG study suggested the involvement of the left sensorimotor cortex and the bilateral frontal opercular regions during music imagery. Previous fMRI studies revealed that the right pars triangularis is sensitive to tonal differences and reflects controlling pitch processing. Extending previous studies, we ascertained that left M1 and right homotopic Broca's area, significantly contributed to pitch decoding.

Disclosures: J. Kwon: None. **J. Kim:** None. **C. Chung:** None.

Poster

PSTR432. Human Perception, Imagery, and Imagination

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Program #/Poster #: PSTR432.05/SS4

Topic: H.02. Perception and Imagery

Support: ANID, Fondecyt de Iniciación N. 11190828

Title: Binocular Rivalry spatiotemporal neuronal dynamics connectivity through nonlinear EEG measures

Authors: *C. SARACINI, A. CANALES-JOHNSON, B. LUCERO;
The Neuropsychology and Cognitive Neurosci. Res. Ctr. (CINPSI Neurocog), Univ. Católica del Maule, Talca, Chile

Abstract: Perceptual multistability offers a privileged possibility to observe neuronal networks at different processing stages as they build up perceptual awareness. Binocular Rivalry (BR) is a special kind of multistable phenomena where each eye is simultaneously presented with an inconsistent image, causing that, at the phenomenological level, the individual starts to experience alternating percepts. The mechanisms through which the neuronal assemblies give rise to the conscious perceptual awareness of each stimulus at a time, temporarily shadowing the other through interocular suppression, are still unclear, with several and somehow opposite theoretical attempts to explain this peculiar phenomenon. Classical linear measures of brain connectivity currently used to explore perceptual multistability are only able to capture some aspects of the activity performed by neuronal assemblies during these processes, leaving others unexplored. Nonlinear analyses of brain connectivity have been shown to assess in a more direct way temporal dynamics of bistable alternations in different perceptual modalities, allowing to shed light on perceptual integration and differentiation at the basis of these phenomena. In this preliminary study, we used superposed red/green grids (with horizontal/vertical lines) to produce BR by means of ophthalmology filters. In an EEG experiment, we registered exogenously (control condition) and endogenously (BR) driven perceptual changes reported by participants, counterbalancing all the stimuli orientation and color and filter/eye association through the experimental blocks within subjects (preliminary sample: N=10 of 30 participants). We isolated response-locked epochs in order to calculate the proper time-window when the change of percept occurs. We conducted a BR analysis through a nonlinear EEG connectivity measure, that is Transfer Entropy (TE). This information-theoretic measure allows to explore how brain connectivity unfolds over time and brain areas (frontal ←→ posterior and right ←→ left hemispheres). Preliminar results reveal a specific directionality of the integrated information before subjects indicate to experience a perceptual switch. If this result is confirmed, it would support feedback accounts of BR. Moreover, specific dynamics in the interhemispheric stream of processing have been found, representing the peculiarity of the BR phenomenon with respect to other multistable phenomena. These results have relevant implications in the actual theoretical debate on the directionality of the neural networks temporal dynamics involved in the conscious perception.

Disclosures: C. Saracini: None. **A. Canales-Johnson:** None. **B. Lucero:** None.

Poster

PSTR432. Human Perception, Imagery, and Imagination

Location: WCC Halls A-C

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Program #/Poster #: PSTR432.06/SS5

Topic: H.02. Perception and Imagery

Title: Mystery of illusory perception: an inspection of 3D impossible objects

Authors: Y.-C. HSIEH[1], K. SUGIHARA[2], H.-H. LIU[3], C.-L. TSAI[4], ***W.-S. LAI**[1];
[1]Natl. Taiwan Univ., Taipei, Taiwan; [2]Meiji Inst. for Advanced Study of Mathematical, Meiji Univ., Tokyo, Japan; [3]Dept. of Clin. Psychology, Fu Jen Catholic Univ., New Taipei City, Taiwan; [4]Natl. Palace Museum, Taipei, Taiwan

Abstract: This study aims to enhance our understanding of illusory perception and sensory processing in 3D illusions by introducing updated task designs and advanced analysis methods, building upon our previous research (Hsieh et al., 2022). While there has been extensive research on 2D optical illusions, empirical investigations into 3D illusions remain scarce. To fill this gap, we collaborated with the second author to transform well-known 2D illusions into 3D impossible objects, which exhibit incongruity between the front view and their reflection in a mirror. To investigate the matching process between prior predictions and observed sensory data, as well as the signaling of sensory prediction errors, we developed the peekaboo task 2.0. Additionally, we designed the perceptual inference task 2.0 to explore the process of perceptual inference on novel objects and identify potential neural correlates. These tasks were accompanied by video clips showcasing authentic 3D impossible objects, providing a more realistic experimental setting. To gain insights into the neural dynamics and visual attention related to illusory perception, we employed two crucial measurement techniques: electroencephalography (EEG) and eye tracking. Through the combined use of EEG and eye tracking, we aimed to unravel the complex interplay between neural activity, visual attention, and illusory perception. These techniques allowed us to examine the temporal dynamics of brain responses and the allocation of visual attention during the processing of 3D illusions. To enhance our analysis, we incorporated two advanced methods: time-frequency analysis and graph-based connectivity analysis. Time-frequency analysis enabled us to explore the spectral dynamics of brain activity, providing insights into the temporal characteristics of neural responses during the perception of 3D illusions. Furthermore, the utilization of graph-based connectivity analysis, enabled us to explore the patterns of connectivity among the brain regions engaged in illusory perception. Through this investigation, we have expanded our understanding of the intricate mechanisms that underlie perceptual illusions in a three-dimensional context. The exploratory results uncover the neural dynamics involved in these illusions and provide valuable insights into the sensory processing of these unique 3D impossible objects.

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Poster

PSTR432. Human Perception, Imagery, and Imagination

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Program #/Poster #: PSTR432.07/SS6

Topic: H.02. Perception and Imagery

Support: Supported by a Georgia State University Brains & Behavior Seed Grant

Title: Atypical belief updating and novelty detection in psychedelic users during saccadic planning task

Authors: *C. L. WEST[1], A. DURAN[1], S. NADEEM[1], N. VAN LEEUWEN[2], J. P. HAMM[1]; [1]Neurosci., Georgia State Univ. Neurosci. Inst., Atlanta, GA; [2]Philosophy, Georgia State Univ., Atlanta, GA

Abstract: To infer the dynamic structure of our world, the human brain must be able to process information within context. One explanation of this function comes from the predictive coding framework, which posits that the brain continually generates and updates internal models of the environment. These models produce predictions of sensory input which derive from top-down prior beliefs, that are then compared to bottom-up sensory information. When stimuli that do not align with internal predictions are present, a prediction error is generated (i.e., novelty detection), signaling that the model needs to update its beliefs. Current research suggests serotonergic psychedelics' therapeutic effects may result from changes to the flexibility of individuals' belief systems, stemming from altered predictive processing. However, this remains untested. In the present study, we hypothesized that psychedelic use alters the distinct neurophysiological processes of belief-updating and novelty detection.

Here, we tested this hypothesis by recording EEG during a saccadic planning task. Twenty control participants and twenty psychedelic participants (those who used serotonergic psychedelics within 30 days) observed different colored stars that appeared in expected locations 80% of the trials and unexpected locations 20% of the trials. The color cues indicated whether the next star would appear at a new location (i.e., surprise update trials) or at the same location as previously (i.e., surprise no-update trials), allowing to experimentally tease apart brain processes related to "novelty detection" and "belief updating." We analyzed typical visual event related potentials (P3A, P3B, vMMN) as well as fronto-visual coherence - an index of top-down modulation, theoretically involved in predictive processing. We found that psychedelic users generated significantly smaller prediction errors in response to novel stimuli in both surprise trial types, as measured through P3A and vMMN. P3B, a potential an index of belief-updating, was modulated by surprise trial type in both groups. These findings suggest that the psychedelic participants were less "surprised" by the novel stimuli, potentially resulting from weaker priors. This research provides a potential mechanism for the effect of psychedelics, suggesting that psychedelics de-weight pathologically over-weighted prior beliefs that underlie various expressions of mental illness. The process of basic, cortical sensory belief updating may share basic neural mechanisms with higher level changes in beliefs that result from psychedelic use, and may underlie these changes.

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Poster

PSTR432. Human Perception, Imagery, and Imagination

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Program #/Poster #: PSTR432.08/SS7

Topic: H.02. Perception and Imagery

Support: Human Brain Project SGA3
CSC scholarship

Title: Contextual feedback signals during amodal completion and visual imagery in the early visual cortex

Authors: *Y. HUANG[1], Y. LAZAROVA[1], A. PATON[1], L. S. PETRO[1], L. MUCKLI[2]; [1]Sch. of Psychology and Neurosci., [2]Inst. of Neurosci. and Psychology, Col. of Medical, Vet. and Lif, Univ. of Glasgow, Glasgow, United Kingdom

Abstract: Amodal completion is representation of the occluded parts of a perceived object for which we receive no sensory stimulation, while visual imagery refers to visual representations or mental images without sensory input. Both amodal completion and imagery can generate visual experiences, which is related to internally-generated top-down signals. To investigate how top-down signals support amodal completion and visual imagery, we conducted a human 7T fMRI experiment with natural scene images combined with a visual occlusion paradigm that allows us to isolate top-down contextual signals in a non-stimulated region of V1 cortex. We recruited 28 healthy human participants to attend the fMRI experiment. In the first half of the fMRI experiment, participants viewed trials of the scene images with a white occluder placed over the lower-right image quadrant, and on other trials they had to try to imagine the full version of the previously seen occluded image. In the second half of fMRI scanning, on separate trials, participants either viewed the full scene image, or imagined the full image. This design allowed us to separate imagery trials where participants imagined image content that they had seen, from imagery trials where they had to generate images of the missing scene information based on the available contextual information. We performed a multi-voxel pattern analysis (MVPA) with a linear support vector machine approach and representational similarity analysis (RSA) for the fMRI data. The MVPA decoding results from the amodal completion condition show that the category information can be decoded in the occluded early visual cortex, replicating our previous findings that non-stimulated early visual areas carry information about surrounding context. The MVPA cross-decoding results between amodal completion and imagery trials show that the classifier cannot cross-decode the category information in amodal completion and imagery, which indicates that the feedback information in these two mental processes is different. The RSA results between imagery based on amodal completion and imagery based on full perception show a dissimilarity. This finding suggests that imagery activity differs if it is based on image-

specific memory, as opposed to being contextually-driven. Our findings, in conclusion, show that the feedback information utilised in amodal completion heavily relies on surrounding contextual information, whereas the feedback information employed in visual imagery might instead draw upon prior knowledge stored in the brain that can be updated by the feedforward information.

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Poster

PSTR432. Human Perception, Imagery, and Imagination

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Program #/Poster #: PSTR432.09/SS8

Topic: H.02. Perception and Imagery

Title: Information-theoretic basis of the physical nature of voluntary movement and perception

Authors: *D. S. GUPTA;
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Abstract: Gupta and Bahmer (2019) had previously proposed that sensoryinput and motor activities during an interaction of the brain with physicalsurroundings lead to an increase in Shannon entropy (a measure of information)measured in the resulting spiking activities of the cortex. This increase inentropy is reduced by increased mutual information, resulting from an increasedprobability of joint activation of pairs of neurons in different parts of thecortex. Rolf Landauer proposed in 1961 that an irreversible change ininformation dissipates a minimum amount of heat to its surroundings. Landauer'sPrinciple was first verified experimentally by Bérut A et al. (2012). Whenеverthere is a successful interaction, there will be an increase in the correlatedactivation of specific pairs of cortical neurons due to the constraints of theinteraction between the brain and physical surroundings. This increase inmutual information, from the correlated activation of neurons, in a successfulinteraction leads to an irreversible change in information. According toLandauer's Principle, this irreversible change in information after asuccessful interaction dissipates a tiny amount of energy to the environment, resultingin an increase in thermodynamic entropy in surroundings. The author argues thatan increase in thermodynamic entropy is consistent with the second law ofthermodynamics, which is responsible for the spontaneous physical nature ofawareness of surroundings and voluntary control of movements. Notethat action potential (AP) at the threshold carries one bit of informationsince there is an equal probability of an AP being triggered or not triggered.According to Landauer's Principle to use a single bit of information, theenergy needed is $3X10^{-21}$ J. Moreover, given an assumption that Na+ions behave as gas molecules during the passage through Na+ channels, with anaverage kinetic energy of $6.41X10^{-21}$ J, and there is an equalprobability that Na+ ions will be entering or exiting through the channels atthe threshold, the author argues Na+ entry or exit will be on average stoppedonce in two movements to prevent or trigger an AP. Thus, on average, the energyneeded to prevent/activate an AP will be $3.2X10^{-21}$

J, which agreeswith the energy needed to use a single bit of information. Thus, when an AP inthe cortex becomes biased by sensory or motor inputs, there is a decrease inthe average uncertainty, which is further decreased by temporal coupling, givena cognitive task, giving rise to perception and voluntary motor control.

Disclosures: D.S. Gupta: None.

Poster

PSTR432. Human Perception, Imagery, and Imagination

Location: WCC Halls A-C

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Program #/Poster #: PSTR432.10/SS9

Topic: H.02. Perception and Imagery

Support: James S. McDonnell Foundation
NSF GRFP

Title: Larger than life: Cartoons drive infant visual cortex more than realistic movies

Authors: *T. S. YATES[1], A. LETROU[1], J. E. TRACH[1], D. CHOI[1], L. BEHM[1], C. T. ELLIS[3], N. B. TURK-BROWNE[1,2];
[2]Wu Tsai Inst., [1]Yale Univ., New Haven, CT; [3]Psychology, Stanford Univ., Stanford, CA

Abstract: The human visual system exhibits sophisticated structure and function even in infancy, including retinotopic organization in early visual areas and category-selective responses in late visual areas. Yet, there is also dramatic plasticity and perceptual learning early in development, and thus it is unclear how the tuning of infant visual cortex relates to adults. For instance, infant visual cortex may be optimized to process realistic input, given the types of visual input it experiences the most. Alternatively, developing sensory systems may respond more to exaggerated features that emphasize diagnostic information. Here, we evaluate this sensory exaggeration hypothesis directly, testing whether cartoonized content drives activity in the human infant visual cortex more strongly and consistently than naturalistic content. In an ongoing study, we collected fMRI data from infants (4-15 months) while they watched two versions of a 3-minute movie: 2-D animated cartoon and 3-D realistic computer-generated imagery. Critically, the two movies were almost perfectly matched in content on a frame-by-frame basis. This allowed us to examine how the infant brain responds to certain combinations of visual features (i.e., shape, contrast, color) while controlling for semantic content and storyline. Infants watched the two movies in a random order, and there was no significant difference across movies in the amount of fMRI data excluded for head motion or looking away. As a baseline, we also collected data from 12 adults who watched the same movies under similar conditions, but for whom we did not expect a preference for cartoons. We performed intersubject correlation analyses in both infants and adults to assess the robustness and reliability of brain activity evoked by the two movie conditions. As expected, visual cortex responded consistently to the movies across adult participants, and there were no significant differences between the cartoon

and realistic conditions. However, the cartoon movie drove more similar spatiotemporal patterns of activity in visual cortex across infant participants, compared to the realistic movie, across the visual hierarchy. To assess how these results relate to certain visual features (e.g., color) or processes (e.g., face detection) in the movie, we are conducting multivariate pattern classification and pattern similarity analyses. These preliminary results suggest that the visual system may be especially attuned to diagnostic visual features in early development. This informs our understanding of infant visual experience and supports the sensory exaggeration hypothesis of perceptual development.

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Poster

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Topic: H.02. Perception and Imagery

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Title: Developmental prosopagnosics have normal visual field coverage in face-selective regions

Authors: *D. A. STEHR[1], Y. ZHANG[1], A. KIDDER[1], K. KAY[2], B. DUCHAINE[1]; [1]Dartmouth Col., Hanover, NH; [2]Ctr. for Magnetic Resonance Research, Dept. of Radiology, Univ. of Minnesota, Minneapolis, MN

Abstract: Developmental prosopagnosia (DP) is a neurodevelopmental condition characterized by extreme difficulty with facial identity recognition despite normal low-level vision and no known brain damage. Preliminary evidence from population receptive field (pRF) modeling suggests that pRFs in face-selective and intermediate visual areas, which normally cover a wide field of view, are strikingly restricted in DPs (Witthoft, 2016, bioRxiv), an organization that could hinder the formation of complex, holistic representations of facial features and their inter-relationships. Although this finding came from a small sample of DPs (n=8), it comports well with reports from DPs that they often base their decisions on slow, effortful piecemeal analysis of featural details. To more thoroughly investigate receptive fields in DP, we measured pRF properties using the compressive spatial summation (CSS) model applied to early, intermediate, and category-selective visual areas in 20 controls and 13 DPs. Participants fixated centrally while colorful, high-contrast images of scenes, faces, limbs, and objects windowed by bar-, wedge-, and wheel-shaped apertures traversed the visual field. In both DPs and controls, we observed sharply defined polar angle and eccentricity maps in early visual areas, typical scaling between pRF eccentricity and size, and strong spatially-modulated responses in face-selective areas with pRF density concentrated just to the contralateral side of the fovea. Absent, however, were reductions in pRF size in early, intermediate, or face-selective regions in DPs. These findings,

using a larger sample of DPs and the latest methods for modeling pRFs, indicate that receptive field size is normal in DP and that their deficits with face recognition result from differences in other neurocomputational characteristics.

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Poster

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Topic: H.02. Perception and Imagery

Support: WARF URI 2021
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Title: Poiesis (psychedelic outcomes: interaction of environment, self-identity, and success) - importance of naturalistic elements and religion/spirituality in a psychedelic dosing space

Authors: *S. LEE[1,2], C. EPLAND[2], R. R. GAJIPARA[2], N. A. KAITZ[2], C. J. WENTHUR[3,2,1]; [1]Univ. of Wisconsin - Madison Neurosci. Training Program, Madison, WI; [2]Sch. of Pharm., UW-Madison, Madison, WI; [3]Psychoactive Pharmaceut. Investigation Program, Univ. of Wisconsin-Madison, Madison, WI

Abstract: Racial and ethnic minorities remain underrepresented in psychedelic-assisted psychotherapy, which has shown promising therapeutic effects for mental illness. Greater inclusion of racial and ethnic minorities may indirectly influence therapeutic outcomes, which are proposed to be mediated through the "set and setting" framework. Since differences in psychotherapy acceptance could possibly be based on self-identity with race and ethnicity, self-identity may also act as a therapeutic filter in the psychedelic dosing room. We hypothesize that the environment, such as artwork, in the psychedelic dosing room influences one's experiences based on racial self-identity connectivity. Online surveys (n = 141; 49% BIPOC) were filled out by members of psychedelic societies based in Wisconsin and Texas. The survey content included demographics (age, gender, race/ethnicity, religion/spirituality) and artwork object connectivity (visual analogue scale) to 15 different objects found in the psychedelic dosing space. Furthermore, 7 focus groups (FG) (n = 1-4; 63% BIPOC) were formed based on demographic information given in the survey. The FGs were split into Black, Latino, Native American, and Non-Hispanic White participants. They were virtually interviewed for perceived connections with dosing room items and the dosing room itself. Multiple regression analyses revealed that racial/ethnic, age, and gender identities has a small impact on perceived connection with the artwork. Religion/spirituality had the largest effect size and is significantly greater than other identity-perceived connections. The major theme that arose in the FGs was a preference for *natural*-like settings and objects, specifically *plants*, in both the dosing room and art.

Additionally, both *physical* and *mental comfort* were another major theme mentioned in both the art objects and the dosing room. While *intention* regarding both the dosing room and art objects was mentioned, it was more prevalent in the latter. Lastly, many individuals from the FGs *narratively engaged* with the art object through *autobiographical*, *religion/spiritual*, and/or *time/mortality* statements. In conclusion, we find that religion/spirituality had a much larger impact on perceived connectivity to art objects than race or ethnicity. One's own comfort and the presence of naturalistic objects were important aspects in the psychedelic dosing room. With increasing movement towards adopting psychedelic-assisted psychotherapy, we recommend crafting the psychedelic dosing space with the themes we identified here.

Disclosures: S. Lee: None. **C. Epland:** None. **R.R. Gajipara:** None. **N.A. Kaitz:** None. **C.J. Wenthur:** 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.; received funding/support from Psilera Inc., and Mike and Mary Shannon for the study of psychedelics.. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); received funding/support from Usona Institute for the study of psychedelics.

Poster

PSTR432. Human Perception, Imagery, and Imagination

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR432.13/SS12

Topic: H.02. Perception and Imagery

Support: NSERC Grant 194517-03

Title: Covert and spontaneous brain to brain communications during memorization of simultaneous images

Authors: *S. CALMELS[1], E. JEULAND[2], M. LENNE[2], F. JARRY[3], J. DEBRUILLE[2]; [1]McGill Univ., Integrated Program in Neurosci., Montreal, QC, Canada; [2]McGill Univ., Montreal, QC, Canada; [3]Univ. de Montréal, Montreal, QC, Canada

Abstract: Many times, science starts by reporting facts that cannot be explained. This is the aim of the present work. With methods that have been refined for 10 years our goal is to see whether one can definitely confirm a surprising hypothesis. In effect, two previous works report that the event-related brain potentials (ERPs) that are evoked by presenting a picture to a participant can be modulated by simultaneously and privately presenting a picture to a partner. A reprocessing of the data of these works showed that these modulations existed only, or mostly, in precise circumstances, which were then copyrighted. A new experiment was thus run only in those circumstances to obtain simple and robust effects. We recorded the ERPs evoked by presenting, at each trial, the photograph of a face. Simultaneously and again, privately, we presented the same face or a different one to the partner. (S)he was in an adjacent room and could not

communicate. The ERPs of these partner-participants were found to strongly depend on the sameness of the two photographs, unbeknownst to them. These joint processing effects (JPEs) confirm that a simple and robust method can be used to study the sensitivity of the human brain to the brain activity of another person. This should help future works answering the many questions raised by this sensitivity, starting with the nature of the physical phenomenon at stake.

Disclosures: **S. Calmels:** None. **E. Jeuland:** None. **M. Lenne:** None. **F. Jarry:** None. **J. Debruille:** None.

Poster

PSTR432. Human Perception, Imagery, and Imagination

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR432.14/SS13

Topic: H.02. Perception and Imagery

Support: JST Moonshot R&D Grant Number JPMJMS2237

Title: Exploring Facial Feature Influence in Masked Face Recognition: Insights from Brain Activity and Eye Fixation Duration on the Face Feature Area

Authors: *I. CHANPORNPAKDI[1], Y. WONGSAWAT[2], T. TANAKA[1];
[1]Tokyo Univ. of Agr. and Technol., Koganei-shi, Japan; [2]Mahidol Univ., Nakhon Pathom, Thailand

Abstract: Objective: During the SARS-CoV-2 pandemic, communication with face masks was common. Nevertheless, people retrain the ability to distinguish and recognize faces. Previous studies showed that the eye might be a crucial component in face cognition, but the face cognition mechanism of the masked face is still under discussion. This study aimed to identify the influence of each face feature using the relationship between brain response and eye-tracking in partial face cognition. We hypothesize that people focus on the eye area the most, and recognizing a full face and a masked face is highly correlated. Method: The experiment was performed on 18 healthy volunteers, nine females (mean age 28.21±2.34, min 23 and max 33). The electroencephalogram (EEG) and eye-tracking were recorded when the participant performed the partial face cognition task. The task consisted of the six face images shown in seven conditions; full and part face, including faces with eyes covered, nose covered, mouth covered, eyes and nose covered, eyes and mouth covered, and nose and mouth covered. Among six face images, one face image was set as the target face, and the participant was asked to identify that target face. To find the relationship between the brain and eye-tracking response, the canonical correlation between event-related potential (ERP) component, calculated by grand averaging the EEG among all participants, and the total fixation duration of the eye-tracking in eyes, nose, mouth, and fixation area was calculated. Results: We found that the ERP amplitude when the eye component was presented was more significant than that when the eyes were absent. Moreover, longer fixation duration was observed in the eye area when the eyes were

visible. We also observed a high correlation between the full face and the masked face-like partial face; the face with mouth covered and the face with nose and mouth covered. Discussion: The results confirmed that the eye component plays the most crucial role in face cognition, and there is a similarity between full face (holistic face) cognition and partial face (masked face) cognition. This could explain how people can recognize masked faces correctly.

Disclosures: I. Chanpornpakdi: None. **Y. Wongsawat:** None. **T. Tanaka:** None.

Poster

PSTR432. Human Perception, Imagery, and Imagination

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR432.15/SS14

Topic: H.02. Perception and Imagery

Support: BBSRC: BB/V003917/1

Title: Dissociating the roles of category selective visual and medial parietal regions during perception and recall of familiar stimuli: an EEG-MRI fusion approach

Authors: *C. L. SCRIVENER, E. H. SILSON;
Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: The interaction between networks facilitating visual perception and recall is not fully understood. It has often been assumed that the same regions are activated in response to both perception and recall of the same stimuli (Dijakstra, 2019) and that reinstatement of the perceived stimuli during recall would result in similar MRI or EEG activity during both states (Xie, 2020). However, category selective regions of medial parietal cortex (MPC) (Silson, 2019) are active during recall, differing from the category selective regions in the visual cortex recruited during perception. We used an EEG-fMRI fusion approach to ask how the similar neural dynamics of category selectivity reported in EEG relates to activity within these visual and MPC regions.
Multivariate pattern analysis (MVPA) was first used to decode the category of personally familiar stimuli (people or places) across all EEG electrodes. During both perception and recall, category information was decoded above chance level throughout the trial, emerging around 140ms after stimuli onset for perception and 200ms for recall. Further, the patterns identified at the emergence of category selectivity in both states successfully time generalized throughout the rest of the trial, indicating a stable representation through time. Using a cross-decoding approach, we also found that category representations identified during perception (around 100ms and 200-300ms) were able to classify stimuli type during the recall trials (between 200ms-1000ms). This indicates a reinstatement of the perceptual classification of category during recall, and adds to the evidence for a link between these two processes (Xie, 2020, Steel, 2023).
How does this reinstatement relate to visual and parietal activation? We used EEG-MRI fusion to correlate representational similarity matrices for all stimuli constructed at each time point in the

EEG with similar matrices constructed across all MRI voxels in the parahippocampal place area, fusiform face area, and people/place memory areas in the MPC. Two peaks of high correspondence were identified in both conditions, occurring between 150-200ms and 350-600ms after stimuli onset for perception, and between 200-300ms and 1000ms-2000ms for recall. Although we expected perception representations to correlate more strongly with visual ROIs, and recall with MPC ROIs, we found similar time courses across all regions. These results suggest the involvement of both visual and parietal regions during perception and recall, with representations emerging at different points throughout the trial. Further work is therefore needed to delineate the exact nature of their contributions

Disclosures: C.L. Scrivener: None. **E.H. Silson:** None.

Poster

PSTR432. Human Perception, Imagery, and Imagination

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR432.16/Web Only

Topic: H.02. Perception and Imagery

Support: Intramural Research Program of the National Institute of Nursing Research
Indiana University
The Ohio State University

Title: Phenotypic clusters of cancer-related cognitive impairment based on subjective reports and objective computerized cognitive function scores.

Authors: *T. GOTO[1], L. SALIGAN[1], P. JUNEAU[2], S. GONSALVES[1], C. RIO[1], L. Y. GRAVES[3], D. VON AH[4];
[1]NIH/NINR, Bethesda, MD; [2]Natl. Inst. of Hlth. Library, Natl. Inst. of Hlth., Bethesda, MD; [3]Univ. of Texas Med. Branch, Sch. of Nursing, Galveston, TX; [4]The Ohio State University, Col. of Nursing, Columbus, OH

Abstract: Cancer survivors experience cancer-related cognitive impairment (CRCI) affecting productivity and quality of life. CRCI is often clustered with conditions (e.g., symptoms, behaviors) and influenced by intrinsic factors (e.g., sociodemographic characteristics). The management of CRCI and its clusters remains challenging because there is no agreed standardized assessment of CRCI. This study identified CRCI clusters based on subjective reports and cognitive function test scores. VARCLUS™ (SAS/STAT; SAS 9.4; Cary, NC) and k-means clustering technique were used based on CRCI reports assessed by the PROMIS® short-form subscales of Cognitive Abilities and Cognitive Concerns and computerized CANTAB Cambridge Cognition® scores (i.e., visuospatial working memory capacity, visual episodic memory, new learning, working memory, executive function, and sustained attention). This clustering technique identified five CRCI cluster groups. Phenotypic characteristics of each

cluster identified associated demographic profiles, physical/ psychosocial factors (i.e., physical function, affect, optimism, social support), and symptoms (i.e., anxiety, depression, fatigue, neuropathic pain, sleep disturbance). Of the 414 participants, 99% were female and 93% self-identified as White. Cluster groups 4 and 5 had the highest cognitive abilities and the lowest cognitive concerns. In terms of demographic factors, participants in cluster group 5 were mostly employed full time and have higher education and income than the other cluster groups. Further, participants in cluster group 5 had the highest physical function, positive affect, optimism, and social support among the cluster groups. Moreover, participants in cluster 5 were less likely to experience severe cancer-related symptoms such as anxiety, depression, fatigue, neuropathic pain, and sleep disturbance than other cluster groups. On the other hand, participants in cluster group 1 showed the worst cognitive function, had worst physical function, low affect and optimism, and less social support with more severe symptoms than the rest of the cluster groups. The clustering approach that we employed in this study using both subjective and objective cognitive function information may provide promising phenotypes that can potentially be clinically relevant to categorize patient CRCI presentations. Validation of the study findings is warranted particularly enrolling a more diverse cohort of participants across demographic populations from a broad spectrum of psychosocial experiences and investigating the influence of social determinants of health on CRCI.

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Poster

PSTR432. Human Perception, Imagery, and Imagination

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR432.17/SS15

Topic: H.02. Perception and Imagery

Support: NIH R01-EY025648

Title: Behavioral and neural correlates of impaired scene perception following saccadic eye movements

Authors: *Y. CHOI[1], T.-Y. CHIU[3], J. D. GOLOMB[2];
[1]Psychology, [2]Dept of Psychology, Ohio State Univ., Columbus, OH; [3]The Ohio State Univ., Columbus, OH

Abstract: People make ballistic eye movements, known as saccades, several times per second to efficiently explore visual environments. Visual input projected onto the retina drastically changes across saccadic eye movements, and it is known that our sensitivity to simple visual stimuli (e.g., Gabor patches) decreases when they are presented around the time of the saccade. However, it is less clear how visual information processing for more realistic and complicated visual stimuli is affected by saccades. Through behavioral and fMRI experiments, we presented scene images

filtered at different spatial frequencies and presented at different times relative to a saccade, to explore how saccadic eye movements influence the processing of visual scenes. In a behavioral experiment, a scene image was flashed for a short duration (50 ms) either immediately (5 ms) after saccadic eye movement or after a delay (500 ms). Subjects were asked to categorize scene images into six categories (e.g. beach, mountain, forest, city, office, and highway). Scene images were filtered through either low- or high-pass spatial frequency filters to further investigate whether post-saccadic scene categorization performance depends on spatial frequency information. For both spatial frequency conditions, we found decreased scene categorization performance for images flashed immediately after the saccade compared to later after the saccade, indicating non-specific impaired visual information processing for realistic scene images. Subsequently, the fMRI study investigated neural correlates of impaired post-saccadic scene perception in the absence of an explicit categorization task, to explore how neural representations of scene category and spatial frequency are influenced by saccades. In the scanner, subjects performed a 1-back task on scene images flashed after a short or long post-saccadic delay. Scenes were drawn from four scene categories (two urban, two nature) and four spatial frequencies (two high SF, two low SF). Consistent with the behavioral findings, preliminary results revealed reliably decreased activity in the scene-selective cortex (PPA) for trials when the scene image flashed within 100 ms after a saccade, compared to when it flashed at least 200 ms after the saccade. Furthermore, multivariate Representational Similarity Analyses were conducted to compare the representation of scene contents and spatial frequency across the post-saccadic delays.

Disclosures: **Y. Choi:** None. **T. Chiu:** None. **J.D. Golomb:** None.

Poster

PSTR432. Human Perception, Imagery, and Imagination

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR432.18/SS16

Topic: H.02. Perception and Imagery

Support: Trinity College Dublin Foundation Scholarship
ERC Consolidator Grant IndDecision, No 865474

Title: Altered perceptual decision-making in aphantasia versus visualisers - a comparative electroencephalograhy analysis

Authors: *F. M. O'SULLIVAN, K. KALOU, R. G. O'CONNELL;
Trinity Col. Dublin, Dublin, Ireland

Abstract: Aphantasia, an inability to voluntarily form visual images, is estimated to affect 300 million people worldwide. Its study has enormous potential to inform several areas of cognitive neuroscience, but due to its recent identification and parsing out from confounding phenomena, aphantasia remains heavily under-researched. Here, we fill a critical neuroimaging gap by

reporting the first electroencephalography (EEG) study explicitly comparing people with aphantasia (n=13) and people who can visualise (n=25), as a control. We utilised a modified contrast discrimination paradigm to assess differences in perceptual decision-making between groups. Both groups received cues to imagine a grating either congruent or incongruent to the direction of a subsequently presented grating. As an exploratory study, we conducted several analyses at each stage of the sensorimotor hierarchy. Differences in sensory signal strength were measured through the amplitude of steady steady visual evoked potentials (SSVEPs), evidence accumulation to a bound through the centro-parietal positivity (CPP) build-up, and motor preparation through lateralized mu/beta band desynchronisation. Aphantasics performed faster, which may fit with the 'alternative strategies' accounts of aphantasia. Our study has implications for both mental imagery and perceptual decision-making research. We integrate our electrophysiological findings with existing fMRI, psychophysics and binocular rivalry studies of aphantasia and mental imagery. We also discuss how our findings inform debate on the differential mechanisms of modulation of perception due to prior expectations, imagery and attention.

Disclosures: **F.M. O'Sullivan:** None. **K. Kalou:** None. **R.G. O'Connell:** None.

Poster

PSTR432. Human Perception, Imagery, and Imagination

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR432.19/SS17

Topic: H.02. Perception and Imagery

Support: NSTC-111-2423-H-002-012

Title: Cortical activations for spatial configuration processing in skilled and poor readers

Authors: *S.-C. HUNG[1], H.-L. WANG[2], C.-C. CHEN[1];
[1]Natl. Taiwan Univ., Taipei, Taiwan; [2]Natl. Taiwan Normal Univ., Taipei, Taiwan

Abstract: Analysis of spatial configuration is important in identifying visual word forms. We investigated the effect of symmetry on character composition, which would require the visual system to analyze the spatial relationships among strokes during visual word form processing in developmental dyslexic (DD) children and chronological aged (CA)-matched typical children. We measured BOLD activations for real-, non-, and scrambled characters with symmetric or asymmetric compositions in a block-design functional magnetic resonance image (fMRI) experiment. Participants were asked to match the presented character with that of the previous trial. Compared to scrambled characters, real-characters elicited activations in the fusiform gyrus for the CA group but in the lingual gyrus for the DD group. Compared to real-characters, non-characters elicited activations in the lingual and fusiform areas for the CA group, whereas this pattern was absent for the DD group. The left fusiform activation in the CA group was consistent with the visual word form areas in the literature. Comparing the symmetric with the asymmetric

conditions, the CA group showed activations in the bilateral fusiform gyri, lingual and inferior occipital areas, whereas the DD group did not. Our findings reveal differential neural evidence between dyslexic and typical children, suggesting that the fusiform gyri and occipitotemporal areas may play a role in analyzing symmetric configurations in orthographic characters. Typical children showed neural responses in the fusiform gyri and occipitotemporal areas during processing symmetric versus asymmetric configurations, but these activations were absent in dyslexic children. Thus, the poor reading performance of dyslexic children may result from impaired spatial configuration processing in identifying visual word forms.

Disclosures: S. Hung: None. **H. Wang:** None. **C. Chen:** None.

Poster

PSTR432. Human Perception, Imagery, and Imagination

Location: WCC Halls A-C

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Program #/Poster #: PSTR432.20/Web Only

Topic: H.02. Perception and Imagery

Support: NINDS Intramural Research Program, Grant No. ZIA NS003144-09

Title: Synchronous and asynchronous spiking states in the human cortex during image viewing

Authors: *V. WANG, K. A. ZAGHLOUL;
Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD

Abstract: Intracranial electroencephalography (iEEG) recordings in the human brain show a spectrum of neural ensemble activity states, but the cognitive drivers behind these empirically observed states are not yet well understood. Such population activity states range from synchronous regimes exhibiting discrete bursts of communal spiking activity to asynchronous regimes lacking clear patterns of neural coordination. To explore the causes differentiating these states, we design an experiment informed by computational neural network simulations suggesting that distinct activity states (synchronous regular, synchronous irregular, asynchronous regular, asynchronous irregular) arise from variations in the strength of external drive to a neural ensemble. Four experimental behavioral conditions—viewing an image, thinking about an image, resting with eyes open, and resting with eyes closed—are used to modulate this external drive to the brain in human subjects, with the hypothesis that increased external input induces more chaotic, asynchronous states, while decreased external input leads to more patterned, synchronous states. During iEEG data collection, implanted microelectrode arrays record activity from individual neurons in the anterior temporal lobe (ATL) of epilepsy patients undergoing a task session that spans the experimental conditions. From the recorded voltage traces, spikes associated with individual units are detected and their timestamps are extracted. To quantify the degree of ensemble spiking synchrony for condition comparison, we calculate a neural synchrony measure based on variances in individual unit and ensemble firing rates. A preliminary analysis in three subjects demonstrates slight decreases in neural synchrony when

moving from the eyes-closed state to the eyes-open state, aligning with our hypothesis. Furthermore, by implementing a probabilistic hidden Markov model with Poisson emissions, we detect “On” (vigorous spiking activity) and “Off” (relative quiescence) episodes within all experimental trials in a systematic manner. This analysis permits the characterization of ensemble spiking behavior in greater detail. Initial pairwise comparisons across experimental conditions reveal statistically significant differences in the mean durations of “On” episodes between viewing-image vs. thinking-about-image, thinking-about-image vs. eyes-open, and thinking-about-image vs. eyes-closed conditions. By applying these mathematical models, we can assess whether empirically observed brain states are experimentally replicated and subsequently elucidate the cognitive modes that underlie them.

Disclosures: V. Wang: None. **K.A. Zaghloul:** None.

Poster

PSTR432. Human Perception, Imagery, and Imagination

Location: WCC Halls A-C

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Program #/Poster #: PSTR432.21/SS18

Topic: H.02. Perception and Imagery

Support: NSF BCS 2022572

Title: Establishing task-general representations and the cues which drive them in spatial perception

Authors: *P. MAHABLESHWARKAR[1], J. PHILBECK[2], D. J. KRAVITZ[3];
[1]The George Washington Univ., District of Columbia, DC; [2]The George Washington Univ., Washington, DC; [3]NIH, BETHESDA, MD

Abstract: Visual representations are remarkable in their ability to integrate multiple cues towards unified percepts and to adapt to various tasks, both of which make them powerful but difficult to study. Here, we leverage natural scene image databases paired with a novel series of pre-registered crowd-sourced behavior studies to discern a task-general representation of egocentric depth and the pictorial cues which drive it. We presented 156 novel scenes with embedded targets and found a remarkable level of scene specific systematicity and generalization across independent groups of participants performing different tasks (metric depth estimation MDE, depth discrimination DD) with distinct stimulus durations (125, 250, 1000ms). In MDE, participants showed not only a strong overall sensitivity to target depth in these 2D scenes, but also highly reliable differences across individual scenes distinct from the general effect of depth (e.g., r^2=.801, $p<10^{-76}$). Moreover, these scene-specific deviations strongly predicted DD performance in an independent group of participants for particular scene pairs (e.g., r^2=.312, $p<10^{-20}$). Overall significant relationships were found that generalized across participants, tasks, durations, and outcome measures, indicating systematic biases for individual scenes independent of those factors. For instance, DD RT predicts MDE RT at every combination of duration (all r >

0.27, all $p<10^{-3}$). Thus, cues within the 2D images must consistently drive 3D spatial perception, enabling in-depth examinations of individual cue contributions (e.g., familiar size, ground plane). Using an unbiased computer vision approach to quantify pictorial information, we uncover a novel relationship between the real-world size of the ground plane and egocentric depth perception even with stimulus presentations as short as 125ms ($F(1,146.62)=34.26$, $p<10^{-7}$). These findings suggest a finely tuned and rapid mechanism for integrating relative depth information, spatial cues, and object information towards depth perception. Further, the success of this method suggests a scalable approach (for all 6 tasks: N=2,178, collection time = 2 weeks, total cost ~$5,500) to quantifying task-general representations which can guide stimulus selection and task design for neurophysiological investigations.

Disclosures: P. Mahableshwarkar: None. **J. Philbeck:** None. **D.J. Kravitz:** None.

Poster

PSTR432. Human Perception, Imagery, and Imagination

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR432.22/SS19

Topic: H.02. Perception and Imagery

Support: NIH MSTP T32 Training Grant
R01 MH132225
R01 MH107797
NSF 1734907

Title: What aspects of the electrophysiological signal contain stimulus information?

Authors: *D. GENG[1], A. ALREJA[3], J. A. COLAN[4], R. M. RICHARDSON[6], T. ABEL[5], A. S. GHUMAN[2];
[2]Neurolog. Surgery, [1]Univ. of Pittsburgh Sch. of Medicine, Carnegie Mellon Univ., Pittsburgh, PA; [3]Carnegie Mellon Univ., Pittsburgh, PA; [5]Neurolog. Surgery, [4]Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA; [6]Neurosurg., Massachusetts Gen. Hosp., Boston, MA

Abstract: Introduction.
Electrophysiological signals can be decomposed in many ways, with different aspects of the signal thought to arise from particular neurophysiological processes. Studies often focus selectively on specific components of a neural signal (e.g., high-frequency broadband [BB], event related potentials [ERP], or spectral decomposition), potentially overlooking critical information. Little is known regarding what aspects of the signal carry the most information about task or stimulus conditions. To address this question, we examined which components of human intracranial electroencephalography (iEEG) signals carry the most informative data about categories of visual stimuli.
Methods.
We analyzed iEEG recordings from 40 participants using 5700+ total electrodes during a six-

category one-back test. We pre-processed raw iEEG signals to extract two neural signal components: ERP and BB components. Furthermore, we explored information distribution among the oscillatory (periodic) and non-oscillatory (aperiodic) components using the FOOOF toolbox. We used multivariate classification performance to measure the amount of information contained within the ERP and BB signal components (or the periodic and aperiodic components). We first identified if the activity in the electrode contained significant information about the stimulus categories in the ERP, BB, or both (or the periodic, aperiodic, or both) components, then compared the relative amount of information contained in these different components.
Results.
Across all categories and patients, we discovered that 3.8% of electrodes contained significant information in the ERP, 6.5% in the BB, and 1.2% in both components. Among these, some electrodes preferentially held information in the ERP (9.0%) and in the BB (13.0%). Notably, there was consistency within participants as to which component held information, with the heterogeneity arising primarily between participants. The aperiodic component of the signal was notably more informative (5.8%) than the periodic component (0.9%), emphasizing the value of non-oscillatory background components of neural signals. We are also analyzing iEEG data from an auditory task to assess the generalizability of these results across sensory domains.
Discussion.
Our findings highlight the need to examine all signal components collectively for comprehensive understanding of neural activity. They also underscore the need for deeper understanding of the neurophysiological basis of the heterogeneity regarding which aspects of electrophysiological signals give rise to information processing.

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Poster

PSTR432. Human Perception, Imagery, and Imagination

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Ministry of Science and Technology of China (STI2030-Major Projects 2021ZD0204202)
CAS Project for Young Scientists in Basic Research (YSBR-071)

Title: Spatiotemporal dynamics of self-generated imagery reveal a reverse cortical hierarchy from cue-induced imagery

Authors: *Y. HU[1,2], Q. YU[1];
[1]Inst. of Neuroscience, Ctr. for Excellence in Brain Sci. and Intelligence Technology, Chinese Acad. of Sci., Shanghai, China; [2]Univ. of Chinese Acad. of Sci., Beijing, China

Abstract: Visual imagery, the ability to generate visual experience in the absence of direct external stimulation, allows for the construction of rich internal experience in our mental world. Most imagery studies to date have focused on cue-induced imagery, namely the to-be-imagined contents were triggered by external cues. It has remained unclear how internal experience derives volitionally in the absence of any external cues, and whether this kind of self-generated imagery relies on an analogous cortical network as cue-induced imagery. Here, leveraging a novel self-generated imagery paradigm, we systematically examined the spatiotemporal dynamics of self-generated imagery, by having participants volitionally imagining one of the orientations from a learned pool; and of cue-induced imagery, by having participants imagining line orientations based on associative cues acquired previously. Our results revealed that: at the behavioral level, self-generated imagery was experienced as less vivid compared to cue-induced imagery; at the neural level, using electroencephalography (EEG) and functional magnetic resonance imaging (fMRI), in combination with multivariate encoding and decoding approaches, cue-induced and self-generated imagery demonstrated largely overlapping neural signatures in both EEG and fMRI; yet, these neural signatures displayed substantially differential sensitivities to the two types of imagery: self-generated imagery was supported by an enhanced involvement of anterior cortex in generating and maintaining imagined contents, as evidenced by enhanced neural representations of orientations in sustained potentials in central channels in EEG, and in posterior frontal cortex in fMRI. By contrast, cue-induced imagery was supported by enhanced neural representations of orientations in alpha-band activity in posterior channels in EEG, and in early visual cortex in fMRI. These results jointly support a reverse cortical hierarchy in generating and maintaining imagery contents in self-generated versus externally-cued imagery.

Disclosures: **Y. Hu:** None. **Q. Yu:** None.

Poster

PSTR433. Decision-Making: Prefrontal Cortex

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR433.01/SS21

Topic: H.03. Decision Making

Support: U19NS123714
U19MH114830

Title: Morphological and transcriptomic diversity of thalamocortical projections

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Abstract: The thalamus is a central hub of the forebrain, and aside from recurrent intratelencephalic connections, is the primary excitatory driver of cortical activity. The thalamus contains several nuclei with structurally and molecularly diverse thalamocortical projection neurons, yet the correspondence between gene expression patterns, cortical projection targets, and subcortical inputs is poorly understood. We are using a combination of approaches to investigate how non-sensory thalamus routes subcortical information to frontal cortex. We use spatial transcriptomics to understand gene expression motifs and transcriptomic cell types within and across thalamic nuclei. Multiplexed error-robust fluorescent *in situ* hybridization (MERFISH) delineates the spatial arrangement of transcriptomic types and gene expression gradients, while projection types are identified using expansion-assisted selective plane illumination microscopy (ExaSPIM) to create single neuron reconstructions on specific genetic backgrounds. We combine this with traditional viral tracing techniques to generate a comprehensive view of circuit inputs and outputs for molecularly defined cell types in the thalamus. Our cell-type resolved anatomical analysis of the thalamus is a foundation for understanding computation in multi-regional circuits with thalamus in the middle.

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Poster

PSTR433. Decision-Making: Prefrontal Cortex

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

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Topic: H.03. Decision Making

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KAKENHI JP22K15618

Title: Differential Roles of the Medial Prefrontal and Sensory Cortices in Integration of Prior Knowledge and Sensory Inputs in Perceptual Decision-Making in Mice

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Abstract: In perceptual decision-making, the integration of sensory inputs and prior knowledge of action outcomes plays a crucial role in optimizing choices. Our previous study (Funamizu, iScience, 2021) examined this integration through an auditory discrimination task in mice. This study investigates the necessity of the auditory cortex (AC) and medial prefrontal cortex (mPFC)

in auditory-perceptual decision-making processes through optogenetic manipulation. Wild-type CBA/J mice were used for the experiments. We injected AAV-CKIIa-stGtACR2-FusionRed and implanted optical fibers bilaterally into either the AC or mPFC of the mice. The delivery of 473 nm photo-illumination through the fibers allowed to activate the stGtACR2 protein and suppress neural activity in the target region (AC or mPFC). The mice performed a tone-frequency discrimination task, associating high- or low-frequency tones with left- or right-spout water rewards. We differenced the reward amounts for the left and right spouts, and interchanged the reward difference for each block of 90 to 100 trials, with each trial presenting either a long- or short-tone. Light stimulation was applied during sound representation in every second trial. The choices were influenced by both the reward amounts and tone durations. Inhibiting mPFC activity led to biased choices, while inhibiting AC activity resulted in variable behavioral changes across sessions. We are going to inhibit the activity during non-sound timings to understand the roles of AC and mPFC in decision making.

Disclosures: K. Ishizu: None. **A. Funamizu:** None.

Poster

PSTR433. Decision-Making: Prefrontal Cortex

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Topic: H.03. Decision Making

Title: Coding by medial prefrontal neurons is not random, but respects the logic of a contextual visual discrimination task

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Abstract: It was reported that the combination of features encoded by individual prefrontal (PFC) neurons is random, but that task features and performance can be detected at the population level. However, assessing whether PFC coding is random requires much larger cell samples than used previously. Thus, we examined the relation between the cluster of features encoded by PFC cells in a contextual visual discrimination task. Rats implanted with Neuropixel probes in the medial PFC were presented with visual stimuli via 2 monitors placed on either side of their heads. 2 epochs of visual stimulation occurred on each trial: (1) when rats step on a platform, triggering the presentation of one of two stimuli signaling context, identical on both sides; (2) when rats nose-poke, during which different stimuli to be discriminated are presented on the left and right. The latter varied along two dimensions (speed & contrast; 5 levels each). Rats had to determine on what side the target dimension is highest, ignoring the left-right difference in distractor levels. Rats reported their decision by pressing a lever on the side where the target dimension is higher. Quantifying explained variance revealed that outcome and response/choice information were encoded by ~50% PFC cells compared to ~25% for context

information and ~12% for stimulus information. To test if the cluster of variables encoded by PFC cells is random, we identified cells showing significant rises (+) or drops (-) in firing rates (within 0.15 s of nose-poking) as a function of speed (S) or contrast (C) on the left (L) or right (R), after balancing the number of trials requiring left or right lever responses. This analysis yielded the following features (SL+,SL-,SLnil; SR+,SR-,SRnil; CL+,CL-,CLnil; CR+,CR-,CRnil), in 81 possible combinations. The number of cells with opposite responsiveness to contrast on left vs. right (CL+ & CR-; CL- & CR+) was 13 times > chance (p=0.001). Opposite responsiveness to speed on left vs. right (SL+ & SR-; SL- & SR+) was 5 times > chance (p=0.003). Moreover, the number of cells with opposite eye dominance for speed vs. contrast was 3.63 times > chance (p=0.007). Finally, we noted that cells with opposite eye dominance for speed vs. contrast rarely exhibited a lever preference compared to cells with eye dominance for only one dimension (1.2% vs. 19.5%). Thus we asked whether cells with eye dominance for only one dimension also exhibited a firing preference for lever on the same vs. opposite side. The number of cells with lever and eye dominance on the same side was 5.6 times > chance (p=0.039). Overall, our results indicate that the clusters of variables encoded by individual PFC neurons reflect the logic the task.

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Poster

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Topic: H.03. Decision Making

Support: SFB1436 Magdeburg

Title: Frontal cortex impacts on exploratory attentional resource allocation in mongolian gerbils: insights from a probabilistic foraging paradigm

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Abstract: In our constantly changing world, it is necessary to continuously adapt choice options to current needs and, if necessary, to change our current behavioural strategy and to explore the environment in order to adaptively reallocate resources. For example, imagine a Mongolian gerbil that forages a desert habitat for distributed food patches. When these patches become exhausted, the gerbil is in an exploitation/exploration dilemma: Should it exploit the current

patch further or should it explore an alternative patch, suffering travel costs but achieving potentially higher food density? Such a foraging example shows a fundamental resource allocation problem. The patch-leaving decision needs to be made based on probabilistic information (how much food is usually available in the alternative patch?) in a potentially changing environment. Previous studies have suggested that the anterior prefrontal cortex (aPFC) plays a decisive role for the neuronal realization of exploratory resource allocation in human and non-human primates (*Daw et al., 2006*). In the present study, we investigated the performance of Mongolian gerbils in a probabilistic foraging paradigm (*adapted from Lottem et al., 2018, Nat Comm.*) while simultaneously recording laminar current source density (CSD) profiles in Frontal field A (FrA) using 32 channel chronic electrodes (Neuronexus probes). We were able to show that activity in the frontal cortex depends on both the received reward as well as the expected probability of the reward. As the latter decreases due to the exponential decrease in reward delivery, activity patterns in frontal cortex reflected an accumulating evidence that accompanied switching from exploitative to explorative search strategies. Using the laminar signal resolution, the goal is to obtain insights into different neuronal circuit elements in frontal cortex that contribute to the optimal selection of exploitative versus explorative search strategies, thereby gaining a better understanding of the neural regulation of attentional resource allocation.

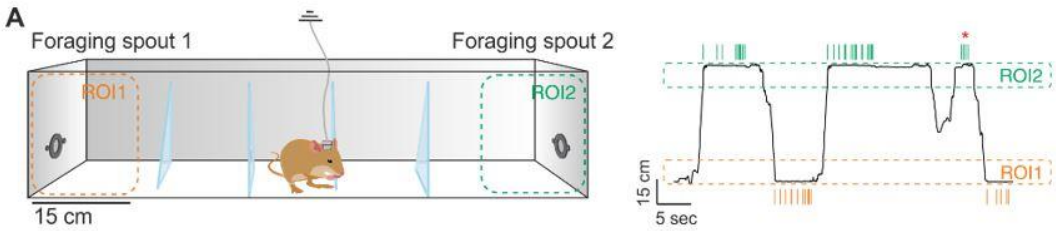

Figure. Foraging setup (left) and patch switches over time (right). Adapted from Lottem et al. (*2018, Nat Comm.*)

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Poster

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Program #/Poster #: PSTR433.05/SS25

Topic: H.03. Decision Making

Support: DANDRITE-R248-2016-2518

Title: History of sensorimotor event representations in mouse medial prefrontal cortex serves reward foraging functions

Authors: J. LOPEZ-YEPEZ, A. BARTA, J. MARTIN, M. MOLTESSEN, T.-F. WOO, ***D. KVITSIANI**;
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Abstract: Prefrontal cortical regions are believed to play a crucial role in integrating information from various sensory, motor, and internal variables to guide decision-making in animals. It has been proposed that neurons in these areas, either individually or as a population, compute a decision variable (DV) that influences an animal's choices. However, representations within the prefrontal regions are complex, encompassing not only DVs but also current and past sensory, motor and internal variables. This complexity is especially apparent when analyzing population-level representations, as they can simultaneously encode different behavioral variables in a high-dimensional space. As a result, a key unanswered question is which representations are utilized by animals out of this diverse and mixed array. To address this question, we conducted experiments involving the recording of neurons in the medial prefrontal cortex (mPFC) of mice engaged in probabilistic reward-based foraging. During the task, animals were required to track both their reward history and choice history, which we refer to as history of sensorimotor events. To quantify the decision variable based on the animals' behavior, we employed a reinforcement learning framework. In our previous studies we have demonstrated that a reinforcement learning model incorporating both reward and choice history performs better than other models of the same class. By analyzing both single neurons and populations of neurons, we found that while neurons represent both the decision variable and the history of sensorimotor events, the representations of sensorimotor events were more pronounced than those of the decision variable. Furthermore, when we manipulated the temporal delay in choices while maintaining the task structure, we observed that representations of sensorimotor history remained robust, whereas the representation of the decision variable degraded. This suggests that the representations in the mPFC primarily serve to encode the history of sensorimotor events rather than directly compute the decision variable. To further test this hypothesis, we inactivated the mPFC and observed behavioral effects only when the relevant sensorimotor events were delayed. Our study provides evidence that neural representations in the mPFC function to maintain a working memory of past events, with little support for the notion that it directly computes the decision variable that guides an animal's choices.

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Poster

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Topic: H.03. Decision Making

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Title: Top-down modulation of autonomic arousal by the anterior cingulate cortex

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Abstract: Hyperactivity of arousal-mediating circuits are implicated in a variety of neuropsychiatric disorders. Therefore, insight into the arousal system of the brain could lead to a better understanding of the neurological basis of these disorders. Subcortical structures such as the locus coeruleus, hypothalamus, and periaqueductal gray (PAG) are known to be involved in the regulation of autonomic arousal but much less is known about the role of higher level cortical circuits. We investigated the role of a major subdivision of the prefrontal cortex, the anterior cingulate cortex (ACC), in modulating autonomic arousal. Pupil size, heart rate, and movement were used as non-invasive measures of arousal. To test how ACC activity contributes to arousal levels, we simultaneously measured arousal and recorded fiber photometry signals from mice virally expressing GCaMP8m. Aligning these signals to the onsets of pupil dilations revealed that pupil dilations were preceded by increases in ACC calcium activity. Further, ACC activity was associated with heart rate increases. To better understand how the ACC can directly modulate arousal, we optogenetically manipulated ACC pyramidal neuron activity. We found that photostimulation of pyramidal neurons in mice expressing CaMKII-ChR2 caused pupil dilations that were time-locked to activation. ACC activation also led to increased heart rate and movement. ACC inactivation via photostimulation of GABAergic neurons caused pupil constrictions. Additionally, closed-loop optogenetic inactivation of the ACC using DeepLabCut Live suppressed ongoing pupil dilations. Vasoactive intestinal peptide-expressing (VIP) interneurons exert disinhibitory control over pyramidal neuron activity by inhibiting other inhibitory cells. We tested whether and how VIP neurons are involved in ACC modulation of arousal. Optogenetic VIP neuron stimulation caused pupil dilations. Next, we investigated a possible ACC to periaqueductal gray (PAG) projection pathway that may be involved in arousal modulation. We used fiber photometry to record calcium activity from ACC axons in the PAG. Aligning calcium activity to onsets of pupil dilations revealed pupil dilations were preceded by increases in ACC-PAG axon activity, similar to what we found in ACC neurons. We also found that optogenetic stimulation of ACC axons in the PAG caused pupil dilations. Together these findings indicate that the ACC modulates autonomic arousal, possibly via projections to the PAG. We are currently evaluating how ACC VIP neurons regulate outflow of information to the PAG for top-down modulation of arousal.

Disclosures: N. Chintalacheruvu: None. **A. Kalelkar:** None. **C. Oh:** None. **C. Tumbokon:** None. **R. Huda:** None.

Poster

PSTR433. Decision-Making: Prefrontal Cortex

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Program #/Poster #: PSTR433.07/TT2

Topic: H.03. Decision Making

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Title: Role of VIP-expressing neurons in the prefrontal cortex in probabilistic reversal learning

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Abstract: The prefrontal cortex (PFC) plays a crucial role in flexible control of behavior. Of various types of neurons in the PFC, vasoactive intestinal polypeptide (VIP)-expressing neurons are thought to exert powerful influences on PFC circuit operations by disinhibitory control of other inhibitory interneurons. To obtain insights on the role of VIP neurons in flexible control of behavior, we investigated modulation effects and activity dynamics of VIP neurons in the medial PFC (mPFC) in mice performing reversal learning under a probabilistic classical conditioning paradigm. We found that chemogenetic or optogenetic modulation of VIP neuronal activity impairs reversal learning as assessed by the animal's cue-dependent anticipatory licking responses. We also found that VIP neurons undergo diverse types of activity change across trials following cue-outcome contingency reversal. During the progress of behavioral reversal (~100 trials since cue-outcome contingency reversal), but not before or after it, VIP neurons conveyed strong signals related to reward history and reward prediction error (RPE). Moreover, there was a significant correlation between the persistence of RPE signals across trials and the number of trials required for each animal to reach the reversal criterion. These findings suggest VIP neurons play a crucial role in behavioral flexibility by modulating mPFC neural circuit activity in response to reward history and reward prediction error. They also suggest the potential significance of VIP neurons in developing interventions for conditions associated with impaired behavioral flexibility.

Disclosures: J. Yi: None. **Y. Yoon:** None. **S. Choe:** None. **M. Jung:** None.

Poster

PSTR433. Decision-Making: Prefrontal Cortex

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Topic: H.03. Decision Making

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VA National Center for PTS
BBRF NARSAD Y.I.
NIH/NINDS R00NS114166

Title: Synergistic dynamics of norepinephrine, dopamine, and serotonin in mouse frontal cortex during naturalistic decision making.

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Abstract: In nature, animals must forage for food under environmental danger to survive. Neuromodulators including norepinephrine (NE), dopamine (DA), and serotonin (5HT) play a fundamental role in this process, enabling flexible switching between motivational drives. The question of how neuromodulators synergistically encode motivational state is thus fundamental to systems neuroscience, yet the interplay between these neuromodulators during naturalistic decision making are not fully understood. Here, we developed a naturalistic approach/avoidance task in mice involving a tradeoff between seeking reward versus safety in the presence of looming predation risk. We utilized whole-brain cFos mapping, multi-fiber photometry, computational behavior tracking, and slice electrophysiology in this task. Mice that experienced looming stimuli showed increased c-fos expression in regions including frontal cortex, locus coeruleus, and ventral tegmental area, but decreased expression in dorsal raphe nucleus. Moreover, by using multi-fiber photometry combined with GPCR-based sensors, we found that cortical NE plays a more prominent role in encoding looming threats while DA represents reward. In contrast, 5HT dynamic negatively correlates to both valences. Furthermore, bath application of 5HT-related treatments increase spontaneous spikes in locus coeruleus NE neurons in an ex vivo slice physiology experiment, suggesting the importance of cross-talk between different neuromodulatory circuits. In conclusion, monoamines such as NE, DA, 5HT can converge in their encoding of naturalistic motivated behaviors as well as dissociate from one another. By utilizing this approach, interactions between innate fear and incentive for food may be delineated in terms of basis in neurochemical signaling events during natural behavior, and may contribute to the understanding of neural mechanisms underlying emotional disorders including anxiety and post-traumatic stress disorder.

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Poster

PSTR433. Decision-Making: Prefrontal Cortex

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Topic: H.03. Decision Making

Support: NIMH 5K08MH116125

Title: Role of prefrontal dopamine in effort-based decision making

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Abstract: Effort-based decision-making (EBD) requires establishing action selection policies that maximize effort-reward tradeoffs and is disrupted in several neuropsychiatric disorders. The anterior cingulate cortex (ACC), a prefrontal subregion, is critical for encoding effort-related value, model-based action selection, resolving uncertainty and other functions relevant to EBD. Our lab has found that the activity of ACC excitatory neurons is necessary for effortful action selection, while a subpopulation of striatal projecting neurons is transiently required to switch from a low to a high effort choice strategy. Dopaminergic inputs strongly modulate function in target regions and striatal dopamine (DA) is heavily implicated in EBD. However, the role of DA in shaping ACC neural activity during EBD remains poorly understood. Here, we deploy temporally precise optical tools to measure and manipulate ACC DA in an effort-based T-maze task. In this assay, mice must choose whether to surmount an effortful obstacle for a large reward vs. taking an unimpeded path to a small reward. Using fiber photometry and GRAB-based fluorescent DA sensors, we observed ramping of ACC DA as mice approach the maze choice point. Distinct DA dynamics emerged during specific epochs of choice trials and were differentially modulated during high vs. low effort choices. Moreover, these DA dynamics correlated with ACC activity patterns as detected by measuring GCaMP signals during the same behavior. In ongoing work, we are now using optogenetic approaches to manipulate DA terminals in ACC to further investigate these relationships.

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Poster

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JSPS Grant-in-Aid for Scientific Research (19H01152, 20H00575 and 23H00522 to MK)
JSPS Grant-in-Aid for Scientific Research (22KJ2945 to TS)

Title: Changes in neuronal activities in the medial prefrontal cortex during decision-making of mice

Authors: *T. SUZUKI[1], D. JOHO[1], H. OKUNO[2], M. KAKEYAMA[3];
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[2]Lab. of Biochem. and Mol. Biol., Grad. Sch. of Med. and Dent. Sci. Kagoshima Univ.,

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Abstract: In the process of decision-making, subjects recognize the external situation, refer to their memory and knowledge to assess the value and cost of an action and alternatives, and decide on a course of action. The prefrontal cortex may play a part in this process in the form of a complex interplay of bottom-up information, driven by the sensory perception of the external environment, and top-down information, driven by retrieval and inference of memories and knowledge encoded in the brain. In rodents, the medial prefrontal cortex (mPFC) is reportedly involved in decision-making; this warrants a detailed analysis at the cellular level. To examine the neural activity during decision-making, we first developed a behavioral task using a touch-screen device. In this task, mice had to make decisions based on visual information, memory and/or knowledge and were given rewards for correct responses. To date, more than 30 mice have been trained and have exhibited a high rate (>80% on average) of correct responses despite a chance level of 50%. To analyze neural activity, GCaMP6f, a genetically encoded calcium indicator, was expressed in the mPFC (n = 3) and primary somatosensory cortex (SSp, n = 2) of the mice. After these mice were trained, calcium signals were measured via *in vivo* Ca^{2+} imaging using a microendoscope during the behavioral task. Task-related cells were assessed using one-way repeated measures ANOVA ($p < 0.0001$) and defined as those whose $\Delta F/F$ before and after visual cue stimulus changed at a consistent time across repeated trials. The results indicated that the mPFC (190/293 cells; 64.85%) had a greater proportion of task-related cells than the SSp (50/199 cells; 25.13%) ($\chi^2(1) = 73.25$, $p < 0.0001$). As temporal variations in the activity of task-related cells in the mPFC were observed, hierarchical cluster analysis was used to classify the cells according to their activity patterns. Interestingly, in addition to activating cells after visual cue stimulus, many deactivating cells were present in the mPFC. Furthermore, choice-specific activity may have occurred in some cell populations of the mPFC along with the initiation of choice. These results suggest that mPFC neurons are involved in decision-making with distinct activity and temporal properties and that some cell populations are part of a network that regulates choice.

Disclosures: **T. Suzuki:** None. **D. Joho:** None. **H. Okuno:** None. **M. Kakeyama:** None.

Poster

PSTR433. Decision-Making: Prefrontal Cortex

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR433.11/TT6

Topic: H.03. Decision Making

Title: Frontal dynamics underlying flexible decision making in mice

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Abstract: Humans and other animals require flexibility to make decisions appropriate for an ever-changing environment. The way mammalian brains achieve flexible decision making is not fully understood. The probabilistic reversal learning (PRL) task is a powerful tool used to assess behavioral flexibility and how positive and negative feedbacks influence decision making. It has been administered to healthy partecipants and patients with a wide range of neurological conditions, including Autistic Spectrum Disorder and Schizophrenia, as well as to animal models. We trained C57BL/6 mice on PRL and conducted high-density single unit recordings using Neuropixels probes from the medial frontal cortex in well-performing animals. The animals had binary choices (e.g., left vs right) associated with different reward probabilities (e.g., 80% vs 20%), which they discovered via trial and error. Once a consistent choice was established, the contingencies were reversed, so the previously highly rewarded side became unfavorable and vice versa. After one month of training, mice were able to flexibly switch contingencies multiple times in one training session. So, as contingency undergoes multiple changes, animals were able to maximize the accumulated reward, via the integration of positive and negative feedback . We have identified the neural correlates for the choice value (i.e., the probability of reward) and the reward prediction error (RPE, i.e., when the obtained reward differs from the value). We will then examine how the choice value and RPE propagate in the mammalian brain by recording from multiple regions simultaneously.

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Poster

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Program #/Poster #: PSTR433.12/TT7

Topic: H.03. Decision Making

Support: NIMH 5K08MH116125

Title: Miniscope calcium imaging of prefrontal dynamics during effort-based decision-making.

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Abstract: Miniscope calcium imaging of prefrontal dynamics during effort-based decision making

Authors*T.A. GUPTA[1], A.Q. KASHAY[1], M. UMAGUING[1], S.A. WILKE[1]
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DisclosuresT.A. Gupta: None. **M. Umaguing**: None. **S.A. Wilke:** None.

Abstract

Effort-based decision making (EBD) requires weighing predicted gains against the physical effort costs required to obtain them, and is disrupted in several neuropsychiatric disorders. The anterior cingulate cortex (ACC) is postulated to compute effort-related value and exert control over action selection policies during EBD. Our lab has found that the activity of ACC excitatory neurons is necessary for effortful action selection, while a subpopulation of striatal projecting neurons is transiently required to switch from a low to a high effort choice strategy. These causal manipulations suggest that ACC neuronal subpopulations may mediate specific functions during EBD. However, little is known about how the computations underlying such effort-related decisions are represented by ACC neural dynamics. Here, we have deployed UCLA Miniscopes to image calcium signals arising from large numbers of ACC neurons simultaneously during EBD. ACC neural activity was recorded in a barrier T-maze task in which mice choose between surmounting an effortful obstacle (climbing a barrier) for a high reward or taking an unobstructed path to a low reward. Calcium signals were extracted using the *Minian* pipeline and aligned to behavior using DeepLabCut and BehaviorDEPOT open-source software. We then used receiver operating characteristic analyses to establish the tuning of ACC neurons to specific behavioral events. We have found that the tuning of ACC neurons during decision making is enhanced when an effort cost is operative. A subset of these neurons exhibited increased or decreased activity that was significantly tuned to approach of the maze choice point. Interestingly, largely non-overlapping subpopulations were tuned to high vs. low effort choices, suggesting that effort-reward tradeoffs drive segregated representations of potential actions in ACC. In ongoing work, we are now using dimensionality reduction and decoding algorithms to further evaluate these relationships.

Disclosures: **T.A. Gupta:** None. **A.Q. Kashay:** None. **S. Wilke:** None. **M. Umaguing:** None.

Poster

PSTR433. Decision-Making: Prefrontal Cortex

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR433.13/TT8

Topic: H.03. Decision Making

Support: NRF-2021M3E5D2A01019542

Title: Inferring cognitive processes of gathering information for decision-making by decoding prefrontal cortical population activity

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Abstract: Value-based decision-making generally entails evaluating and comparing options before choice. Often, it requires integrating multiple pieces of information to determine the optimal choice in which the prefrontal cortex (PFC) plays a major role. However, how PFC neuronal populations accumulate evidence during evaluation and comparison of options are relatively less explored. Based on decoding analysis, this study aimed to examine the neural correlates of evidence accumulation in the PFC of two non-human primates during information gathering for decision-making. We decoded the outputs of various cognitive processes, such as valuation and comparison, from neuronal populations at multiple PFC regions including dorsal lateral PFC (DLPFC), orbitofrontal cortex (OFC) and anterior cingulate cortex (ACC). The analysis on decoding outcomes across trials revealed that each subject exhibited a distinct level of neural representation of evidence accumulation for each process. A subject with less clear neural representation of accumulated evidence tended to gather more information compared to another with clearer neural representation of accumulated evidence. Furthermore, we calculated an association index to estimate the likelihood of decoding outcomes from preceding processes being associated with current processes through information gathering in a trial. The result showed a significant association between the decoding outcomes of the current and preceding processes. We also observed that a subject with neural representation of more evidence exhibited a stronger association index between temporally adjacent cognitive processes, while the opposite was true for the other with less evidence. These findings highlight the relationship between neural representations of evidence accumulation in PFC and decision-making behavior via information gathering.

Disclosures: S. Lee: None. **J. Sohn:** None. **M. Kim:** None. **L.T. Hunt:** None. **S.W. Kennerley:** None. **S. Kim:** None.

Poster

PSTR433. Decision-Making: Prefrontal Cortex

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

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Topic: H.03. Decision Making

Support: T32 MH115886
R01 MH112688

Title: Network physiology metrics reveal a dynamic balance between excitatory and inhibitory functional connections in rodent prefrontal cortex during decision making

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Abstract: The balance between excitatory and inhibitory inputs is critical for proper network function, but this balance is thought to change as behavior changes. Measuring Excitatory:Inhibitory (E:I) balance in vivo has thus far remained difficult, limiting the ability to

probe E:I dynamics during behavior. Recent work has argued that examining spectral power of local field potentials (LFP) and specifically the exponential decay rate of the underlying aperiodic component (**χ**;; of 1/f^**χ**;;) can provide a window into E:I balance in vivo (Gao et al., 2017; Donoghue et al., 2020). In concert, spike timing cross-correlation histograms (CCHs) have long been identified as a means of measuring direct monosynaptic connectivity and the functional strength of local connections (Csicsvari et al., 1998). In addition, concurrent spiking between cells (within 1ms) provides a lens into functional network activity and has been found to depend on NMDA receptor functionality (Zick et al., 2021).

Using these methods, we evaluated recordings from the medial prefrontal cortex (mPFC) of rats as they performed the economic decision task Restaurant Row. In this task, rats must make a series of decisions whether or not to wait out temporal delays to receive food rewards based on presented information. Critically, this task involves multiple distinct behavioral phases, with distinct cognitive demands and decision processes. Evaluating the aperiodic component of the LFP spectrum we found that aperiodic decay rates varied dynamically throughout the task, implying changes to E:I balance. Notably, there was increased excitatory drive as rats entered the Offer Zone where they were presented with information to make their decisions, and decreased excitation during the Wait Zone when rats must hold their decisions in working memory and wait to receive a reward. In examining CCHs and monosynaptic connections between mPFC cells, we found a complementary picture. Local connections were weaker in the Offer Zone and stronger in the Wait Zone. These findings suggest parallel brain states during the Offer and Wait phases of the RRow task. In the Offer Zone, excitatory information arrives into the mPFC from other brain areas while local communication is muted. In the Wait Zone, there is less external input and instead mPFC is engaged in local processing. Finally, we combined these two methods to evaluate if E:I balance, as measured from LFP, influenced the coupling strength of co-spiking pairs. Interestingly, we found that when the spectrum was flatter (implying more excitation) there was stronger coupling between co-spiking pairs of cells.

Disclosures: G.W. Diehl: None. **A.D. Redish:** None.

Poster

PSTR433. Decision-Making: Prefrontal Cortex

Location: WCC Halls A-C

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Program #/Poster #: PSTR433.15/TT10

Topic: H.03. Decision Making

Support: NEI: R01 EY033430-01A1

Title: Rotational dynamics help stabilize cortex

Authors: *T. BATABYAL, S. L. BRINCAT, E. K. MILLER;
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Abstract: The cortex requires stability to recover after disruption. Events such as distractions, eye movements, sensory inputs, and more can trigger strong neural responses that have the potential to disrupt cortical processing. It is crucial for the cortex to "regain its focus" and return to the task at hand, reverting to its previous state prior to the disruption.
We found that this may be explained by rotational dynamics. We examined spiking in the prefrontal cortex during working memory tasks in which there was a disruption during the memory delay (a saccade or a distractor stimulus). The disruption led to the emergence of organized rotational structures in subspace dynamics. This effectively restored the system to its pre-disruption state.
The rotations were correlated with behavioral performance. When trials were performed correctly, the rotational structure was more likely to complete a 360 deg rotation. On incorrect trials, trajectories failed to complete a full rotation.The rotations changed with expectation. Rotational velocity was inversely correlated with the expected delay duration. The velocity was faster on shorter delays as if the cortex was "rushing" to complete the rotation before the delay ended.
We found some evidence that the rotation through subspace may, in fact, be rotations in cortex itself. Spiking organized into traveling waves that showed rotational dynamics in rotational Principal Component Analysis (jPCA) space. We also observed rotating traveling waves in local field potentials.
Neural stability is crucial for cognition and consciousness. Our results suggest that it may be attributed to brain rhythms rotating the brain back to its previous state.

Disclosures: T. Batabyal: None. **S.L. Brincat:** None. **E.K. Miller:** None.

Poster

PSTR433. Decision-Making: Prefrontal Cortex

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Program #/Poster #: PSTR433.16/TT11

Topic: H.03. Decision Making

Support: Howard Hughes Medical Institute

Title: Encoding of structured knowledge in the medial prefrontal cortex

Authors: *M. PROSKURIN[1], **M. MANAKOV**[1], H. WANG[1], E. KULESHOVA[1], A. LUSTIG[1], R. BEHNAM[1], S. DRUCKMANN[2], D. G. TERVO[1], A. Y. KARPOVA[1];
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Abstract: One of the most interesting, and mysterious, aspects of biological brains is that they form representations of environments that are richer than an interlinked series of associations, and more "conceptual" than partitions in a multidimensional representation of the sensory input. Our best intuition about how this happens is that brains have evolved to pick up on, and exploit, the ubiquity of structure in the natural world and in the types of tasks that animals might have to

solve over their life span, efficiently forming structured relational models of the animal's world that can be used for planning and action. But even with that intuition, we know little about how structured knowledge is acquired or updated, especially when the extraction of the relevant structure must happen incidentally, without explicit instruction or feedback. Here, by examining neural ensemble activity in rats that sample different sequences of binary choices in a self-guided search for latent task structure, we reveal a relationship between the incidentally acquired structured knowledge and the neural activity in the medial prefrontal cortex is strong enough that we can begin to decode it with confidence on single trials. Going forward, this puts us in a position to probe how structured knowledge about the world is acquired by the brain, and how it is updated with experience.

Disclosures: M. Proskurin: None. **M. Manakov:** None. **H. Wang:** None. **E. Kuleshova:** None. **A. Lustig:** None. **R. Behnam:** None. **S. Druckmann:** None. **D.G. Tervo:** None. **A.Y. Karpova:** None.

Poster

PSTR433. Decision-Making: Prefrontal Cortex

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR433.17/TT12

Topic: H.03. Decision Making

Title: The ventrolateral prefrontal cortex underlies decision making based on structured sequences

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Abstract: Humans are capable of understanding a sequence of events based on the structure of the sequence. The sequence structure indicates how components of the sequence relate to one another and how they should be integrated for decision making. The neural mechanism underlying sequence processing is, however, not well understood. Therefore, we trained two male macaque monkeys to perform a sequence decision-making task. They viewed eight centrally-presented visual stimuli sequentially, which represented a math expression consisting of four signed numbers and four operators that indicated whether each number needed to be added or subtracted. The monkeys were required to report whether the resulting value was positive or negative by making a saccade towards either a red (positive) or a green (negative) peripheral target. Following the monkeys' successful learning of the task, we conducted recordings of single-unit activities from the ventrolateral prefrontal cortex (vlPFC). Neurons in the vlPFC encoded both the operators (addition or subtraction) and the signed numbers in the stimulus sequence. In addition, vlPFC neuronal activities reflected the intermediate result indicated by the stimuli during the stimulus presentation period. Finally, after the sequence was presented and when the fixation point vanished, which served as a cue for the monkeys to initiate

a saccade towards the target, the population responses in the vlPFC was transformed into a binary encoding of the eye movement direction. Together, these results suggest that the vlPFC plays an important role in processing sequence information for decision making.

Disclosures: Y. Bai: None. **T. Yang:** None.

Poster

PSTR433. Decision-Making: Prefrontal Cortex

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Program #/Poster #: PSTR433.18/TT13

Topic: H.03. Decision Making

Support: NIH Grant MH118925

Title: Prefrontal and Striatal activity during a Strategy Board Game

Authors: *M.-Y. PARK[1], M. OEMISCH[1], B. VAN OPHEUSDEN[2], K. OSBORNE[1], H. LIANG[1], M. FERGUSON[1], W. MA[3], D. LEE[1];
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Abstract: Planning for sequential movements in a social context is challenging, because the agents must anticipate the actions of others. Although many previous studies have investigated computational algorithms applicable in such iterative social interactions, how the values of self and opponent's actions are computed and represented in the brain remains poorly understood. In this study, we trained rhesus monkeys to play a competitive strategy board game called a four-in-a-row. During this game, the monkeys placed their stones one at a time using a joystick on a 4-by-9 board while taking turns with a computer opponent. Each player could win by placing 4 pieces in a row horizontally, vertically, or diagonally, and the game outcome could be a win, loss, or tie for the animal, which was rewarded only for wins. We recorded the activity of individual neurons from the dorsomedial prefrontal cortex (dmPFC), the dorsolateral prefrontal cortex (dlPFC) and the caudate nucleus of one monkey using two 64-channel silicone probes simultaneously in two of these areas in a given session. During the recording experiment, the computer opponent always initiated the game, and probabilistically alternated between defensive and offensive strategies while making its move randomly with a small probability so that the probability of winning for the animal did not become too low. We used a linear regression model to evaluate the neural signals related to the value of the board position chosen by each player, the ordinal position of the move in a game, and whether the given move connected 4 stones and therefore led to winning. The value of the chosen position was approximated by the maximum number of stones continuously connected by the current move of each player. Neurons in these brain areas modulated their activity more frequently according to the board positions chosen by the animal than in response to the positions chosen by the computer opponent. In addition, whereas many of the neurons changed their activity according to the ordinal position of the move

throughout the game, the signals related to the number of stones connected by the animal and the computer opponent were both present in these brain areas. This suggests that the prefrontal cortex and the striatum might play an important role in computing the action values of the choices made by the self and others during iterative social interactions. Furthermore, many neurons modulated their activity when either player won the games, indicating that these brain areas play multiple roles in learning and decision making during rule-based social interactions.

Disclosures: **M. Park:** None. **M. Oemisch:** None. **B. van Opheusden:** None. **K. Osborne:** None. **H. Liang:** None. **M. Ferguson:** None. **W. Ma:** None. **D. Lee:** None.

Poster

PSTR433. Decision-Making: Prefrontal Cortex

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR433.19/TT14

Topic: H.03. Decision Making

Title: The role of the macaque anterior cingulate cortex in reward expectancy and/or goal attainment

Authors: *P. KUSMIEREK[1], J. PARK[2], B. M. BASILE[3], E. A. MURRAY[2];
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Abstract: The anterior cingulate cortex (ACC) has been proposed to be involved in cognitive control of action selection, including that based on expected reward value (e.g., Chudasama et al, *Cereb Cortex*, 2013; Shenhav et al, *Nat Neurosci*, 2016). Neural activity consistent with representations linking reward outcomes to motor actions have been found in the region (e.g., Shima & Tanji, *Science*, 1998; Matsumoto at al, *Science*, 2003), and activity indicative of reward expectancy has been described as well (Shidara & Richmond, *Science*, 2002). Here we assessed the ACC's causal contribution to the expression of reward expectancy via an autonomic measure. Three rhesus macaques received bilateral neurotoxic lesions of ACC. Together with four unoperated controls (CON), they were presented with a Pavlovian procedure whereby visual stimuli predicted reward delivery, independent of the animal's actions. Mildly thirsty monkeys sat in a primate chair facing a monitor screen. In each trial, two blue circles (1° diam) were presented 8° away from the screen center opposite each other in one of four cardinal/intercardinal orientations. The circles slowly moved towards each other for 15 seconds until they touched, disappeared, and fluid reward was delivered. A 15-s intertrial interval ensued, during which the screen was blank. Pulse signal was acquired with a plethysmography sensor placed on the ear, and heart rate was extracted off-line. We found a group difference in heart rate in anticipation of reward. Whereas CON monkeys exhibited a gradual heart rate increase while the circles moved closer together, monkeys with ACC lesions did not. This implies that the autonomic reflection of reward expectancy was lost as a result of the ACC lesion. In contrast, we observed a significant heart rate change after reward delivery in all monkeys, controls and

monkeys with ACC lesions alike, suggesting that the autonomic response to receipt of reward was intact after ACC lesions. Taken together, the results support a role for the ACC in reward expectancy, but not in the reaction to reward receipt.

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Poster

PSTR433. Decision-Making: Prefrontal Cortex

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR433.20/TT16

Topic: H.03. Decision Making

Title: Neurophysiological investigation of effort monitoring and decision-making dynamics in the anterior cingulate cortex in macaque

Authors: *A. LESPART[1], S. BOURET[2];
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Abstract: Understanding cerebral processes leading our behavior toward efficient choices and performance in effortful situations is a central topic in neurobiology of decision making. The anterior cingulate cortex (ACC) is involved in at least 2 aspects of effort: effort as a decision variable and effort as an amount of energy invested to overcome a difficulty. The dynamic neurophysiological processes underlying these functions, however, remains unclear. To address this issue, we used a neurophysiological approach in rhesus monkeys. We recorded 160 single units from the ACC in 1 macaque performing a choice task, which manipulates both effort and reward, and where monkeys must produce the chosen effort by pressing a grip at each trial in order to obtain the chosen reward. The monkey's choices were clearly influenced by both reward and effort levels, and the animal applied the required force on the grip, showing appropriate option valuation (effort discounting of reward value) and appropriate mobilization of resources for action. In addition, the latency between cue onset and first saccades toward the chosen option was highly dependent upon the choice difficulty (absolute difference in value between the 2 options), whereas latency from this saccade to action onset was not. This suggests a sequential relationship between decision and action processes, but further analysis is necessary to further understand the dynamics of effort processing in this task. Preliminary analysis indicates that ACC units show a stronger sensitivity to the side chosen, the reaction time and errors compared to benefits and costs (reward and force proposed). This result suggest that ACC is more involved in the implementation of effort in an effector specific way than in the processing of effort as a decision variable. In addition, a dynamic analysis of the firing patterns in the task suggests that ACC single units are not only related to the dynamics between decision and action, but also show change of states outside choice epoch that are strongly related to behavioral variables. Together these preliminary results provide a new insight into the dynamic implication of ACC neurons in effort, and they are in line with the proposed role of the ACC in monitoring

action/outcome as a function of expected costs and benefits, to facilitate the adaptation of goal-directed behavior.

Disclosures: **A. Lespart:** None. **S. Bouret:** None.

Poster

PSTR433. Decision-Making: Prefrontal Cortex

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Topic: H.03. Decision Making

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Title: Dynamics of population activity in macaque prefrontal cortex predict impact of prior expectation during perceptual decision-making

Authors: T. LANGLOIS[1], J. CHARLTON[2], *R. GORIS[3];
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Abstract: Perceptual decisions are informed by the present sensory input and by expectations, or "priors", that reflect previously experienced statistical regularities in the environment. The neural mechanisms that integrate sensory signals with priors that are specific to a given context likely engage a network of association areas. However, it is not known how neural circuits perform this integration. To shed light on this matter, we developed an analysis that relates the dynamics of population activity in the prefrontal cortex to perceptual decisions on a trial-by-trial basis. We used multi-electrode arrays to record neural population activity in the prearcuate gyrus of two macaque monkeys as they performed a perceptual orientation discrimination task. The animals judged the orientation of drifting grating stimuli under two contexts, each associated with a different distribution of stimulus orientation, as described in Charlton & Goris (2022 - bioRxiv). The choices of both monkeys were biased in a context-specific manner, reflecting the impact of their priors on their perceptual interpretations. To study the neural correlate of this behavior, we decoded a time-varying decision variable (DV) from jointly recorded neural responses. We previously reported that this decoded DV reflects the formation of a perceptual choice, and differs across the two task-contexts (Charlton & Goris, 2022). To determine the functional significance of these differences, we measured the DV's initial value and dynamic range on a trial-by-trial basis. We found that the initial value on average differed across the task contexts, but by itself, did not predict choice outcome. In contrast, the dynamic range on average did not differ across the two task contexts, yet it accounted for systematic differences in the choice behavior. On trials in which the DV exhibited a small dynamic range, the animals tended to be more biased by the context-specific expectation compared to trials in which the DV exhibited a

large dynamic range. These findings reveal how prefrontal circuits integrate prior stimulus expectations and incoming sensory signals at the behaviorally relevant timescale of the single trial.

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Poster

PSTR433. Decision-Making: Prefrontal Cortex

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Topic: H.03. Decision Making

Support: NIH Grant 1RF1NS128974-01

Title: Flexible representations of prior expectations and sensory evidence in the macaque dorsolateral prefrontal cortex

Authors: *L. W. THOMPSON[1,2], V. SUBRITZKY-KATZ[1,2], K. SCHAPIRO[3], A. DALLSTREAM[1,2], J. ZWEIGLE[1,2], J. I. GOLD[1,2];
[1]Neurosci., [2]Computat. Neurosci. Initiative, Univ. of Pennsylvania, Philadelphia, PA; [3]Brandeis Univ., Waltham, MA

Abstract: Successful decision-making relies on our ability to accumulate sensory evidence in a context-dependent manner. For example, our previous choices and learned expectations can modulate the amount of sensory evidence needed to guide future decisions. Here we used an adaptive oculomotor-delayed response (AODR) task to study the role of working-memory representations in the macaque dorsolateral prefrontal cortex (dlPFC) to flexible, sensory- and expectation-dependent decision-making. The task required the monkeys to choose which of two visual saccade targets would provide a reward on each trial based on two factors: (1) sensory evidence, indicated by the proximity of a flashed cue to each target; and (2) prior expectations, or the probability that the rewarded target is switched from the previous trial (i.e., hazard rate). Thus, the AODR task provides a common spatial framework to assess how representations of expectations, evidence, and choices interact within populations of dlPFC neurons to help guide behavior.
Monkeys performed the AODR task in two separate blocks: (1) a low hazard-rate condition (H = 0.05), where the rewarded target is usually the same as on the previous trial and thus the sensory evidence can be largely ignored; and (2) an intermediate hazard-rate condition (H = 0.5), where the rewarded target is independent from trial to trial and thus the sensory evidence is maximally informative. We estimated the contributions of both sensory evidence and expectations to the monkeys' decisions by fitting logistic psychometric functions to choice data with respect to the strength of the sensory evidence (position of the flashed cue) that the reward target switched from the previous trial for each hazard-rate condition. The monkeys behavior was roughly consistent with an ideal observer model, where the relative contributions of expectations and

sensory evidence were hazard-rate dependent. We found that this flexible decision-making strategy was reflected in neuronal activity in the dlPFC, which included sensory, memory, and/or saccade-related activity that for many neurons was also modulated by the hazard-rate condition to enhance or suppress the representation of sensory information and its persistence into working-memory. These preliminary results suggest that the macaque dlPFC likely contains spatial working-memory representations that interact with sensory and motor signals to support flexible decision-making behavior.

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Poster

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Brain and Behavior Research Foundation

Title: Cortical State Dynamics in the Frontal Eye Field (FEF) during Spontaneous Activity

Authors: *G. CHAN[1], Y. SHI[2], X. CHEN[3], T. MOORE[4], T. ENGEL[2];
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Abstract: Neocortical activity is permeated with endogenous fluctuations. The pattern of spontaneous activity reflects variations in global cortical states, which form a continuum of spectra from sleep to wakefulness [1,2]. These spontaneous cortical-state fluctuations are also modulated locally by cognitive factors such as spatial attention [1,2], however these local cortical state dynamics during perception have only been studied previously in the occipital cortex. In this study, we investigated cortical state dynamics within the primate prefrontal cortex, specifically within the frontal eye field (FEF), a cortical area causally involved in deployment of visual attention. We recorded spiking activity across layers of multiple cortical columns in the FEF of monkeys during a fixation task. Using Hidden Markov Models (HMMs), we found that population neural activity in FEF spontaneously fluctuated between phases of vigorous (On) and faint (Off) spiking synchronously, similar to On-Off dynamics in the visual cortex. Next, we used gap statistics analysis to identify cortical columns within and across recordings based on correlations of receptive fields. On-Off dynamics were shown to be confined within local cortical columns, with synchrony gradually decreasing with the increase of lateral spatial distance.

Finally, we compared On-Off dynamics and fluctuations in local field potentials (LFPs) to identify the sequence of propagation of local On-Off activity along the cortical surface. By using wave signal detection methods, we find the time lag of On-Off phases is correlated with spatial distance, which suggests that the propagation of On-Off dynamical patterns across cortical columns takes the form of a traveling wave, specifically at the times when the phase transition occurs within one of the cortical columns. Combined, our results reveal columnar On-Off fluctuations in prefrontal cortex and their spatio-temporal propagation patterns across columns. The columnar synchrony and spatial coordination of cortical dynamics extend our understanding of the operating regime in cortical networks.
References:[1] Harris, K. D., & Thiele, A. Nature reviews neuroscience (2011). [2] Engel, et al, Science (2016).

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Poster

PSTR433. Decision-Making: Prefrontal Cortex

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Program #/Poster #: PSTR433.24/TT19

Topic: G.03. Motivation

Support: R01MH130608
T32NS115705

Title: Prefrontal Cortical Afferents Send Parallel, Discrete Inputs to Perigenual and Subgenual Anterior Cingulate in Nonhuman Primates

Authors: *D. C. MYERS[1], J. L. FUDGE[2];
[1]Neurosci., Univ. of Rochester Sch. of Med. and Dent., Rochester, NY; [2]Neuroscience, Psychiatry, Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: In humans and monkeys, prefrontal (PFC)-amygdala connections have a specialized role in decisional responses during social interactions. In the medial PFC, the perigenual (pgACC) and subgenual (sgACC) anterior cingulate cortex are adjacent sites that provide strong 'top-down' modulation. The pgACC and sgACC mediate conflict monitoring (including to social cues) and sustained arousal to salient stimuli, respectively. We previously showed that pgACC inputs to the amygdala always co-project with sgACC inputs, but only in select 'hotspots'. Here, we asked whether general PFC networks influencing the pgACC and sgACC are segregated or overlapping. We used neuronal tracing of each circuit in the same animal (n=2). pgACC injections resulted in fewer labeled cells in a more restricted number of PFC regions compared to sgACC injections. Surprisingly, PFC inputs to pgACC and sgACC nodes in the same animal were highly segregated. Following pgACC injections, labeled cell bodies were mainly in areas 9, 46, 8, 45, 12l, 12o, and 24b,c. In contrast, the sgACC injections resulted in labeled cells in broadly distributed regions including areas 10, 11m, 12o, 13a/b, 32, 24a/b, 14r/c. Area 12o was

unique in projecting to both pgACC and sgACC. Analysis of intrinsic connections indicated a one-way (top-down) flow of information from pgACC to sgACC. Taken together, we conclude that pgACC and sgACC act largely as separate hubs, conveying 'on-line' conflict monitoring and emotional valuation of sensory information, respectively. In specific amygdala 'hotspots', inputs from these distinct nodes are highly convergent, co-modulating common pyramidal neuron ensembles.

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Poster

PSTR433. Decision-Making: Prefrontal Cortex

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR433.25/TT20

Topic: H.03. Decision Making

Support: CI 1902 2021

Title: Von Economo neurons are present throughout the medial surface of the human prefrontal cortex

Authors: *D. ORTIZ-MUÑOZ[1], C. A. GONZALEZ-ACOSTA[1], O. A. PLAZA-PATIÑO[2], E. BURITICA-RAMIREZ[1], **L. V. BECERRA-HERNANDEZ**[1];
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Abstract: Von Economo neurons (VENs) are large spindle-shaped soma cells located in the Vb sublamina of the cerebral cortex of large mammals such as elephants, whales, and higher primates including humans. In the latter, VENs have been found in the anterior cingulate, the frontoinsular, the dorsomedial prefrontal, and the medial frontopolar cortices. Due to their morphology, laminar and cortical locations, they have been linked functionally to social cognition, and their alteration has been linked to diverse neuropsychiatric pathologies. **Objective:** To explore the presence of VENs in other areas of the human medial prefrontal cortex and to confirm its presence in the precuneus. **Materials and Methods**: nissl staining and anti-NeuN immunohistochemistry were performed in the areas of Brodmann 11m, 14r, 10m, 10r, 25, 32s, 32p and precuneus on the medial surface of both hemispheres from postmortem subjects (4M:1F) without a history of neurological, or psychiatric diseases or brain damage from the National Institute of Forensic Medicine and Forensic Sciences in Cali, Colombia. 288 tissue sections (50 μm) per subject to histological observation with a light microscope ZEISS Scope.A1. After checking the cytoarchitectural of areas and searching the VENs throughout the Vb layer, according to the two observers' expertise. In the microphotographs 10x, we determined the VEN/pyramidal ratio and the differences between the crest and walls of the same gyre, while in the microphotographs 40x, determined and compared the size of the cell body of the pyramidal neurons and VENs. These counts and measurements were made with the assistance of

the Fiji program. **Results:** We found VENs in all areas studied, although were present in all subjects, they were not present in all processed tissue sections nor were they evenly distributed on the crest and walls of each section, because they are very scarce. A higher proportion of VENs was found in the crests than in the walls. We confirmed the interhemispheric asymmetries by finding more VENs in the studied cortical areas of the right hemisphere. Additionally, the inter-area differences in the size of the VENs, are larger in areas with fewer of them and also found a greater number of VENs in the areas closest to the anterior cingulate cortex. 354 VEN/57.186 Pyramidal **Conclusions:** These findings give further support to the hypothesis that VENs could establish a bridge between cognition and its emotional expression, given that all these cortical areas and their connections have been related to the control of attentional and motivational processes. as well as with the symptomatology in pathologies that compromise them.

Disclosures: D. Ortiz-Muñoz: None. **C.A. Gonzalez-Acosta:** None. **O.A. Plaza-Patiño:** None. **E. Buritica-Ramirez:** None. **L.V. Becerra-Hernandez:** None.

Poster

PSTR433. Decision-Making: Prefrontal Cortex

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Topic: H.03. Decision Making

Support: NIH Grant R01 MH121509

Title: Causal evidence of directed interactions supporting cognitive control via transcranial magnetic stimulation of the lateral prefrontal cortex

Authors: *A. MEYER, D. E. NEE;
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Abstract: The frontoparietal control network (FPCN) supports the flexible integration of demands of the external environment with internal goals. Recent research has shown the FPCN can be delineated into distinct control sub-subsystems organized along a present to future axis across sensorimotor-proximal to sensorimotor-distal areas (Nee, 2021). Moreover, effective connectivity modeling has suggested asymmetries in influences among areas suggesting 1) a future-oriented sensorimotor-distal sub-system of the FPCN excites itself, but suppresses other FPCN sub-systems (Nee, 2021), and 2) a mid-dorsolateral prefrontal cortex (mid-DLPFC) area within the future-oriented sub-system is particularly influential (Nee & D'Esposito, 2016; Badre & Nee, 2018). However, these models require causal validation. Here, we contrasted the effects of continuous theta burst stimulation (cTBS) to two areas within the future-oriented control sub-system: the lateral frontal pole (FPl) and the mid-DLPFC, as well as a control site (S1) in 34 healthy human adults while they performed a task designed to manipulate present- and future-oriented cognitive control (Nee & D'Esposito, 2016). Activation changes attributable to cTBS

were examined with post-cTBS fMRI during the task in a within-subjects, cross-over design. cTBS was predicted to downregulate efferent influences providing a causal test of models of effective connectivity (Bergmann & Hartwigsen, 2021). First, we observed cTBS to the future-oriented sub-system produced numerically reduced activation locally, but increased activation in other sub-systems of the FPCN. This suggests cTBS dampens within-system excitation and between-system suppression (i.e. disinhibition). Second, cTBS to the mid-DLPFC uniquely elevated activation in conditions requiring future-oriented control with increases most pronounced in the present-oriented control sub-system. Interestingly, these activation changes were paralleled by improved performance selectively in conditions requiring future-oriented, but not present-oriented control. These results suggest the increases in activation help to boost performance in a compensatory manner. Moreover, these activation changes and behavioral improvements were unique to mid-DLPFC-cTBS suggests the mid-DLPFC is a particularly influential area within the FPCN. Collectively, these data indicate downregulatory cTBS paradoxically increases activation in a manner consistent with models of effective connectivity. Improvements in behavior indicate these changes need not be disruptive, but can facilitate performance in some situations, which may have future clinical utility.

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PSTR433. Decision-Making: Prefrontal Cortex

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Topic: H.03. Decision Making

Support: Hong Kong Research Grants Council (15600619)

Title: Causal role of lateral frontopolar cortex in decomposing complex choice information

Authors: ***C.-K. LAW**, N. H. L. WONG, J. J. WONG, B. K. H. CHAU;
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Abstract: Digesting complex choice information is often essential for making important decisions in daily life. Our recent neuroimaging study revealed that lateral frontopolar cortex (FPl), a region that is uniquely well-developed in the human brain, has a specific role in decision making when complex information is involved (Law et al., 2023). Particularly, it is suggested that FPl decomposes complex information and passes this information to posterior cingulate cortex (PCC) for guiding decision making. Nonetheless, based on correlational neuroimaging data, the frontopolar cortex is implicated in a wide range of cognitive functions, surprisingly, few of these links receive support from data that establish casual relationships. Hence, in this study, we tested the causal role of FPl in digesting complex choice information using transcranial magnetic stimulation (TMS). Healthy human participants (n=27, 12 females, aged 18-37) had their FPl disrupted by continuous theta burst stimulation and then performed a two-stage

decision making task. In Stage 1 they chose between two options that involved complex information and in Stage 2 between two simple options. Our results revealed disruption of FPl, but not the control vertex region, led to poorer choices only with complex options in Stage 1, but not with simple options in Stage 2. Previous studies have demonstrated that TMS does not only influence a local region, but also extends its effect on functionally connected regions that are distant (Fox et al., 2012). Hence, we focused on the FPl-PCC functional connectivity, which is involved in decision making with complex information. We found that participants with stronger resting-state FPl-PCC functional connectivity also showed poorer decision making with complex information after FPl-TMS. Next, we scrutinized the specific aspects in decision making that were modulated by FPl-TMS. This was achieved by fitting nine computational models with variable computational complexities. We found that after FPl disruption participants tended to employ simpler heuristics for integrating complex information. Our findings support the causal role of FPl in integrating complex information during decision making.

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Topic: H.03. Decision Making

Support: ZIA DA000642

Title: Formation of an abstract task representation for generalization

Authors: *A. R. VAIDYA[1], N. MOUSSA[2], D. BADRE[3], T. KAHNT[4];
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Abstract: Prospectively considering the consequences of actions is key to goal-directed behavior. This capacity is believed to depend on an internal model of the world that can be used for planning and making inferences about the outcomes of choices. Failure to utilize or build such an internal model is thought to contribute to failures of goal-directed behavior in substance use disorders and other neuropsychiatric conditions. We are investigated the processes involved in the formation of such an internal model, as well as its use for making novel inferences. Over multiple days, healthy young participants completed a task where they learned that the stimulus-reward associations of multiple contexts were equivalent while undergoing fMRI scanning. After acquiring new stimulus-reward associations in a subset of these contexts, participants were tested on these new stimulus-reward associations in the held-out contexts without feedback. Importantly, this manipulation requires that participants infer the current value of these stimuli based on an abstract representation of the task. We will examine how neural representations of

task conditions change over multiple sessions as this abstract task structure is discovered, and whether different networks are involved in uncovering this structure and implementing abstract rules for controlling behavior.

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Poster

PSTR433. Decision-Making: Prefrontal Cortex

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Topic: H.03. Decision Making

Support: SNSF grant CR13I1_162720/1
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Title: Frontal mechanisms underlying primate calls recognition by humans

Authors: *L. CERAVOLO[1], C. DEBRACQUE[2], E. POOL[3], T. GRUBER[4], D. GRANDJEAN[5]; [1]Swiss Ctr. For Affective Sci., Geneve, Switzerland; [2]Univ. of Geneva - CISA - NEAD, Geneve, Switzerland; [3]Univ. de Geneve, Geneve, Switzerland; [4]Univ. of Geneva, Geneva, Switzerland; [5]Neurosci. of Emotion and Affective Dynamics Lab., Geneva, Switzerland

Abstract: The ability to process verbal language seems unique to humans and relies not only on semantics but on other forms of communication such as affective vocalisations, that we share with other primate species—particularly great apes (*Hominidae*). To better understand these processes at the behavioural and brain level, we asked human participants to categorize vocalizations of four primate species including human, great apes (chimpanzee and bonobo), and monkey (rhesus macaque) during MRI acquisition. Classification was above chance level for all species but bonobo vocalizations. Imaging analyses were computed using a participant-specific, trial-by-trial fitted probability categorization value in a model-based style of data analysis. Model-based analyses revealed the implication of the bilateral orbitofrontal cortex and inferior frontal gyrus *pars triangularis* (IFG_{tri}) respectively correlating and anti-correlating with the fitted probability of accurate species classification. Further conjunction analyses revealed enhanced activity in a sub-area of the left IFG_{tri} specifically for the accurate classification of chimpanzee calls compared to human voices. Our data—that are controlled for acoustic variability between species—therefore reveal distinct frontal mechanisms that shed light on how the human brain evolved to process non-verbal language.

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Poster

PSTR433. Decision-Making: Prefrontal Cortex

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Topic: G.03. Motivation

Support: NIMH Grant MH119422

Title: Insula target cells within the prelimbic cortex are necessary for social affective behaviors

Authors: *A. DJERDJAJ[1], Z. BHATHENA[1], A. CALLEN[1], T. MATULIS[1], J. P. CHRISTIANSON[2];
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Abstract: The ability to detect, appraise, and respond to another's emotional state is an essential component of social affective behavior. This is mediated by a network of brain regions that include subcortical and cortical structures and is responsible for integrating external cues with internal states to yield appropriate behavioral responses. Dysfunction in the connectivity of this network contributes to abnormal social behavior that occurs in several neuropsychiatric disorders, including autism and schizophrenia. The insula, a site for sensory integration, and prelimbic (PL) cortex, a site involved in decision-making, are reciprocally connected nodes within this network. Here, we investigated the functional role of insula-innervated neurons in the PL in a social affective preference (SAP) test in which experimental rats presented with two age-matched conspecifics, one naïve to treatment and one stressed via 2 footshocks, exhibit approach to stressed juveniles but avoidance of stressed adults. In separate experiments, a transsynaptic viral genetic transfer technique was used to specifically inhibit target neurons within the PL that receive input from either the anterior or posterior insula. Male test rats received bilateral infusions of AAV1-hSyn-Cre into either the anterior or posterior insula and AAV5-DIO-hM4Di into the PL. Prior to SAP testing with either juvenile or adult conspecifics, test rats received an injection of saline or clozapine-N-oxide (CNO, 3 mg/kg) to inhibit insula target cells within the PL. Inhibition of both anterior and posterior insula target cells of the PL abolished preference for stressed juveniles and naïve adults. To determine whether these cells are specific to more complex social interactions, a SAP test was performed with adult male and female conspecifics. Inhibition of target cells had no effect on male test rats' preference for interactions with the opposite sex, indicating that these cell populations may contribute specifically to emotionally-motivated interactions. Future studies aim to identify the cell-type of these PL populations and define their neural activity in response to different social interactions. Findings presented here identify a tract by which social emotional information can be shared between cortical regions and shape behavioral responses to others.

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Poster

PSTR434. Prefrontal Mechanisms of Executive Functions II

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Topic: H.04. Executive Functions

Support: Brain/MINDS
KAKENHI 22H05160
Funakoshi Ryuta Award

Title: Flexible adaptation of mPFC neuronal dynamics before, during and after auditory fear conditioning

Authors: *H. KONDO, R. KIM, R. SHIMODA, R. YAMASHITA, J. KIM, L. S. BREBNER, H. SONG, K. INOKUCHI, Y. KONDO, M. OKAMURA, K. OTA, H. FUJII, H. BITO;
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Abstract: The medial prefrontal cortex (mPFC) is one of the neural substrates that underlie cognitive and emotional functions in mammals. In particular, the mPFC has been suggested to guide animals to adaptive behavior by processing a novel external stimulus that triggers attention and/or a sensory stimulus that elicits emotion. While a topographic map for stable responses to sensory stimuli is maintained as a whole in the sensory cortices, whether the information representation of the sensory attributes also remains stable in prefrontal cortex or are dynamically influenced by external stimuli has not been investigated at single cell resolution. Using repetitive two-photon calcium imaging on head-fixed awake mice, we longitudinally monitored the single-cell dynamics of mPFC neuronal populations responding to trials of auditory cue exposures, over several days. In the first and second series of trials (Day 1 and Day 2), an auditory stimulus of a single frequency (CS) was repeatedly presented. Then, in a third series of trials (Day 3), the same CS was presented but an aversive foot-shock was paired five times at the end of the CS presentation epoch. Control experiments demonstrated that under these conditions, the CS-US pairings caused an auditory fear conditioning in a context distinct from the microscopic stage (freezing change 15.2%, N= 3 mice, p= 0.030). Finally, during a fourth series of trials (Day 4) an identical auditory stimulus (CS) was presented again, as a cue for fear memory recall. We found that the CS-triggered neural activity of the excitatory neurons in the mPFC gradually and significantly decreased in a stepwise manner during repeated presentation of the auditory CS in a neutral context (-55%, CS 2^{nd} response vs 1^{st} CS response, $p< 0.05$; -117%, 5^{th} CS response vs 2^{nd} CS response, $p< 0.01$, N= 9 mice; n= 347 neurons). This decrease persisted on Day 2. When the mice were subjected to auditory fear conditioning on Day 3, however, the proportion of up-regulated responsive neurons significantly increased as the animal underwent paired CS-US exposure, and augmented responsiveness persisted on Day 4 when CS only was presented as a cue for fear memory recall. Finally, using a linear discriminant analysis, we found that encoding accuracy of CS was significantly increased after the CS-US pairings. These results suggest that the mPFC neurons can dynamically adapt their responsiveness to auditory cues in a bidirectional manner depending on the immediate affect. Elucidating the origin of this flexible activity changes at the neural circuit level will shed light on fundamental cognitive processing in the mPFC.

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Poster

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Topic: H.04. Executive Functions

Support: M.E.C.H recieved a Posdoctoral Fellowship from Universidad Panamericana Research Vicepresidency

Title: Identifying the relationship between prefrontal executive function, eating habits, eating behavior, and academic achievement in university students

Authors: *M. CHAVEZ HERNANDEZ;
Univ. Panamericana, Mexico, Mexico

Abstract: In recent decades, diet has changed increasing availability of ultraprocessed foods, which contributes to high levels of overweight and obesity. Studies have shown that there is a bidirectional relationship between prefrontal executive function (PEF) and overweight/obesity, and that there is a cognitive effect derived from poor diet quality, skipping breakfast, and consumption of ultraprocessed foods. University life represents a change in an individual's development, and studies indicate that only 17.4% of university students maintain a healthy diet; additionally, academic load and poor time management in college students have been linked to diet quality and weight gain. The aim of the present study was to evaluate the relationship between PEF, eating habits, eating behavior, and academic achievement in college students, considered a vulnerable population. A cross-sectional correlational study was carried out in a group of undergraduate students from public and private universities within Mexico City. Eating habits were measured by a frequency questionnaire including healthy food intake, ultraprocessed food intake and meal skipping; the Executive Functions in University Students Questionnaire was used to evaluate PEF; eating behavior was measured with the Eating Behavior and Physical Activity Scale; and academic achievement was measured with the self-reported GPA in a 0-to-10-point scale. A sample of 1903 undergraduate students was included in the study (1070 women, 911 men); the mean age was 20.91 years (SD ±2.10). Spearman's rho correlation analysis revealed that there are significant correlations between all variables of interest (rs range -0.20 to 0.36). Specifically, there is a positive relation between academic achievement and PEF, healthy food intake and PEF, and emotional undereating and PEF; also, a significant negative correlation was found between PEF and emotional overeating, with ultraprocessed food intake, and with meal skipping. These results provide a broad vision and are consistent with previous reports that indicate that there is a significant correlation between PEF, eating habits, eating behavior, and academic achievement.

Disclosures: M. Chavez Hernandez: None.

Poster

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Topic: H.04. Executive Functions

Support: NIH R01MH124849

Title: Neural circuits underlying learning and adjustment to dynamic changes in the expected value of cognitive control

Authors: *I. GRAHEK, Y. DONG, A. OGBAA, J. KIM, A. SHENHAV;
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Abstract: People calibrate the amount of effort they invest in a task based on the amount of reward they expect to accrue, and the extent to which they think their effort matters for achieving that reward (versus, e.g., being determined at random). Recent work has explored the neural and computational mechanisms by which people combine information about these two components of motivation, reward and efficacy, to determine the overall expected value of investing mental effort (cognitive control), but how they learn this information in the first place remains unknown. To uncover the mechanisms by which people learn and dynamically adjust to the expected value of control in their current environment, we had 38 human subjects perform a control-demanding Stroop task while measuring BOLD fMRI. Over the course of the experiment, we varied the probability of obtaining a large vs. small monetary reward and the probability of those rewards being based on performance vs. random chance (high vs. low efficacy). Thus, participants had to continually update reward and efficacy estimates based on feedback, and were sporadically asked to report their current estimates of each. We show that these subjective estimates are best captured by a reinforcement learning model that assumes independent updating of reward and efficacy estimates based on respective feedback. Model-based analyses showed that participants integrated reward and efficacy estimates to determine the expected value of control, and adjusted control allocation accordingly. This resulted in them investing the most effort and achieving the best performance (fastest and most accurate) when they believed that rewards were likely to be (a) large and (b) determined by their performance. When participants received feedback for a given trial, we found signatures of signed reward prediction error within the ventral striatum. Notably, we found evidence that distinct frontoparietal regions tracked unsigned estimates of reward and efficacy prediction errors, providing neural evidence for separate learning of reward and efficacy, prior to the integration of this information into the expected value of control. While participants were performing the task, we found widespread encoding of model-based estimates of the value of control, including across regions of frontal and parietal cortex, consistent with behavioral findings suggesting concomitant increases in effort allocation. Our findings chart the

neural and computational mechanisms through which components of motivation are learned, integrated, and used to guide the allocation of cognitive effort.

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Poster

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Topic: H.04. Executive Functions

Support: P20NS123151

Title: Evoked frontal rhythms in Parkinson's Disease during interval timing task performance

Authors: *F. TABASI[1], R. C. COLE[2], A. ROHL[3], K. JOHARI[4], N. S. NARAYANAN[5], J. D. GREENLEE[6];
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Abstract: Parkinson’s disease is commonly associated with deficits in cognitive functions, including cognitive control, an ability that exerts top-down regulation on executive functions enabling adaptive behavior. Cognitive control dysfunction can appear in the early or late stages of the disease. However, the underlying pathophysiologic mechanism of impaired cognitive control in PD patients is unclear. Prefrontal low-frequency activities are shown to be engaged in cognitive control. Previous studies showed that cue-evoked mid-frontal 4-Hz rhythm is attenuated in PD patients with cognitive impairments, predicting increased timing variability indicative of cognitive decline (Singh *et al.* npj Parkinson Disease 2021; Singh A, *et al*. J Neurol Neurosurg Psychiatry 2023). Here, we used electrocorticography recordings from the prefrontal cortex in eight PD patients and nine essential tremors (ET) patients (all without cognitive problems) during awake deep brain stimulation implantation while performing a simple interval timing task, in which patients needed to estimate short (1 s) and long (3 s) temporal intervals. Timing abilities depend on higher-order cognitive functions, including cognitive control. We compared cue-evoked and response-induced frontal activities in PD and ET. The timing variability was higher in PD compared to ET. Also, we observed differences in cue- and response-related prefrontal activities between the two groups. These observations will enhance our understanding of changes in prefrontal activities related to cognitive control in patients with the PD.

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Title: Medial prefrontal cortical amplification of Reuniens output

Authors: *G. VANTOMME, G. DEVIENNE, J. M. HULL, J. R. HUGUENARD;
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Abstract: The neuronal circuit interconnecting medial prefrontal cortex (mPFC), thalamic Reuniens nucleus and hippocampus is central for memory and executive functions. A critical gap exists between known structural and behavioral aspects of this network compared to the paucity of knowledge regarding functional connectivity, especially in mouse models. We applied a combination of electrophysiological recordings and optogenetic manipulation both *in vitro* and *in vivo* to reveal the consequences of Reuniens activity. Beyond direct excitatory monosynaptic connections and feedforward inhibition targeting both pyramidal cells in mPFC and CA1, Reuniens output shows a strong recruitment of feedforward excitation specifically in mPFC. This feedforward excitation was strongly enhanced upon pharmacological blockade of GABA receptors, could induce burst discharge of action potentials in mPFC neurons (8/12 cells), which may constitute a mechanism underlying the capacity of Reuniens inputs to synchronize activity in mPFC. Optogenetic activation of Reuniens *in vivo* evoked heterogeneous responses in mPFC neurons, as detected by unit responses recorded with NeuroPixel probes. Responses included increases in firing (12/22 units), decreased firing (2/22 units) and delayed firing responses (14/22 units) consistent with the observations made *in vitro* of delayed firing, and confirming that mPFC can amplify Reuniens output through delayed burst firing. Feedforward activation of cortical circuits was more prominent than what has been reported with activation in sensory thalamic regions, suggesting that intracortical amplification of Reuniens output is a specialized feature of this subsystem underlying memory/cognition. Our results also show that imbalance in inhibition/excitation can lead to excessive delayed activity, which might be a mechanism underlying hyper synchrony during seizures. Initial observations in the *SCN8A* mouse model of absence seizure revealed that Reuniens activation of mPFC neurons resulted in an increase in excitation/inhibition ratio as measured with composite evoked synaptic response recorded intracellularly and compared to wild-type littermates, and a slight increase in the amplitude of the sink in deep layers when recorded with LFP. Altogether, these data demonstrate the fundamental synaptic properties underlying the function of the mPFC-Reuniens-hippocampal circuit. They also suggest that mPFC network uniquely amplifies Reuniens output, which may be one of the mechanisms underlying normal hippocampal prefrontal cortical circuit function and abnormal synchronization in the *SCN8A* mouse model of absence seizure.

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Poster

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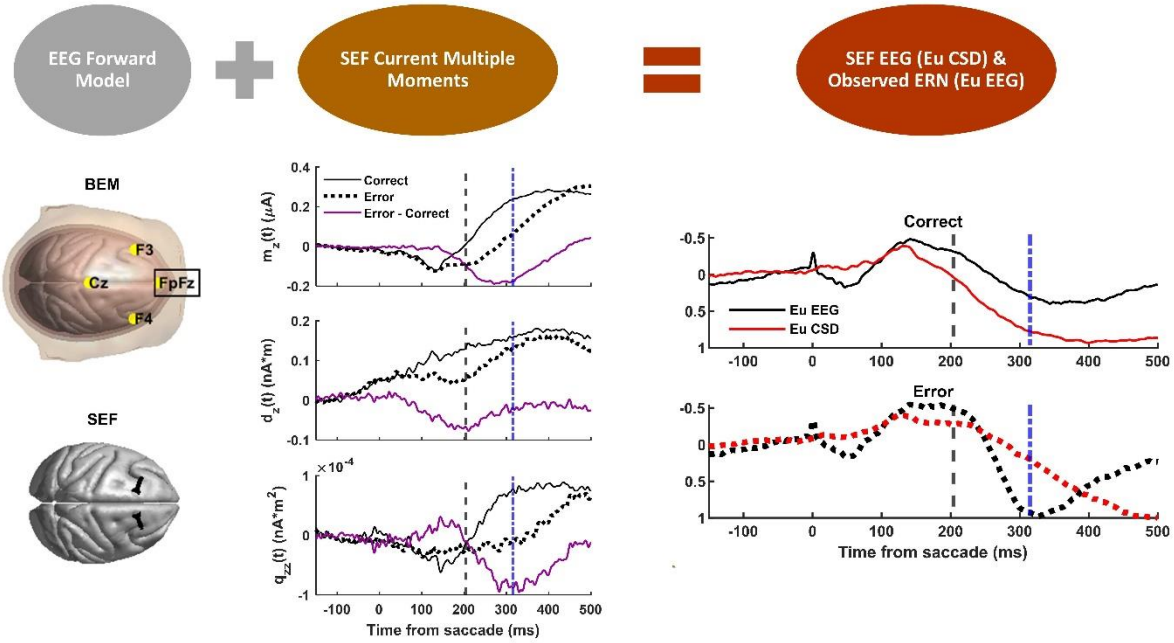
Title: The neural basis of the error-related negativity: A biophysical modeling study

Authors: *B. HERRERA[1], A. SAJAD[3], S. P. ERRINGTON[4], J. D. SCHALL[5], J. J. RIERA[2]; [2]Biomed. Engin., [1]Florida Intl. Univ., Miami, FL; [3]Psychology, Vanderbilt Univ., Nashville, TN; [4]Dept. of Neurosci., Washington Univ. Sch. of Med., St. Louis, MO; [5]Biol., York Univ., North York, ON, Canada

Abstract: The error-related negativity (ERN) is a biomarker of psychiatric disorders such as ADHD, OCD, and schizophrenia. However, the utility of ERN as a biomarker depends on our understanding of how it is generated at the cellular and circuit levels. Although ERN is known to originate from medial frontal areas such as the supplementary eye field (SEF), little is known about the underlying neuronal mechanisms. Here, we investigate the cortical origin of the ERN employing a multiscale approach that links cellular-level dynamics, local field potentials (LFPs), and scalp potentials. Recently, we demonstrated that *in-vivo* laminar current source density (CSD) maps can be used to accurately predict EEG reflections. In this study, we utilized our methodology to predict the contribution of SEF to the ERN from the *in-vivo* laminar field potentials. We recorded neuronal spiking and LFPs across all layers of SEF in two macaque monkeys performing a saccade countermanding stop-signal task. The observed laminar CSD comprised multipolar components, with dipoles explaining ERN features and quadrupoles reproducing those for the post-error positivity or Pe. Forward modeling of the estimated SEF current multiple moments explained the ERN generation but not the Pe, confirming the involvement of other areas, such as the anterior cingulate cortex. Additionally, we employed optimized biophysical models of error layer 3 (L3) and layer 5 (L5) pyramidal cells to evaluate their contribution to SEF CSDs, which was negligible. The CSD derived from L3 error pyramidal cells could not explain the observed association between their error-related spiking modulation and the ERN. This last finding suggests that error pyramidal cells are not the main

neuronal generators of the ERN but instead drive the activity of the performance monitoring circuit in SEF. These results provide the most advanced explanation of the cellular mechanisms generating the ERN.



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Poster

PSTR434. Prefrontal Mechanisms of Executive Functions II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR434.07/UU4

Topic: H.04. Executive Functions

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Title: Practice-dependent effects of dorsolateral prefrontal cortex representational geometry on context-dependent behavior

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Abstract: Control representations in human dorsolateral PFC (dlPFC) have been implicated in flexible mapping of inputs to outputs in context-dependent tasks. Recent work has shown that

dlPFC representational geometry is organized into orthogonal subspaces for each context, and each subspace preferentially encodes context-relevant stimulus dimensions. The importance of dlPFC geometry for behavior remains poorly understood. Here, we examine how dlPFC representational geometry relates to behavior, and how this relationship changes with practice. We hypothesized that practice would shift the representational geometry to one that was more separable, thus contributing to making behavior more efficient, but also more sensitive to task-irrelevant information. We investigated representational geometry in dlPFC using fMRI and pattern analysis as participants (n=20) performed a context-dependent categorization task over 5 sessions. Participants were presented with stimuli with 3 features. The auditory feature (context) determined which of the two visual features was relevant for categorization on the trial. In a region of dlPFC encoding task decisions, we trained linear classifiers to identify the context-specific coding axes for each stimulus feature and estimated the location of multi-voxel patterns on each trial along these axes. Neural coding strength for a particular stimulus was defined as the absolute distance of the trial pattern from the relevant, context-specific classification hyperplane. Using mixed-effects models, we regressed trial-by-trial measures of task performance on estimates of neural coding strength for each task feature. Trial-by-trial variance in response time was explained by the strength of neural coding for task context, as well as the context-relevant and context-irrelevant stimulus features. Stronger coding of context, and the context-relevant stimulus feature, predicted faster responses, while stronger coding for the context-irrelevant feature predicted slower responses, providing evidence for a role of dlPFC representational geometry in behavior. Practice transformed the influence of the context-irrelevant feature on behavior such that in later scanning sessions, stronger coding of the context-irrelevant feature predicted faster responses. Our results suggest that dlPFC geometry contributes to context-dependent behavior, and this contribution is transformed with practice.

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Poster

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Title: Distinct population architecture for cognitive flexibility in frontal and parietal cortex

Authors: *Y. OSAKO[1], Y. SAITO[2], Y.-N. LEOW[3], G. R. HELLER[3], S. K. ÄHRLUND-RICHTER[3], Z. WANG[3], G. T. DRUMMOND[3], T. OSAKI[3], M. SUR[3];
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Abstract: Cognitive flexibility, a vital ability for mammals to adapt to diverse situations and environments, is hindered by the curse of dimensionality in neuronal activity. To address this challenge, leveraging shared information among cognitive operations within the system to reduce information dimensionality is advantageous. However, the underlying mechanisms of such cognitive operations remain elusive. In this study, we trained mice on a behavioral task involving distinct cognitive operations and recorded neuronal activity simultaneously in the anterior cingulate cortex (ACC) and posterior parietal cortex (PPC), regions thought to be associated with cognitive flexibility. Mice were trained on a delayed match-to-sample (DMS) task, requiring them to discern whether two consecutive stimuli were matched. During the delay period between stimuli, the stimulus information was maintained as working memory (WM). Subsequently, a second delay period served as an action planning (AP) period, allowing mice to make decisions and prepare for the behavior. This task enabled investigation of different cognitive processes (WM and AP) driven by identical stimuli. Our findings revealed that both the ACC and PPC represented WM and AP using linearly separable neural decoders. Moreover, the neuronal subspaces corresponding to these representations were independent (orthogonal) in PPC, while partially overlapping in ACC. Notably, this population architecture correlated with animal behavior, collapsing during error trials. Additionally, recurrent neural networks trained on the same task exhibited latent representations akin to those observed in the ACC, implying an efficient geometric structure for achieving cognitive flexibility. These results suggest that the ACC represents WM and AP in a more abstract manner within a higher-dimensional space to facilitate cognitive flexibility, while the PPC achieves flexibility by utilizing distinct neural subspaces. Future experiments will explore potential interactions between these geometric structures in efficiently representing cognitive flexibility.

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Poster

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Topic: H.04. Executive Functions

Support: DFG Grant VO 1432/22-1
ESF Grant 100342331

Title: Physical and cognitive training interventions to improve everyday-like dual-task driving behavior in older adults

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Abstract: Driving is an important everyday behavior in older adults that heavily relies on various cognitive and motor functions. Engaging in additional tasks while driving (i.e., dual-tasking), such as interacting with in-vehicle devices, places extra demands on neurocognitive resources and may lead to compromised driving performance. Enhancing neurocognitive capacity through structured cognitive and/or physical training may provide support in performing such complex everyday tasks. This study therefore aimed to investigate the effects of physical, cognitive, and combined physical-cognitive training on dual-task driving performance in healthy older adults. A total of 106 older adults (age: M = 69.15 years ± 2.71, 59 female) participated in a simulated car driving scenario. Participants followed a lead car at a constant speed (70 km/h) on a slightly winding rural road. While driving, they engaged in two additional tasks. Additional tasks, i.e. typing 3-digit numbers and stating arguments for/against public issues, were presented visually or auditorily at intervals of about 15-25 s. Dual-task driving performance was assessed by measuring average velocity (speed control) and variability in lateral position on the road (lane keeping) during a 0-10 s interval after each additional task onset. Brain activity was recorded using functional near-infrared spectroscopy (fNIRS) in frontal and parietal brain regions. After pre-test, participants were randomly assigned to one of three training groups: physical, cognitive, or combined physical-cognitive training. Training sessions lasted for 45 min and were conducted twice a week for twelve weeks (24 sessions or 1080 min in total). The effect of the different training programs on dual-task driving performance was analyzed by use of linear mixed models (Time x Training Group x Task Type). We observed no significant main or interaction effects for speed control, although all training groups exhibited slightly improved speed control at post-test (p = .097, ηp^2 = .15). For lane keeping, the triple interaction was significant (p = .044, ηp^2 < .01), indicating that only during the typing task, lane keeping benefited from training, mainly in the physical-cognitive training group. In summary, effects of training effects on dual-task driving performance appear to be small and specific to the outcome parameter and type of additional task. Combined physical-cognitive training showed the greatest effectiveness, possibly due to the additive advantages of physical and cognitive training. Whether this effect is moderated through an improvement of multitasking skills or motor/cognitive performance needs to be further investigated.

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Poster

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Topic: H.04. Executive Functions

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R01-EY019882
P30-EY008126
Canadian Institutes of Health Research Postdoctoral Fellowship

Title: Microcircuitry of performance and outcome monitoring in the supplementary eye field and mid-cingulate cortex

Authors: *A. SAJAD[1], S. P. ERRINGTON[2], P. THIRUNAVUKKARASU[3], J. D. SCHALL[3]; [1]Vanderbilt Univ., Nashville, TN; [2]Dept. of Neurosci., Washington Univ. Sch. of Med., St. Louis, MO; [3]Biol. Dept., York Univ., North York, ON, Canada

Abstract: Successful interaction with the environment requires the monitoring of performance and outcomes and exerting adjustments on behavior. The medial frontal cortex (MFC) is critical to these functions; however, the precise neural mechanisms remain unclear. To address this, we recorded laminar neural data using linear vector arrays (164 penetrations) from the Supplementary Eye Field (SEF) and the dorsal (dMCC) and ventral (vMCC) banks of the Mid-Cingulate Cortex, in two monkeys. Here, we report the laminar organization of different error signals in SEF and dMCC in a stop-signal task. Fluid reward was earned for generating a saccade to a target and for inhibiting it when a stop-signal appeared. The target location cued whether the upcoming reward value was high or low; this target-reward mapping changed across blocks unpredictably. Following the response, two distinct auditory feedback tones indicated whether or not reward would be provided. On ~10% of correct trials, an unexpected negative feedback tone was presented. This design enabled the identification of signals related to internally generated errors (saccades made after stop-signal appearance), feedback, feedback prediction error (FPE), and reward prediction error (RPE). These signals were largely observed in non-overlapping neuron populations. Overall, error neurons (SEF: n = 142; dMCC: n = 54) in SEF had an earlier modulation onset than those in the dACC. In the SEF, the error signal initially emerged in the middle layers (L3 and L5) and subsequently the upper (L2/3) and lower (L5/6) layers. In contrast, in the dMCC, error neurons were present across all layers, with the earliest modulation in L2/3. Negative feedback in both SEF and dMCC resulted in facilitation of neurons (SEF: n=40, dMCC: n = 56) largely confined to L2/3 and suppression (SEF: n=25, dMCC: n = 20) of neurons mainly in L5/6. FPE was first encoded by neurons (SEF: n=36, dMCC: n = 64) in L5/6 in SEF, in contrast to the dMCC where it first appeared in L2/3. Finally, positive (SEF: n=24, dMCC: n = 28), negative (SEF: n=35, dMCC: n = 28), and unsigned (SEF: n=33, dMCC: n = 39) RPE signals were found across all layers in both areas, with a larger proportion of negative RPE neurons in L2/3. These results illustrate similarities and differences in laminar organization of complementary error monitoring signals in SEF and dMCC, and contribute to future models of cortical microcircuitry and MFC function.

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Poster

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Program #/Poster #: PSTR434.11/UU8

Topic: H.04. Executive Functions

Title: Brain Oscillations Related to Prolonged Reaction Times in Go/No-go Tasks with Different Meanings of Stimulus Color

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Abstract: *Background*: Color has meaning in particular contexts, and the meaning of color can impact behavioral performance. For example, the meaning of color about traffic rules (blue/green and red mean “go” and “stop” respectively) influences reaction times (RTs) to signals. Specifically, in a Go/No-go task, RTs have been reported to be longer when responding to a red signal and withholding the response to a blue signal (Red Go/Blue No-go task) than when responding to a blue signal and withholding the response to a red signal (Blue Go/Red No-go task). However, the neurophysiological background of this phenomenon has not been fully understood. The purpose of this study was to investigate the brain oscillatory activity associated with the effect of meaning of color on RTs in the Go/No-go task.
Methods: Twenty participants performed a Blue simple reaction task, a Red simple reaction task, a Blue Go/Red No-go task, and a Red Go/Blue No-go task. We recorded responses to signals and electroencephalogram (EEG) during the tasks and evaluated RTs and changes in spectral power over time, referred to as event-related synchronization (ERS) and event-related desynchronization (ERD).
Results: The behavioral results were similar to previous studies. The EEG results showed that frontal beta ERD and theta ERS were greater when signals were presented in blue than red color in both simple reaction and Go/No-go tasks. In addition, the onset of theta ERS was delayed in the Red Go than Blue Go trial in the Go/No-go task.
Discussion and Conclusion: The enhanced beta ERD may indicate that blue signals facilitate motor response, and the delayed onset of theta ERS may indicate the delayed onset of cognitive process when responding to red signals as compared to blue signals in the Go/No-go task. Thus, this delay in cognitive process can be involved in the slow response in the Red Go/Blue No-go task.

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Poster

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Title: Juxtaposed regions in dorsolateral prefrontal cortex differentially support domain-specific scene construction and domain-flexible cognitive control

Authors: *L. DINICOLA, W. SUN, R. L. BUCKNER;
Harvard Univ., Cambridge, MA

Abstract: Primate prefrontal cortex (PFC) is heterogenous, and the roles that PFC regions play in higher-order functions remain debated. One debate surrounds the degree to which PFC regions support domain-flexible cognitive control functions or are specialized for domain-specific information processing. Here, we tested the hypothesis that side-by-side regions of PFC, linked to distinct parallel association networks, differentially support domain-flexible control or domain-specialized processes. Using networks estimated from functional connectivity MRI, we identified three separate PFC regions of distinct parallel networks within each of 9 repeatedly-scanned individuals. These regions included a dorsolateral PFC (DLPFC) region of default network A (DN-A), a broader PFC region of a frontoparietal network (FPN-A), and an inferior PFC region of a language network (LANG). Using independent task data, we explored the response properties of these three side-by-side regions. Results revealed a significant triple dissociation. Within DLPFC, DN-A regions were preferentially recruited by an Episodic Projection task contrast. Juxtaposed FPN-A regions were preferentially recruited by working memory (N-Back) task contrasts and in a domain-flexible way (i.e., across multiple categories). The inferior PFC region of the LANG network was preferentially recruited for sentence processing. Post-hoc trial-level analyses further supported that the DLPFC region linked to DN-A specifically responded to task demands involving scene construction and not cognitive effort. Our findings demonstrate that DLPFC possesses multiple differentially specialized regions that are spatially near to one another but support diverse aspects of cognitive function. These results also raise the possibility that PFC regions gain processing properties through segregated anatomical projections, including a specialized region in DLPFC that is connected to the hippocampal formation and distinct from more commonly studied domain-flexible regions supporting cognitive control.

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Poster

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Topic: H.04. Executive Functions

Support: NIH R01 MH125497
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Title: Error monitoring is modulated by activation of conjunctive representations of uncommitted correct actions

Authors: A. KIKUMOTO[1], D. BUYUKYAZGAN[1], S. POELLNITZ[1], A. PEETZ ALIO[1], M. CRUZ[1], T. NISHIO[2], N. KAWAMURA[2], M. KAMINOKADO[2], Y. KANEKO[2], K. SHIBATA[2], ***D. BADRE**[1];
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Abstract: Monitoring and detecting action errors allows cognitive control systems to adjust behavior promptly and learn from mistakes. Thus, understanding the neurophysiological mechanisms of the subjective perception of errors is important for cognitive control theory. However, the link between standard event-related potentials (ERPs) of error processing in humans (e.g., error-related negativity and positivity) and error monitoring and awareness has been surprisingly inconsistent. One possibility is that the geometric and dynamic properties of encoded task representations, which are typically missed in conventional ERP approaches, directly inform error monitoring decisions, as well as influencing ERP markers of error processing. In this study, we applied time-resolved multivariate analyses to EEG data to track representations of uncommitted correct actions and committed erroneous actions and related them to error monitoring. Participants performed a rule-based response selection task with a response deadline that provided variable time to prepare before compelling a response. Deadlines were calibrated to set expected error rates at approximately chance levels. Following a response of the main task, participants pressed a key to report when they detected an error before the next trial. During action selection of error trails, conjunctive representations of uncommitted correct actions did not differ between detected and undetected errors. However, the difference emerged immediately following error commissions, coinciding in time with error-related negativity. Surprisingly, stronger activation of conjunctions of uncommitted correct actions resulted in more failures of error detection. These results suggest that error awareness is directly modulated by the retrieval of conjunctive task representations.

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Poster

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Topic: H.04. Executive Functions

Support: R01 MH053851
T32 NS082145

Title: Role of central medial thalamus to orbitofrontal cortex pathway in reversal learning

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Abstract: Stress-related psychiatric disorders, such as major depressive disorder and anxiety disorders, have cognitive flexibility deficits that persist even after other symptoms of these disorders go into remission. Reversal learning, a form of cognitive flexibility necessary to adapt to a changing environment, is disrupted in stress-related psychiatric disorders. The orbitofrontal cortex (OFC) mediates reversal learning, and hyperactivity in the OFC is associated with depression and obsessive-compulsive disorder in humans. Using a reward-based discrimination digging task to assess reversal learning in rodents, we have previously reported that chronic stress impairs reversal learning and potentiates responses to excitatory input in the OFC, and that inducing long-term depression in the mediodorsal thalamus to OFC pathway reverses these deficits, indicating that increased activity in projections to the OFC is detrimental to reversal learning. However, the circuit-level mechanisms underlying stress-induced reversal learning deficits are not well established. Preliminary data using Fos immunohistochemistry, showed a significant decrease in Fos in the lateral OFC following reversal learning, and a significant increase in Fos induction in the central medial (CM) thalamus. Other preliminary data suggest that the CM projects to the OFC and converging evidence suggests the CM activates inhibitory interneurons in the OFC which are crucial for reversal learning. Therefore, we next tested the hypothesis that excitatory input from the CM to the OFC is essential to reversal learning. We used an adeno-associated virus to deliver an inhibitory (Gi) DREADD, an excitatory (Gq) DREADD, or GFP control into the CM under the control of the CaMKII promoter, and implanted guide cannulae into the lateral OFC for pathway specific in/activation. Animals received microinjections of the DREADD agonist clozapine-N-oxide (300 μM, i.c. 0.75 μL) directly preceding the reversal learning task. Inactivating the CM-OFC pathway in non-stressed animals significantly impaired reversal learning. Based on these results we hypothesize that activating the CM-OFC pathway in chronically stressed animals will reverse stress-induced deficits in reversal learning, these experiments are ongoing. These results suggest that activation in the CM-OFC pathway is important for reversal learning and that it is a potential target for reversing stress-induced deficits. This implicates a circuit not yet investigated in the role of chronic stress in disruptions of cognitive flexibility.

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Poster

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Topic: H.04. Executive Functions

Support: NIMH R01MH122613

Title: Eeg decoding of task switching effects on hierarchical task representations

Authors: *H. MORROW[1], K. HWANG[2];
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Abstract: Adapting responses flexibly based on contextual circumstances is a fundamental cognitive ability that is thought to be mediated by hierarchical task representations (Badre & Nee 2018). Prior research has shown that task-switching cost is likely associated with reconfiguration of task representation, as indicated by a decrease in decoding accuracy from fMRI and EEG data. However, most studies switched between simple stimulus-response mappings, without considering the multi-faceted, hierarchical structure of task representations. In the present study, we developed a hierarchical control task that necessitated subjects switching between tasks by taking into account multiple task contexts and features. Importantly, the task encompasses three critical components of task representations: context, feature, and response rule. Thus, during task switching, not only was the response rule altered, but the context and features of the task also underwent changes. Our goal is to determine which constituent representation of such hierarchical task representation is affected by task switching. We used encephalography (EEG) based linear discriminatory analysis to decode task representations. The rational being that when trained a classifier to decode task representations, decoding accuracy will decrease when the to-be-decoded representation is affected by task switching. We collected EEG data from 36 healthy participants. From the time-frequency decoding analysis, we found that participants had higher decoding accuracy when trials kept the same context-dependent rules as the previous trial, and lower decoding accuracy when the context of the current trial was switched from the previous trial. This difference in decoding accuracy is most prominent in the alpha-to-beta high frequency range, indicating a potential neural signal reconfiguring contextual representation. Notably, decoding accuracy of other task features and response rules did not show significant difference when switched between tasks. Our results indicate reconfiguration of context representations in a hierarchical task representation likely has the strongest contribution to task-switching cost.

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Poster

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Title: Neurons in prefrontal cortex respond to abstract pattern violations

Authors: *K. E. CONEN, T. M. DESROCHERS;
Neurosci., Brown Univ., Providence, RI

Abstract: The ability to identify patterns in events around us shapes our expectations and helps us adapt to changing environments. One example of pattern recognition is the detection of abstract sequences - higher-order relationships that remain consistent across stimuli (e.g., AAAB, &&&*). Both humans and nonhuman primates can detect abstract sequences and respond when these patterns are violated. Recent work in our lab identified specific subregions of macaque LPFC with robust BOLD responses to pattern deviants during awake sequence viewing. However, it is not clear how these signals translate to neuronal responses, or whether specific features of LPFC responses distinguish it from other regions. We performed fMRI-targeted neuronal recordings in LPFC and orbitofrontal cortex (OFC) while monkeys performed a no-report sequence viewing task. Monkeys fixated on a central point as fractals appeared in four-item sequences. Each recording session included multiple five-block runs (4-20 runs/session). Within a run, sequences were defined by a standard pattern (e.g. AAAB). Images were drawn pseudorandomly from a set of fractals generated daily. Each run consisted of a habituation block (30 trials, standard sequence), followed by four blocks with deviant sequences on 20% of trials. Deviants were either new images in the standard pattern (NI) or pattern deviants (PD), and were drawn from a separate pool of fractals. Juice was delivered on a graduated schedule contingent on fixation and independent from sequence presentation. We recorded ~500 neurons from the LPFC and ~400 from the OFC of one monkey and analyzed a subset of these neurons. Using two-way ANOVAs, we examined how activity in each neuron varied with ordinal position, reward, sequence type (standard, NI, or PD). While the two regions contained a comparable fraction of reward-sensitive neurons (LPFC: 15%; OFC: 17%; chi-square test $p = 0.59$), effects of sequence-type were more prevalent in LPFC (LPFC: 23%, OFC: 13%; chi-square test $p = 0.008$). Notably, most sequence-type responses in LPFC showed the highest activity during pattern deviants (PD: 53%; NI: 33%, standard: 14%). In contrast, OFC neurons were most likely to respond to NI sequences (PD: 33%, NI: 42%, standard 24%), suggesting that they are more sensitive to rarity of the images rather than the abstract sequence pattern. These results provide preliminary evidence that LPFC networks preferentially respond to abstract sequence patterns. Future analyses will test how these responses change with task demands and examine whether they contribute to a generalizable representation of abstract sequences.

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Poster

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Title: Investigating the effect of transcranial alternating current stimulation paired with cognitive exercises on cerebral blood flow with functional near-infrared spectroscopy

Authors: *S. AZARBARZIN;
Biomed. Engin., Univ. of Manitoba, Winnipeg, MB, Canada

Abstract: ***Introduction:*** Transcranial alternating current stimulation (tACS) is a promising approach to modify brain oscillations, as it enables precise entrainment of cortical oscillations at specific frequencies. ***Method:*** The present double-blind cross-over study aims to evaluate the effect of tACS treatment at 40 Hz paired with cognitive exercises on Alzheimer's Disease (AD). Study participants received either real or sham tACS daily (excluding weekends) for 4 weeks, followed by the opposite treatment after a 2-month wash-out period. Participants were randomly assigned to two groups based on age and cognitive level. An eight-channel fNIRS (functional near-infrared spectroscopy) device was used to measure participants' blood oxygenation levels in their prefrontal cortex to investigate treatment efficacy. These measurements were taken before and after each treatment cycle in combination with a verbal fluency task. Previous studies have indicated that individuals in the early stages of AD exhibit increased oxygen consumption in their prefrontal cortex while performing specific cognitive tasks as a compensatory mechanism. In contrast, healthy individuals do not show such patterns. Therefore, it was hypothesized that a lower oxygenated blood flow would present in patients' post-intervention as compared to baseline as a result of real tACS treatment. ***Results:*** A total of 15 older adults (50-95 years old) completed the study as of January 2021. The data of all who received real tACS versus those who received sham tACS was averaged regardless of treatment order. The difference of blood oxygenation changes between real and sham treatment was statistically significant using a non-parametric test ($p = 0.05$). Results further indicated a statistically significant decrease in blood oxygenation level (by a mean difference of 0.09μM) after real tACS when compared to its corresponding baseline ($p = 0.04$). There were no significant changes in the blood oxygenation level between pre and post sham tACS treatment. These results confirm our hypothesis although the power of the test is low due to small sample size.***Conclusion:*** Active tACS treatment can potentially enhance cognitive abilities in patients with AD, as evidenced by reduced oxygenated blood flow measured with fNIRS.

Disclosures: **S. Azarbarzin:** Other; Samaneh Azarbarzin, Department of Biomedical Engineering, University of Manitoba, Natasha Jacobson, Department of Biosystem Engineering, University of Manitoba, Zahra Moussavi, Department of Biomedical Engineering, University of Manitoba, Department of Electrical and Computer Engineering, University of Manitoba, Riverview Health Centre.

Poster

PSTR434. Prefrontal Mechanisms of Executive Functions II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR434.18/UU15

Topic: H.04. Executive Functions

Support: Grant-in-Aid for Early-Career Scientists 21K13743

Title: Combined mindfulness-based intervention with non-invasive brain stimulation for metacognitive processing

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Abstract: Our previous study which combined repetitive transcranial magnetic stimulation (rTMS) with electroencephalogram (EEG) demonstrated the effectiveness of using specific task-relevant stimulation frequency and target location for the modulation of cognitive and behavioral performance. In the previous study, we initially investigated the correlations between the EEG oscillations and cognitive giving-up processes during problem-solving tasks and found that the frontal theta rhythm is associated with the giving-up processes. We then conducted online rTMS to examine the frequency-dependent stimulation effects of rTMS on the performance of problem-solving tasks and ongoing oscillations by applying rTMS to induce theta and alpha amplitudes in the frontal area. The results showed that theta-frequency rTMS application induced an increase in theta amplitudes and shortened the giving-up response, while a control alpha-frequency rTMS application induced an increase in alpha amplitudes, but not the giving-up responses. Giving-up behaviors, such as intentionally quitting problem solving or disengaging from goals that are too difficult to attain, are considered adaptive and important for mental health. For instance, it has been suggested that rumination, a risk factor for the onset and maintenance of depressive states, plays a role in hindering adaptive giving-up behaviors, due to impairments in metacognition, the higher-order cognitive skills that help individuals to monitor and control their information processing. In this preliminary study, to find an effective approach to reduce rumination, we examined the effect of online rTMS with a mindfulness-based cognitive intervention on metacognitive processing. Performance of sustained attention to response task and EEG data were obtained the pre- and -post intervention. We found that the task performance was associated with the frontal theta amplitude and our results suggest the

neuromodulatory effectiveness of combining non-invasive stimulation with psychological approach on cognitive function.

Disclosures: **E. Miyauchi:** None. **M. Kawasaki:** None.

Poster

PSTR434. Prefrontal Mechanisms of Executive Functions II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR434.19/UU16

Topic: H.04. Executive Functions

Support: R01 MH126971-01A1

Title: Mental representations of latent states in the human brain

Authors: *F. BOUCHACOURT[1], L. YU[2], A. R. VAIDYA[4], A. AKHMETZHANOVA[2], S. BRUINSMA[3], M. NASSAR[2];
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Abstract: We don’t always need to relearn behavioral policies from scratch when the environment changes. When appropriate, we build mental representations of the hidden outcome contingencies (“latent states”) and we flexibly use these representations to retrieve a previous behavior more rapidly. The prefrontal cortex, orbitofrontal cortex and dorsolateral prefrontal cortex in particular, have been argued to be the locus of the neural representations of latent states, however with a limited characterization of their implementation. To examine the formation and reuse of latent states in the brain, we designed a human task that dissociates latent states from action-outcome contingencies, so that we can quantify behaviorally when a latent state is being reused. At each trial, participants were asked to move the position of a colored cue in a circular arena. Five color cues were used, and participants were required to learn and switch between two distinct patterns. A pattern was defined by the association between the five cues to different locations on the screen, and participants received supervised feedback about the expected location at each trial. Two sessions were run and counterbalanced between participants. In one session, the switch between patterns was signaled on the screen ; in the other session, it was not. Looking at behavior after the initial learning phase, we found evidence for single trial reuse of the patterns after switches. In the unsignaled session, this is seen by looking at the retrieval of the four remaining cue-location associations from knowing the location of the color cue that was first presented after a switch. This means that participants in both conditions learned the two patterns and retrieved them when needed as two separate latent states. However, the degree to which they do so varied across participants. Using computational modeling and model-driven analysis of fMRI data allowed us to study the mechanism of the formation of latent states in this task and to explain individual differences in behavior.

Disclosures: **F. Bouchacourt:** None. **L. Yu:** None. **A.R. Vaidya:** None. **A. Akhmetzhanova:** None. **M. Nassar:** None.

Poster

PSTR434. Prefrontal Mechanisms of Executive Functions II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR434.20/UU17

Topic: H.04. Executive Functions

Support: Friends of BrainHealth

Title: The role of trait mindfulness in self-regulatory processing during an affective Stroop task

Authors: *G. BATCHALLI MARUTHY[1], L. HIMES[2], B. P. RYPMA[3];
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[3]Behavioral & Brain Sci., Univ. of Texas At Dallas, Dallas, TX

Abstract: Trait mindfulness (TM) is the dispositional ability of an individual to be attentive in the present moment, with a nonjudgmental, curious, and accepting attitude. Mindfulness practice aims at enhancing one's TM. The mechanisms underlying the salutary benefits of mindfulness are thought to involve modification of cognitive and emotion regulation processes. If so, individual differences in TM should be related to the neural processes that occur during cognitive and affective processing. To assess the brain bases of TM and emotion regulation, we examined associations between TM and Blood Oxygen Level Dependent (BOLD) signal while participants performed an emotional Stroop task. During scanning, participants responded to the font color of emotional words shown on a screen via button press. Words were chosen from the Affective Norm for English Words (ANEW; Bradley and Lang et al., 1999) and were either positive or negative in valence ratings. Words were equivalent in length, arousal, and frequency ratings. Functional echo planar images were acquired on a 3T Prisma SIEMENS scanner. Anatomical MPRAGE T1-weighted images were also obtained. Responses to words did not differ on accuracy or Reaction time (RT) to font color. fMRI data were preprocessed with slice-timing correction, realignment, co-registration, normalization to Talairach space, and smoothing. Functional connectivity analysis revealed that, for the negative words, higher TM was related to higher connectivity between a Posterior Cingulate Cortex (PCC) seed and regions attributed to self-regulatory processes including the right medial frontal gyrus, left insula, right anterior cingulate and right inferior frontal gyrus. These results provide support for the idea that the mechanisms underlying mindfulness, in fact, might be related to neural processes of both cognitive and emotion regulation. They suggest that a core network of regions interact to bring about the present-moment non-judgmental awareness that characterizes TM.

Disclosures: **G. Batchalli Maruthy:** None. **L. Himes:** None. **B.P. Rypma:** None.

Poster

PSTR434. Prefrontal Mechanisms of Executive Functions II

Location: WCC Halls A-C

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Program #/Poster #: PSTR434.21/UU18

Topic: H.04. Executive Functions

Support: DFG Grant VO 1432/22-1
ESF Grant 100342331
Ghana Scholarship Secretariat 510082

Title: Multitasking: does task-switching add to the effect of dual-tasking in everyday activities?

Authors: P. A. G. ASUAKO[1], R. STOJAN[1], M. MACK[2], O. L. BOCK[3], *C. VOELCKER-REHAGE[1];
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Abstract: The investigation of potential negative effects of performing multiple tasks simultaneously or switching between multiple tasks has gained significant attention in various research fields. Most studies focus on task interference as the reason for the observed performance decrement when performing multiple tasks. Task interference is usually examined by asking subjects to perform two tasks either concurrently, known as dual-tasking (DT), or sequentially, known as task-switching (TS). However, little is known about the interplay effect of TS and DT as observed in everyday activities, such as car driving. Car driving is a complex activity that often requires switching between two or more concurrent activities, such as driving while conversing with a passenger and switching to manipulating in-vehicle stereo. In this scenario, we expect that the cost of dual-tasking will be further compounded by task-switching, leading to a stronger deterioration in driving performance due to the increase in cognitive load required to handle multiple task-sets. We conducted an experiment with 43 young adults (age: 23.62 ± 2.51, 26 females) in a driving simulator. Participants were instructed to follow a lead car driving at a constant speed (70km/h) through a rural landscape while concurrently performing two additional tasks (stating arguments, typing) modelled after realistic driver activities. The additional tasks were presented through visual and auditory modalities. We presented the additional tasks either in separate blocks to simulate conventional dual-task conditions (condition A) or intermixed them to replicate task-switching conditions (condition B). We quantified the average velocity and standard deviation of the lateral lane position within 0 to 10 seconds after the additional task onset. We performed 2x2x2 repeated measures ANOVA with Condition (within: separate, mixed) Tasks (within: argument, typing) and Modality (within: auditory, visual) as factors. None of these variables showed significant differences between conditions (all $p > 0.05$), although our statistical power was sufficient to detect a moderate difference between conditions ($f = 0.25$, a = 0.05, 1-ß = 0.95, 4 repetitions -> required n = 34). The results do not support the hypothesis that in realistic multitasking, the costs of dual-tasking are compounded by the costs of task switching. That is, our findings suggest that the cognitive load of handling multiple sets of additional tasks rather than just one additional task does not significantly degrade driving performance of young adults.

Disclosures: **P.A.G. Asuako:** None. **R. Stojan:** None. **M. Mack:** None. **O.L. Bock:** None. **C. Voelcker-Rehage:** None.

Poster

PSTR434. Prefrontal Mechanisms of Executive Functions II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR434.22/UU19

Topic: H.04. Executive Functions

Support: NIH Grant R01 HD100383-04

Title: Set-shifting function predicts dynamic balance in people with Parkinson's disease

Authors: *G. R. HARKER, C. SILVA-BATISTA, A. RAGOTHAMAN, J. ELLISON, A. PREWITT, P. CARLSON-KUHTA, F. B. HORAK, M. MANCINI;
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Abstract: Background: Parkinson's disease (PD) is associated with cognitive, as well as motor, impairments. Cognitive function is essential for balance performance. Balance is composed of at least 4 different domains, as measured by the Mini Balance Evaluation System Test (Mini-BESTest). Recent laboratory studies have shown that cognitive assessments (e.g., Montreal Cognitive Assessment [MoCA]) are related to standing balance in people with PD, but it is unknown whether specific executive functions are related to different balance domains. Here, we aimed to investigate if executive function, assessed with a Tablet-based Cognitive Assessment Tool [TabCAT], can predict variations in Mini-BESTest total score and its subscores among people with PD. We hypothesize that Set-Shifting executive function will predict dynamic balance control during gait, which may require cognitive flexibility to create appropriate balance and postural responses. **Methods:** A total of 39 individuals, diagnosed with idiopathic PD (age=69.4 years, disease duration=9.5 years, MDS-UPDRS-III=36.4 score, MoCA=26.4 score) took part in the study. The Mini-BESTest and TabCAT were assessed in the laboratory. The TabCAT included assessments of visuospatial function (the Line Orientation task modeled on the Benton Judgement of Line Orientation task) and executive function (the Set-Shifting and Flankers tasks). **Results:** The multiple linear regressions showed that Set-Shifting was a predictor for the Mini-BESTest total score (R^2=0.35, $p<0.001$), as well as the Reactive Postural Control (R^2=0.17, p=0.009), Dynamic Gait (R^2=0.24, p=0.001), and Anticipatory Postural Adjustments subscores (R^2=0.20, p=0.004), but not the Sensory Orientation subscore. The Flanker and Line Orientation TabCAT tasks did not predict Mini-BESTest total score or its subscores. **Conclusions:** Our novel results show that better performance in instrumented set-shifting, assessing cognitive flexibility, is related to better dynamic balance during gait but not to static balance during standing in people with mild-to-moderate PD. These results suggest that people with PD lose ability to selectively switch attention to generate appropriate reactive, anticipatory and gait postural control, which seem not to be required for static balance control.

Disclosures: **G.R. Harker:** None. **C. Silva-Batista:** None. **A. Ragothaman:** None. **J. Ellison:** None. **A. Prewitt:** None. **P. Carlson-Kuhta:** None. **F.B. Horak:** A. Employment/Salary (full or part-time):; APDM Wearable Technologies, a Clario Company. **M. Mancini:** None.

Poster

PSTR435. Encoding and Retrieval in Human Long-Term Memory: Psychology and Deficits

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR435.01/UU20

Topic: H.07. Long-Term Memory

Support: JSPS KAKENHI Grant Number JP20H05802
JSPS KAKENHI Grant Number JP21K03128

Title: Neural representation modulated by impression of trustworthiness generated from social interaction in memory for other persons

Authors: *A. KAMO, M. MIHARA, T. TSUKIURA;
Kyoto Univ., Kyoto, Japan

Abstract: Memory for other persons is modulated by impression of trustworthiness, which is mainly categorized into stimulus-dependent trustworthiness from faces and context-dependent trustworthiness formed in social interaction with others. Functional neuroimaging studies have demonstrated that the insula and amygdala are involved in the effect of stimulus-dependent trustworthiness on memory for other persons. However, little is known about the neural mechanisms underlying the effect of context-dependent trustworthiness on memory for other persons. To investigate this issue, using fMRI, we scanned healthy young adults during the encoding of objects associated with trustworthy, intermediate or untrustworthy persons, whose impression of trustworthiness was formed in the Trust Game before fMRI scanning. In the multivariate pattern analysis (MVPA), we investigated activity patterns represented in a region-of-interest (ROI) reflecting the social brain network including the medial prefrontal cortex (mPFC), precuneus/posterior cingulate cortex (PCC), anterior temporal lobe (ATL), and temporoparietal junction (TPJ). In addition, MVPA was applied to analyze activity patterns in the amygdala and insula ROIs related to the processing of stimulus-dependent trustworthiness. MVPA used three support vector machine (SVM) binary classifiers of other person's trustworthiness: Trustworthy vs. Intermediate, Intermediate vs. Untrustworthy, and Untrustworthy vs. Trustworthy, which were trained and tested using a leave-one-run-out cross-validation. Classification accuracies in MVPA for the social brain network ROI were significant in all classifiers, whereas activity patterns in the amygdala ROI significantly discriminated between Untrustworthy and Trustworthy. In the insula, activity patterns significantly differentiated Untrustworthy from the other conditions. These findings suggest that activity patterns in the social brain network represent the social knowledge related to impression of trustworthiness generated from social interaction with others. In addition, amygdala activity

could represent the socioemotional information associated with trustworthy and untrustworthy persons, whereas insular activity could represent the socioemotional information to specify untrustworthy persons.

Disclosures: **A. Kamo:** None. **M. Mihara:** None. **T. Tsukiura:** None.

Poster

PSTR435. Encoding and Retrieval in Human Long-Term Memory: Psychology and Deficits

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR435.02/UU21

Topic: H.07. Long-Term Memory

Support: JSPS KAKENHI Grant Number JP20H05802
JSPS KAKENHI Grant Number JP21K03128

Title: Neural mechanisms underlying the effect of prediction errors in facial attractiveness between masked and unmasked faces on face memories in young and older adults

Authors: *M. MIHARA, R. IZUMIKA, T. TSUKIURA;
Kyoto Univ., Kyoto, Japan

Abstract: The COVID-19 pandemic has forced people to predict facial attractiveness from partially covered faces. Given that facial attractiveness is processed as a social reward, differences between the predicted and observed facial attractiveness are defined as reward prediction error (RPE) in a social context. The age-related decline of RPE-dependent learning in monetary rewards has been explained by the impaired mechanisms including the RPE-related ventral striatum (VS) and memory-related hippocampus in older adults (OAs). However, little is known about the age-related difference in activation during the encoding of faces with social RPE derived from facial attractiveness. To elucidate this, using fMRI, we scanned healthy male young adults (YAs) and healthy male OAs during the encoding of female faces. In each encoding trial, participants rated the predicted attractiveness of a face covered except for around the eyes (prediction phase) and then rated the observed attractiveness of the face without any cover (outcome phase). The difference in ratings between these phases was defined as RPE in facial attractiveness, and RPE was categorized into positive RPE (increased RPE from the prediction to outcome phases), negative RPE (decreased RPE from the prediction to outcome phases), and non-RPE (no difference in RPE between the prediction and outcome phases). During retrieval, participants were individually presented with faces seen and unseen in the encoding trials, and judged whether or not each face had been seen in the encoding trials. In behavioral data, OAs showed significantly higher hit rates vs. false alarm (FA) rates in positive RPE than in negative RPE, whereas there was no significant difference in hit rates vs. FA rates among the RPE categories in YAs. Univariate fMRI activity in the VS and precuneus reflected a linear increase with increased RPE in facial attractiveness in YAs but not in OAs. Significant activation

reflecting linear decrease with increased RPE or increase with both positive and negative RPE was not identified in either YAs or OAs. These findings suggest that encoding-related activation underlying the processing of social RPE changes with age and that the age-dependent change is modulated by the RPE categories in face-based social rewards.

Disclosures: **M. Mihara:** None. **R. Izumika:** None. **T. Tsukiura:** None.

Poster

PSTR435. Encoding and Retrieval in Human Long-Term Memory: Psychology and Deficits

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR435.03/UU22

Topic: H.07. Long-Term Memory

Support: NIH Grant R01NS132872

Title: Memory decisions modulate ongoing performance and brain state engagement

Authors: *J. WHEELOCK, N. M. LONG;
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Abstract: We frequently encounter demands to switch between learning new information (encoding) and access-ing stored information (retrieval), yet how moment-to-moment fluctuations in these processes manifest in the brain is not well understood. Memory encoding and memory retrieval constitute neurally dissociable brain states which rely on distinct configurations of subcortical and cortical activity and connectivity patterns. Memory brain states tradeoff such that both encoding and retrieval cannot be engaged simultaneously and impact downstream processing and behavior. Although prior behavioral work has shown that memory judgments can have a lingering impact on subsequent judgments, these effects have not been directly linked to memory brain states. Our hypothesis is that memory brain states are modulated by memory judgments, and that these brain states persist for several hundred milliseconds, impacting subsequent memory judgments. To test this hypothesis, we conducted a recognition memory experiment in participants undergoing scalp electroencephalography (EEG) recording. Our critical manipulation was the inter-stimulus interval (ISI) between each recognition trial and we expected greater impact of memory states on subsequent judgments on trials with faster ISIs. We measured memory state engagement using a multivariate pattern classifier trained on an independent dataset in which participants were biased towards either encoding or retrieval. Replicating past work, we find that memory judgments influence subsequent judgments whereby successful recognition of targets (items that were studied; hits) improves memory accuracy for subsequently presented targets and decreases memory accuracy for subsequently presented lures (items that were not studied). Likewise, we find that correct rejection of lures improves memory accuracy for subsequently presented lures and decreases memory accuracy for subsequently presented targets. We expected to find greater retrieval state engagement on hits compared to

correct rejections and that memory states would persist for several hundred milliseconds after a memory judgment was made. However, we find that the memory retrieval state is more strongly engaged during correct rejections than hits and quickly decreases after a response is made. Our interpretation is that explicit, top-down demands inherent in the recognition task as well as preparation for the rapidly presented trials strongly influence the recruitment of memory brain states. This work enhances our understanding of how memory processes unfold on a moment-to-moment basis.

Disclosures: J. Wheelock: None. **N.M. Long:** A. Employment/Salary (full or part-time):; University of Virginia.

Poster

PSTR435. Encoding and Retrieval in Human Long-Term Memory: Psychology and Deficits

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR435.04/UU23

Topic: H.07. Long-Term Memory

Support: NIH/NCATS Grant UL1TR003015
NIH/NCATS Grant KL2TR003016

Title: Age effects on the encoding and retrieval of overlapping events

Authors: *I. L. MOORE, D. E. SMITH, N. M. LONG;
Univ. of Virginia, Charlottesville, VA

Abstract: Healthy older adults typically show impaired episodic memory, memory for when and where an event occurred, but intact semantic memory, knowledge for general information and facts. We hypothesize that these effects can be explained by an increased tendency to enter into and remain in a ‘retrieval state,’ a brain state in which attention is focused internally in an attempt to access prior knowledge. Engaging in a retrieval state can lead to impairments in subsequent memory, potentially because the retrieval state trades off with an ‘encoding state,’ a brain state in which attention is focused externally. To test our hypothesis, we conducted multivariate pattern analyses of scalp electroencephalographic (EEG) data while participants were explicitly directed to encode or retrieve object images. We find that both young and older adults can flexibly engage in memory brain states as directed. However, whereas young adults' memory state engagement gradually increases throughout the stimulus interval, older adults’ memory state engagement plateaus early in the stimulus interval. These findings suggest that the temporal dynamics of encoding and retrieval states differ across the lifespan, with possible implications for the ability to maintain versus flexibly shift between memory states.

Disclosures: I.L. Moore: None. **D.E. Smith:** None. **N.M. Long:** None.

Poster

PSTR435. Encoding and Retrieval in Human Long-Term Memory: Psychology and Deficits

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR435.05/UU24

Topic: H.07. Long-Term Memory

Support: NIH Grant NS132872

Title: Response-locked theta power tracks successful retrieval independent of memory goals

Authors: *D. E. SMITH, N. M. LONG;
Univ. of Virginia, Charlottesville, VA

Abstract: Test-phase theta power (4-8Hz) is greater for identification of previously studied items (hits) compared to rejection of novel lures (correct rejections, CRs). In our preliminary work, we find that this theta power dissociation between hits and CRs 'flips' following a response. As theta power is greater following negative relative to positive outcomes across cognitive control tasks, the post-response theta 'flip' that we have identified in our preliminary work may represent a positive feedback signal in response to successful retrieval. This hypothesis is consistent with neuro-imaging work showing that reward-related regions (e.g. striatum) are more active during hits compared to CRs in the absence of explicit reward. However, reward signals during hits may instead reflect goal attainment; in a typical recognition experiment, the subjects' goal to identify old items is confounded with successful retrieval. To adjudicate between these hypotheses, we conducted two recognition memory experiments (E1, E2) in participants undergoing scalp electroencephalographic recording. The critical manipulation was the test-phase instructions. Subjects' goal was either to successfully retrieve study items (E1) or to detect new items (E2). If feedback signals reflect successful retrieval, the same post-response signals should dissociate hits vs. CRs regardless of memory goals. We find decreased theta power following hits vs. CRs in both E1 and E2. These preliminary findings are consistent with the hypothesis that successful retrieval is intrinsically rewarding.

Disclosures: D.E. Smith: None. **N.M. Long:** None.

Poster

PSTR435. Encoding and Retrieval in Human Long-Term Memory: Psychology and Deficits

Location: WCC Halls A-C

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Program #/Poster #: PSTR435.06/UU25

Topic: H.07. Long-Term Memory

Support: National Natural Science Foundation of China (62293550, 62293551, 61977008)

Title: The neurocognitive mechanism of knowledge construction during on-line teaching

Authors: *X. XU, X. HE, X. FENG, S. ZHOU, C. LU;
Beijing Normal Univ., Beijing, China

Abstract: It has been long-debated that knowledge will be constructed into a hierarchical architecture in the human brain to facilitate encoding and later recall. However, there are few studies investigating this issue when learning knowledge with complex hierarchy, particularly during a naturalistic teaching process. To address this issue, 80 healthy adults were scanned using functional magnetic resonance imaging (fMRI) while they learnt a complex physical knowledge through video teaching. The knowledge taught by the video were either with an intrinsic hierarchy (intact condition) or not, i.e., the conceptual events of the video were temporally scrambled (structure-scrambled condition) or the words of the video were temporally scrambled (word-scrambled condition). First, a representational similarity analysis was conducted to determine the neural underpinnings of the content and hierarchy representation in the learner's brain. The results of the intact condition showed that the precuneus was significantly associated with the representation of knowledge hierarchy. Interestingly, the precuneus also had a significant role in representing knowledge hierarchy even no explicit structure existed in the video teaching process (i.e., the structure-scrambled condition). However, the precuneus did not appear in the word-scrambled condition. Most importantly, it seemed that learners reconstructed the structure-scrambled knowledge into a well-organized hierarchy that was consistent with the intrinsic hierarchy of the knowledge. Finally, the representation of hierarchy in the precuneus significantly predicted subsequent behavioral performance of knowledge recall. Together, these findings provide novel and important support for the knowledge construction theory, and suggest that the default mode network is involved in both content and hierarchy representation of high-level naturalistic stimuli during on-line teaching.

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Poster

PSTR435. Encoding and Retrieval in Human Long-Term Memory: Psychology and Deficits

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Program #/Poster #: PSTR435.07/UU26

Topic: H.07. Long-Term Memory

Support: NIH Grant AG065255
NIH Grant AG071263
Center for Mind, Brain, Computation and Technology Fellowship, Stanford Wu Tsai Neuroscience Institute

Title: Effects of spontaneous lapses of attention on goal representation and episodic memory retrieval

Authors: *D. MILLER[1], S. T. SCHWARTZ[2,3], T. T. TRAN[2,4], J. E. RATHMANN-BLOCH[2], J. PARK[2,4], A. ROMERO[2,4], J. SHENG[2,4], H. YANG[2], E. C. MORMINO[4,3], A. D. WAGNER[2,3]; [1]Stanford Univ., Palo Alto, CA; [2]Psychology, [3]Wu Tsai Neurosciences Inst., [4]Dept. of Neurol. & Neurolog. Sci., Stanford Univ., Stanford, CA

Abstract: Episodic memory is a complex process, involving the interaction of multiple systems, including the medial temporal lobe as well as frontoparietal networks of attention and cognitive control, and midbrain structures subserving fluctuations in arousal and sustained attention. While extant data indicate that attention at the time of retrieval can affect memory performance, less is known about how fluctuations in sustained attention in the moments just prior to a retrieval attempt impact neural mechanisms of memory and performance. Here, we investigated trial-to-trial fluctuations in preparatory sustained attention and the brain networks supporting goal-state processes and episodic retrieval in younger adults (18-30 yrs) as they completed a goal-directed associative memory task. At encoding (not scanned), participants made conceptual (living/non-living) and perceptual (bigger/smaller) judgments on images of everyday objects and animals. At retrieval and concurrent with functional MRI, participants performed one of two source memory tasks (i.e., one of two retrieval goal states), indicating whether they remembered test probes as having been encountered in the perceptual or conceptual encoding task. Associative memory was assayed at the trial-level (i.e., associative hits vs. misses) and at the individual-level using associative memory d'. During fMRI, participants also underwent concurrent pupillometry to assay moment-to-moment tonic fluctuations in preparatory sustained attention. Initial analyses revealed classifier evidence of retrieval goal-state representation correlated to performance on the associative memory task. Furthermore, associative memory performance varied with pupil size (an assay of arousal/sustained attention) in the pre-goal-cue and pre-stimulus periods just prior to attempts to remember. Additionally, participants completed a gradual-onset continuous performance task (i.e., the gradCPT) across two sessions (separated by up to one month) to assay sustained attention. Predicted analyses suggest trait-level sustained attention varies with associative memory *d'*. Together, these results highlight the impact of "readiness-to-remember" preparatory mechanisms on retrieval, further advancing understanding of the mechanisms of memory expression.

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Poster

PSTR435. Encoding and Retrieval in Human Long-Term Memory: Psychology and Deficits

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR435.08/UU27

Topic: H.07. Long-Term Memory

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Center for Mind, Brain, Computation and Technology at the Stanford Wu Tsai Neurosciences Institute (to Shawn Schwartz). T.T. was supported by a National Institute on Aging NRSA (F32AG071263), and the Alzheimer's Association (AARFD-21-852597)

Title: Effects of white matter integrity and amyloid-β on sustained attention in cognitively unimpaired older adults

Authors: *S. SANKARASUBRAMANIAN[1], A. ROMERO[1,2], J. E. RATHMANN-BLOCH[1], J. PARK[1,2], S. T. SCHWARTZ[1,3], D. S. MILLER[1], T. T. TRAN[1], H. VOSSLER[2], A. N TRELLE[2], E. C. MORMINO[2,3], A. D. WAGNER[1,3];
[1]Dept. of Psychology, [2]Dept. of Neurol. & Neurolog. Sci., [3]Wu Tsai Neurosciences Inst., Stanford Univ., Palo Alto, CA

Abstract: The ability to attend to relevant event features, and sustain that attention from moment-to-moment, contributes to episodic memory encoding and retrieval. Sustained attention is posited to decline in older adulthood, with individual differences in sustained attention potentially accounting for variability in episodic memory performance. Given that aging is marked by changes in structural connectivity in the brain (e.g., white matter degradation) and some cognitively unimpaired older adults demonstrate an increased presence of amyloid plaques (biomarkers of Alzheimer's disease (AD) pathology), we sought to understand how white matter integrity and amyloid-β burden relate to the ability to sustain attention. Trait-level sustained attention was behaviorally assessed using a gradual-onset continuous performance task (i.e., the gradCPT, FC Fortenbaugh et al 2015) in a large cohort of cognitively unimpaired (CU) older adults (60-90 yrs.) enrolled in the Attention Memory and Aging Study at Stanford (AMASS) and the Stanford Aging and Memory Study (SAMS). We collected structural MRI, diffusion-weighted MRI, and amyloid PET scans. Initial results from a sample of 27 CU older adults suggest that global white matter integrity measured by mean diffusivity (MD) and fractional anisotropy (FA) mediated the relationship between age and trait-level sustained attention (as measured by gradCPT d'). Subsequent analyses focused on structural integrity of the superior longitudinal fasciculus (SLF), a tract posited to support attention, with the integrity of corticospinal tract (CST), serving as a control. Both FA and MD values of the SLF, but not of the CST, significantly mediated the relationship between age and trait-level sustained attention. For further analysis, we plan to include data from the larger cohort, and investigate how amyloid and tau pathology interact with structural connectivity measures and jointly contribute to the observed variability in sustained attention capabilities. Collectively, these data add to the growing understanding of how structural connectivity changes and early-stage AD pathology partially explain trait-level attentional differences in CU older adults.

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Poster

PSTR435. Encoding and Retrieval in Human Long-Term Memory: Psychology and Deficits

Location: WCC Halls A-C

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Topic: H.07. Long-Term Memory

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We also received support from the Center for Mind, Brain, Computation and Technology at the Stanford Wu Tsai Neurosciences Institute (to Shawn Schwartz).
T.T. was supported by a National Institute on Aging NRSA (F32AG071263), and the Alzheimer's Association (AARFD-21-852597).

Title: Effects of white matter integrity and amyloid-β on episodic delayed recall performance in cognitively unimpaired older adults

Authors: J. PARK[1,3], ***J. E. RATHMANN-BLOCH**[1], S. SANKARASUBRAMANIAN[1], S. T. SCHWARTZ[1,2], D. S. MILLER[1], T. T. TRAN[1], A. ROMERO[1], H. VOSSLER[3], A. N. TRELLE[3], E. C. MORMINO[3,2], A. D. WAGNER[1,2];
[1]Dept. of Psychology, [2]Wu Tsai Neurosciences Inst., Stanford Univ., Stanford, CA; [3]Dept. of Neurol. & Neurolog. Sci., Stanford Univ. Sch. of Med., Stanford, CA

Abstract: Episodic memory performance varies across cognitively unimpaired (CU) older adults, with multiple potential underlying causes. Possible sources of variance in memory function include changes in structural connectivity in the brain (e.g., white matter degradation) as well as the detrimental effects of early-stage Alzheimer's disease (AD) pathology (e.g., amyloid plaques and neurofibrillary tau tangles). Extant data further suggest that AD pathology correlates with deterioration in white matter integrity, particularly within the medial temporal lobe (Kantarci et al., 2017). Here, we examined the relationships between episodic memory performance, white matter integrity, and early-stage AD pathology (specifically, amyloid-β burden) in CU older adults (60-90 yrs.) enrolled in the Attention Memory and Aging Study at Stanford (AMASS), Stanford Aging and Memory Study (SAMS), and the Stanford Alzheimer's Disease Research Center (ADRC). An episodic memory composite score was computed from delayed recall performance on the (1) logical memory subtest of the Wechsler Memory Scale, (2) Hopkins Verbal Learning Test-Revised, and (3) Brief Visuospatial Memory Test-Revised. Participants underwent 3T amyloid-PET/MR imaging, which included diffusion-weighted imaging to evaluate white matter integrity. Early-stage AD pathology was assessed using amyloid and tau PET scans and cerebrospinal fluid assays. Based on prior literature, we identified the cingulum cingulate gyrus (CGC) and the fornix as tracts that might play a role in

episodic memory performance and the corticospinal tract (CST) as a control (Alm et al., 2022). Initial analyses on a sample of 27 CU older adults revealed that there was a trend-level association between mean diffusivity (MD) in the right CGC and episodic memory performance. Moreover, global DTI white matter integrity metrics (e.g. MD) and amyloid status jointly mediated the relationship between age and episodic memory performance. Collectively, these data suggest that the integrity of structural connectivity, in tandem with AD biomarker pathology, explain variability in episodic memory performance in CU older adults.

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Poster

PSTR435. Encoding and Retrieval in Human Long-Term Memory: Psychology and Deficits

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR435.10/VV1

Topic: H.07. Long-Term Memory

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NIH Grant AG071263
NIH Grant AG074625
Alzheimer's Association Grant AARFD-21-852597
Center for Mind, Brain, Computation and Technology Fellowship, Stanford Wu Tsai Neurosciences Institute

Title: Effects of attention, goal representation, and amyloid burden on episodic retrieval in cognitively unimpaired older adults

Authors: *S. T. SCHWARTZ[1,2], D. S. MILLER[1], T. T. TRAN[1,3], J. E. RATHMANN-BLOCH[1], J. PARK[1,3], A. ROMERO[1,3], H. VOSSLER[3], J. SHENG[1,3], H. YANG[1], J. R. WINER[3], E. C. MORMINO[2,3], A. D. WAGNER[1,2];
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Abstract: Episodic memory decline is prevalent in aging, yet the severity of age-related memory change is markedly variable across older adults. Differential structural and functional integrity in the medial temporal lobe, frontoparietal networks of attention and cognitive control, and midbrain structures subserving fluctuations in arousal and sustained attention likely partially explain individual differences in episodic memory and executive function in cognitively unimpaired (CU) older adults. The extent to which these changes co-occur with the early (asymptomatic) stage of the Alzheimer's disease (AD) pathophysiological cascade remains unknown. As such, there remains limited understanding of how moment-to-moment and

individual differences in sustained attention and goal-state representation explain variability in memory in CU older adults, and their relations with AD pathology. Here, we investigated age-related functional changes in the neural networks supporting episodic memory, goal-state processes, and trial-to-trial fluctuations in preparatory sustained attention as well as their relationship to amyloid beta (Aβ) biomarker status (assayed through Aβ-PET). CU older adults (65-80 yrs; half Aβ+/Aβ-) and younger adults (18-30 yrs) completed a goal-directed associative memory task. At encoding (not scanned), they made conceptual (living/non-living) and perceptual (bigger/smaller) judgments on images of everyday objects and animals. At retrieval and concurrent with functional MRI, participants performed one of two source memory tasks (i.e., one of two retrieval goal states), indicating whether they remembered test probes as having been encountered in the perceptual or conceptual encoding task. Associative memory was assayed at the trial-level (associative hits vs. misses), and at the individual-level using associative memory *d'*. During fMRI, participants also underwent concurrent pupillometry to assay moment-to-moment tonic fluctuations in preparatory sustained attention. As predicted, associative *d'* was lower and retrieval decision reaction times were slower in older compared to younger adults. Initial analyses revealed age-related differences in frontoparietal cognitive control network activation and classifier evidence of retrieval goal-state representation. Furthermore, associative memory performance varied with pupil size (an assay of arousal/sustained attention) in the pre-goal-cue and pre-stimulus periods just prior to remembering. These initial findings set the stage for understanding age-related changes in an ensemble of neural mechanisms that collectively orchestrate whether and how we remember.

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Poster

PSTR435. Encoding and Retrieval in Human Long-Term Memory: Psychology and Deficits

Location: WCC Halls A-C

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Program #/Poster #: PSTR435.11/VV2

Topic: H.07. Long-Term Memory

Support: NIH Grant R01AG048076
NIH Grant R01AG074339
NIH Grant K99AG075184

Title: Category-level neural dedifferentiation is associated with memory and CSF biomarkers of Alzheimer's disease in cognitively unimpaired older adults

Authors: *J. SHENG, A. TRELLE, A. ROMERO, J. PARK, E. MORMINO, A. WAGNER; Stanford Univ., Stanford, CA

Abstract: Extensive human neuroimaging evidence indicates that neural responses evoked by stimuli from different visual categories (e.g., faces vs. scenes) are less distinctive in older relative to younger adults, a phenomenon termed *neural dedifferentiation*. While neural dedifferentiation has been consistently reported to relate to poor task performance in older adults, especially in episodic memory, the role of Alzheimer's disease (AD) pathology in neural dedifferentiation and its impacts on memory are poorly understood. In cognitively unimpaired (CU) older adults, two cerebrospinal fluid (CSF) assays -- a decrease in β-amyloid ($A\beta_{42}$; i.e., higher amyloid burden) and increase in phosphorylated tau_{181} ($p\text{-}tau_{181}$) -- are some of the earliest detectable biomarkers of the AD pathophysiological cascade. Here, we examined the relationships between neural selectivity during face vs. scene encoding and $A\beta_{42}/A\beta_{40}$, $p\text{-}tau_{181}$, and memory performance in a large sample of CU older adults (n = 166; 60-88 yrs) enrolled in the Stanford Aging and Memory Study (SAMS), with age, sex, and years of education as covariates. fMRI data were acquired as participants first encoded word-face and word-scene associations and then engaged in an associative retrieval task. Data were preprocessed with fMRIPrep (version 23.0.0) and then submitted to surfaced-based analysis. Category-level neural selectivity was measured by contrasting the amplitude of the blood-oxygen-level-dependent (BOLD) response to encoding trials that included faces vs. scenes, thus revealing a vertex's/region's preferred and non-preferred stimulus categories. Initial analyses examining how selectivity varied with factors of interest revealed that place-selective neural activity in the bilateral superior parietal lobule (SPL) decreased with age. Subsequently, initial examination of the relationships between neural selectivity and CSF (n = 121) $A\beta_{42}/A\beta_{40}$ and $p\text{-}tau_{181}$ revealed that place-selective neural activity in left occipital and left superior parietal cortex showed a significant positive association with CSF $A\beta_{42}/A\beta_{40}$. Finally, place-selective neural activity in the left SPL positively correlated with associative memory performance (i.e., associative *d'*). Taken together, these findings suggest that preclinical $A\beta_{42}$ burden is associated with neural dedifferentiation in CU older adults, pointing to one route through which early AD processes may impact memory performance.

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Poster

PSTR435. Encoding and Retrieval in Human Long-Term Memory: Psychology and Deficits

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR435.12/VV3

Topic: H.07. Long-Term Memory

Support: NIH R56AG068149

Title: Eye movements are associated with neural differentiation in scene-selective and object-selective cortical regions

Authors: *S. SROKOVA, N. S. SHAHANAWAZ, M. D. RUGG;
The Univ. of Texas at Dallas, Richardson, TX

Abstract: Neural differentiation in cortical regions which are selectively responsive to distinct visual stimulus categories has been shown to have a critical role in episodic memory function. Specifically, neural differentiation during encoding is predictive of subsequent memory performance, and differentiation has also been shown to decline in older age (a phenomenon known as 'age-related neural dedifferentiation'). The neurobiological and behavioral mechanisms underlying the relationship between neural differentiation and episodic memory performance are, however, currently unknown. A wealth of behavioral research highlights the functional significance of eye movements in memory function, but it is yet to be examined whether eye movements and neural differentiation are related. In the present experiment, 24 healthy young adults (males and females, 18 - 30 years old) underwent fMRI with simultaneous eye-tracking as they viewed images of scenes and objects. The relationship between neural differentiation and eye-movements was examined with complementary within- and across-subject approaches, focusing on the scene-selective parahippocampal place area (PPA) and the object-selective lateral occipital complex (LOC). Trial-wise estimates of scene- and object-selectivity were obtained from multivoxel pattern similarity analyses, and linear mixed effects models were employed to determine whether trial-wise variability in neural differentiation covaried with the number and duration of gaze fixations during each trial. In the PPA, more gaze fixations and shorter fixation durations during scene viewing were predictive of greater scene selectivity. A similar, albeit trending only, pattern emerged in the LOC, where object selectivity also covaried positively with gaze fixations and negatively with gaze durations during object viewing. For each participant, we then computed their average number and duration of fixations across all scene and object trials, along with a univariate metric of differentiation. These metrics were then entered into a set of zero-order, across-participant correlations. Analogously to the within-participant analyses, object selectivity in the LOC correlated positively with the number of gaze fixations, and negatively with gaze durations. No across-participant relationships were evident in the PPA. These findings indicate that neural differentiation for scene and object stimuli are systematically associated with eye movements. Thus, the relationship between neural differentiation and memory performance is likely a reflection of the efficiency with which study items are visually sampled at the time of encoding.

Disclosures: S. Srokova: None. **N.S. Shahanawaz:** None. **M.D. Rugg:** None.

Poster

PSTR435. Encoding and Retrieval in Human Long-Term Memory: Psychology and Deficits

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR435.13/VV4

Topic: H.02. Perception and Imagery

Support: ZIAMH002909

Title: Linking brain activity during movie viewing and recall through gaze behavior

Authors: *M. NAU[1], H. TARDER-STOLL[2], A. GREENE[4], J. LOSSIO-VENTURA[5], F. PEREIRA[5], C. BALDASSANO[3], J. CHEN[7], C. I. BAKER[6];
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Abstract: When imagining your living room, your brain's activity patterns are similar to when you last saw it (known as cortical reinstatement), and your eyes tend to move as if you were looking at it (known as gaze reinstatement). Here, we hypothesized that cortical- and gaze reinstatement are deeply interrelated phenomena that jointly reflect the retrieval of memorized events. If so, gaze patterns and brain activity should be event-specific, and gaze-dependent brain activity should overlap between viewing and recall. To test these predictions, we combined eye tracking, spoken-recall recordings, and fMRI data acquired while participants watched and recalled an episode of the BBC show Sherlock. First, language modeling of participants' spoken recall showed that the movie was recalled accurately, and it allowed segmenting the movie into 48 narrative events. Second, gaze patterns and brain activity during movie viewing were indeed event-specific and consistent across participants. Third, by relating the eyeball multi-voxel pattern to brain activity we found substantial overlap in gaze-dependent activity between viewing and recall. Finally, we used a hidden-Markov model to capture the dynamics of gaze patterns during viewing, and found preliminary evidence that these patterns were sequentially reinstated during recall. Taken together, our findings suggest that gaze behavior is intrinsically linked with the process of remembering past events, and that cortical- and gaze reinstatement jointly support the recall of episodic memories.

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Poster

PSTR435. Encoding and Retrieval in Human Long-Term Memory: Psychology and Deficits

Location: WCC Halls A-C

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Program #/Poster #: PSTR435.14/VV5

Topic: H.07. Long-Term Memory

Support: National Science and Technology Innovation STI2030-Major Project (2021ZD0204103 to H.L.)
National Natural Science Foundation of China (31930052 to H.L.)

Title: Direct suppression and thought substitution engage dissociated oscillatory neural mechanisms to achieve active forgetting

Authors: *S. CHEN[1,2,3], Y. WU[1,3], J. LI[1,2,3], H. LUO[1,2,3];
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Abstract: Active forgetting is crucial for emotion regulation and psychological well-being. This could be achieved via two strategies: direct suppression (DS) and thought substitution (TS) yet their underlying neural mechanisms remain elusive. Here we recorded electroencephalography (EEG) activities on 49 human subjects while they were instructed to use DS or TS strategies in different trials to inhibit previously memorized word associations. Behavioral results show that both DS and TS strategies efficiently disrupt memories, displaying gradual reductions in intrusive memories and decreased recall performance for both the trained and independent probe word cues. Most importantly, we demonstrate dissociated oscillatory neural mechanisms for DS and TS strategies. First, DS elicits stronger sustained alpha-band (8-11 Hz) activities in the parietal region while TS shows stronger theta-band (3-6 Hz) activities in the frontal region, indicating their respective inhibitory and excitatory characteristics. Second, the decrease of alpha-band power across blocks is accompanied by a similar decline in intrusive memories in the DS condition, suggesting alpha-band inhibition may be required to facilitate forgetting. Third, the theta-band power during TS condition is correlated to individual executive control function measured in independent ANT tasks and could also predict subsequent forgetting. Taken together, we present new evidence for the dissociative neural mechanisms underlying different active forgetting strategies. While the DS strategy employs alpha-band inhibitory modulation to suppress intrusive memories, TS strategy relies heavily on theta-band frontal executive control activities to enable the formation and replacement of new and old memories, respectively.

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Poster

PSTR435. Encoding and Retrieval in Human Long-Term Memory: Psychology and Deficits

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Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR435.15/VV6

Topic: H.07. Long-Term Memory

Support: NIMH R01MH119099

Title: The neural dynamics of mental time travel in the stream of spontaneous thoughts

Authors: *H. LEE, S. BORN, X. LI, C. J. HONEY, J. CHEN;
Johns Hopkins Univ., Baltimore, MD

Abstract: When our minds wander, we often engage in remembering the past or imagining the future. What cognitive and neural mechanisms initiate and shape such spontaneous mental time travel? To explore this question, we conducted an fMRI experiment in which subjects verbally described any thoughts that entered their stream of consciousness for 10 minutes. Each subject's

speech was manually segmented into individual thought units (mean duration = 11 s) and categorized as episodic recall, future thinking, semantic knowledge, or current experiences. Episodic recall and future thinking on average accounted for 44% of thoughts and exhibited higher activation in the default mode network (DMN), known for its association with mental simulation, compared to current experiences. Mental time travel was not preferentially preceded by any specific thought category, including episodic recall or future thinking itself, more than expected by chance. Instead, mental time travel (e.g. imagining adopting a dog) was preceded by semantically associated thought content (e.g. other thoughts about dogs), irrespective of category. In general, thoughts that were nearby in time were more semantically similar than temporally distant thoughts. Independent observers detected boundaries when consecutive thoughts were semantically dissimilar. These semantic boundaries evoked transient responses across multiple cortical areas in fMRI subjects, generating an established DMN activation pattern associated with major transitions in mental context (Lee & Chen, 2022, eLife). The semantic boundary pattern was not observed during thought category changes or non-boundary periods within individual thoughts. Additionally, we found that individuals with greater functional connectivity between the hippocampus and the medial temporal cortical area in the DMN generated less tightly connected thought networks, indicative of more divergent and varied thinking. Greater functional connectivity between the hippocampus and DMN cortical areas was also associated with personality traits related to exploration and curiosity. Overall, these findings demonstrate that the DMN tracks the dynamics of mental time travel in the stream of spontaneous thoughts, and semantic connections may serve as a major trigger and organizing principle of spontaneous thought in general.

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Poster

PSTR435. Encoding and Retrieval in Human Long-Term Memory: Psychology and Deficits

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR435.16/VV7

Topic: H.07. Long-Term Memory

Title: Structured encoding in the orbitofrontal cortex facilitates long-term associative memory

Authors: *C. GONG, X. WAN;
Beijing Normal Univ., Beijing, China

Abstract: Individuals are required to encode substantial amounts of information and events on a daily basis. One effective approach to achieve such demanding tasks is organising large amounts of items into concise structures according to task demands, which can save cognitive resources and facilitate the integration of new information with prior knowledge or schema. The extent to which such structure impacts individuals' long-term memory has not yet been quantitatively measured, and the underlying neural representations remain unclear. In this study, we modified

the delayed colour estimation task to quantitatively measure participants' long-term associative memory performance towards a set of purposely structured stimuli inside the fMRI scanner (N=44). The results showed that both individuals' short-term (tested immediately after encoding) and long-term (tested 24 hours after encoding) memory errors displayed a pattern consistent with the designed stimuli structure. Items that were more likely to be encoded according to structures were also less prone to memory decay compared with items that were less likely to be encoded following structures. Such superiority of memory performance was absent after removing stimuli structure (control group, N=22). The activity in the OFC during encoding can predict the decay of memory performance over 24 hours, with stronger OFC activation linked with less memory decay. Importantly, activities in the OFC during encoding also displayed structural distinctions toward stimuli. More specifically, OFC showed greater differentiation between items within the same category than items from different categories. The observed differentiation in OFC activity during encoding might be a mechanism to facilitate the encoding of highly similar items by providing a foundation for forming distinct memory traces and ultimately helping mitigate systematic categorical interference. The results of the current study confirm the advantages of structural encoding for retaining long-term memory and reveal the role played by the OFC in forming abstract knowledge structures to assist better memory.

Disclosures: C. Gong: None. **X. Wan:** None.

Poster

PSTR436. Hippocampal–Prefrontal Cortex Circuit

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR436.01/VV8

Topic: H.08. Learning and Memory

Title: The role of prefrontal spatial coding in supporting a contextual association task

Authors: *A. CUMPELIK, J. L. CSICSVARI;
IST Austria, Klosterneuburg, Austria

Abstract: The medial prefrontal cortex (mPFC) has a broad role in decision making, cognitive flexibility, and executive function, as well as consolidation of long-term memories. These distinct functions are reconciled by the mPFC’s role in context-dependent decision making, which requires the evaluation and selection of representations that are relevant for a particular task or goal.
The hippocampus and mPFC interact via direct and indirect pathways. Coordinated activity between these regions occurs both during hippocampal theta and sharp-wave ripples (SWRs) and is correlated with performance during tasks that rely on spatial information for correct decisions. The mPFC has been shown to encode spatial information and mPFC assemblies reactivate during sleep coincidentally with hippocampal SWRs. Moreover, the mPFC has recently been shown to replay temporally organized spatial sequences in well-trained animals during awake immobility while performing a spatial rule-switching task. Our work aims to identify the emergence of

spatial coding and trajectory replay in the mPFC and correlate this with performance in naive animals.
We use 32-tetrode microdrives to record from the hippocampus and mPFC while rats learn to associate a particular food cue with a specific reward location in an 8-arm maze. Two paired cue-reward associations are learned in parallel. To find the reward, the rats must flexibly adapt their behavior based on which cue is presented, i.e. which "context" they find themselves in. During the acquisition of the behavioral data, we observed a sudden jump in performance after 6-7 days of training. This shift may coincide with the emergence or refinement of spatial representations and trajectory replay in the mPFC.
Determining the time course of mPFC spatial representations and trajectory replay during the learning process will provide insight into how behavioral demands may drive the refinement of task-relevant information in the mPFC.

Disclosures: A. Cumpelik: None. **J.L. Csicsvari:** None.

Poster

PSTR436. Hippocampal–Prefrontal Cortex Circuit

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR436.02/VV9

Topic: H.08. Learning and Memory

Support: NIH Grant R01 MH112661
NIH Grant R01 MH120228

Title: Hippocampal-prefrontal dynamics during a spatial rule-switching task

Authors: *M. DING[1], S. P. JADHAV[2];
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Abstract: The hippocampus (HPC) and medial prefrontal cortex (mPFC) play distinct roles in memory processing, and cooperatively support cognitive functions. Prior research indicates that mPFC neuronal activity corresponds with behavioral strategy switches in rats (Rich and Shapiro, 2009), and such transitions lead to dynamic changes in the hippocampus (Hasz and Redish, 2020). Nevertheless, the initiation of representational changes and the influence of HPC-mPFC interactions on action evaluation and planning, particularly after reward outcomes, remain elusive. Our research aims to explore the evolution of HPC and mPFC representations as animals transition between variations of a spatial working memory task, with a particular emphasis on offline replay activity. We trained animals on a rule-switching W-maze task where the task structure was kept constant, but the identities of trajectories were changed. Simultaneous high-density tetrode recordings were conducted in the rat hippocampal CA1 and mPFC during task execution. We observed a gradual drift in the joint CA1-mPFC population representations over rule switches during active running, mirroring improvements in behavioral performance. Our

ongoing work further investigates the shifts in content of hippocampal replay and coordinated CA1-mPFC replay as animals adjust to changing rules, which could elucidate the complex interplay between these brain regions during spatial memory tasks.

Disclosures: M. Ding: None. **S.P. Jadhav:** None.

Poster

PSTR436. Hippocampal–Prefrontal Cortex Circuit

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NIH/NIMH R01 MH112661 Role of Physiological Patterns in Hippocampal-Prefrontal Interactions
NIH/NIMH R01 MH120228 Multiple Mechanisms of Neural Coordination for Associative Memory Processes

Title: Subiculum and CA1 coordination in rats during learning of a novel complex navigation task

Authors: *J. M. OLSON, C. W. REES, S. P. JADHAV;
Brandeis Univ., Waltham, MA

Abstract: Complex cognitive abilities such as decision-making and spatial navigation are built upon basic cognitive building blocks of memory formation and recall. A working hippocampus is necessary for successful memory encoding and retrieval, and coordination with extrahippocampal regions through rhythmic network patterns such as sharp-wave ripples and theta oscillations play important roles in spatial learning and decision-making. During sharp-wave ripples, hippocampus “replays” memory sequences, reactivating neural activity patterns originally experienced in behavioral timescales into a timescale amenable to Hebbian plasticity.Subiculum (SUB) and CA1 are the two main outputs of the hippocampus. SUB, the less studied of the two, receives strong inputs from CA1 and entorhinal cortex, and its outputs largely mirror CA1. SUB also has outputs to key decision-making brain regions such as prefrontal cortex and nucleus accumbens. Despite the extensive anatomical connectivity, little is known regarding CA1/SUB coordination during memory-guided navigation. We hypothesize that during rhythmic network activity, neurons in SUB and CA1 with overlapping spatial firing fields will be active, jointly “replaying” previous experience and linking categorical SUB representations with CA1 ensembles encoding specific experiences. Here, we recorded dorsal CA1 and SUB single cell activity using *in vivo* electrophysiology while adult male Long-Evans

rats navigated a novel complex environment. Through the use of dynamic barrier locations, we adapted the available paths to rewards over learning. SUB and CA1 ensembles do indeed show coordination during sharp-wave ripples during learning. Differences in field quantities and distribution between SUB and CA1 neurons were observed. Overall, this work adds to a growing body of evidence that SUB and CA1 must be considered together when understanding hippocampal output during navigation.

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Poster

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Topic: H.08. Learning and Memory

Support: NIH/NIMH R01 MH112661
NIH/NIMH R01 MH120228

Title: Hippocampal-prefrontal neural correlates of schema formation during a transitive inference learning

Authors: *B. S. PORTER[1], C. SHI[2], E. KOZLOVA[2], S. P. JADHAV[2];
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Abstract: Inferential reasoning is the process of animals making novel associations between parts of previously learned information. Memory schema, or relational networks of knowledge, may enable inferential reasoning. While previous studies have indicated that the hippocampus and prefrontal cortex are necessary for both inference and schema formation, the neural mechanisms supporting schema formation for inference are poorly understood. During learning, episodic information may be generalized over experiences and linked by common elements to create schema. We hypothesize that hippocampal sharp-wave ripples (SWRs) may replay past experiences that have common elements with the current experience. Such a mechanism can link distinct experiences together by their shared content and thus create memory schemata. Indeed, SWRs have been shown to be critical for memory consolidation between the hippocampus and cortex. To test our hypothesis, we recorded hippocampal and medial prefrontal neuronal ensembles in adult rats (Long-Evans, male and female, 3-12 months old, N = 5) while they learned a spatial transitive inference task (A>B>C>D>E). We found distinct populations in the hippocampus that represented each item in the transitive inference set. In contrast, prefrontal ensembles were more generalized and reflected the item that should be chosen first regardless of item identity. Furthermore, we identified SWR events that reactivated hippocampal neurons selective to different items together. Overall, these results indicate that SWR-mediated reactivation during learning may aid in forming schema through the retrieval of memories related to the current experience.

Disclosures: **B.S. Porter:** None. **C. Shi:** None. **E. Kozlova:** None. **S.P. Jadhav:** None.

Poster

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Topic: H.08. Learning and Memory

Support: NINDS Intramural Research Program (ZIA NS003168)

Title: Reshaping hippocampal input-prefrontal interneuron interactions in wildtype and 22q11.2 deletion-relevant model mice.

Authors: *E. VAUGHAN[1], T. T. CLARITY[1], S. E. SILVERSTEIN[1], M. S. DESHPANDE[1], R. M. MIKOFSKY[1], M. V. MYROSHNYCHENKO[1], M. S. BOWEN-KAUTH[1], M. HSIANG[2,1], J. A. GORDON[3,1], D. A. KUPFERSCHMIDT[1];
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Abstract: Functional connectivity between rodent ventral hippocampus (vHPC) and medial prefrontal cortex (mPFC) supports many cognitive functions and is disrupted in the Df(16)$A^{+/-}$ mouse model of the schizophrenia-predisposing 22q11.2 deletion syndrome. Inhibition of vHPC inputs to mPFC or select mPFC interneuron (IN) populations in wildtype mice induces vHPC-mPFC dysconnectivity and cognitive deficits that mimic phenotypes observed in Df(16)$A^{+/-}$ mice. This phenotypic convergence raises three questions: (1) How do vHPC inputs to mPFC interact with mPFC INs *in vivo*? (2) Are these interactions disrupted in Df(16)$A^{+/-}$ mice? and (3) Are these interactions plastic, offering a means to correct circuit dysconnectivity? We characterized *in vivo* activity dynamics and plasticity of discrete mPFC IN population responses to vHPC input stimulation in adult wildtype and Df(16)$A^{+/-}$ mice. We expressed ChrimsonR in vHPC neurons and GCaMP6f in mPFC somatostatin (SST), vasoactive intestinal polypeptide (VIP), or parvalbumin (PV)-expressing INs (n=8-13). We delivered red light pulses to mPFC to excite vHPC inputs and monitored postsynaptic GCaMP6f Ca^{2+} responses using fiber photometry. SST-IN responses to vHPC input stimulation were initially weak in wildtype and Df(16)$A^{+/-}$ mice, but progressively increased with minimal, periodic stimulation over 50 days ($p<0.001$). This potentiation was blunted in Df(16)$A^{+/-}$ mice ($p<0.001$), but partially recovered with additional high-frequency vHPC input stimulation. Conversely, VIP- and PV-IN responses to vHPC input stimulation were initially robust but were rapidly and persistently depressed by repeated high-frequency stimulation in all mice ($p<0.001$). vHPC input stimulation also enhanced and diminished non-evoked, endogenous Ca^{2+} dynamics in SST- and VIP-INs, respectively ($p<0.05$). Ongoing work is characterizing synaptic, neuronal ensemble, and cognitive behavioral impacts of this plasticity. These studies reveal divergent and malleable *in vivo* responses across mPFC IN classes to vHPC input stimulation. Beyond their implications for optogenetic study design and interpretation, these findings demonstrate cell-type-specific

connectivity and plasticity within intact vHPC-mPFC networks that may be used to influence cognition-relevant circuit function and dysfunction.

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Poster

PSTR436. Hippocampal–Prefrontal Cortex Circuit

Location: WCC Halls A-C

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Program #/Poster #: PSTR436.06/VV13

Topic: H.08. Learning and Memory

Support: NINDS Intramural Research Program (ZIA NS003168)

Title: Keep me(dial PFC) in the loop: Closed-loop optogenetic stimulation on mouse hippocampal-prefrontal communication and spatial working memory performance

Authors: A. NTAMATUNGIRO[1], M. V. MYROSHNYCHENKO[1], A. A. DUIN[1], *D. KUPFERSCHMIDT[1], J. A. GORDON[1,2];
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Abstract: Spatial working memory (SWM) is the ability to temporarily store and manipulate spatial information about one's environment to guide behavior. In mice, direct projections from ventral hippocampus (vHPC) to medial prefrontal cortex (mPFC) support SWM and vHPC-mPFC oscillatory synchrony. However, the causal contributions of oscillatory synchrony to SWM performance remain unclear. To explore these contributions, we developed a closed-loop optogenetic stimulation paradigm aimed at manipulating endogenous vHPC-mPFC oscillatory synchrony. We injected adult mice with viruses encoding ChR2 (n=8) or GFP (n=5) in bilateral vHPC and implanted them with local field potential (LFP) wires in unilateral mPFC and vHPC, stereotrodes in unilateral mPFC, and optical fibers in bilateral mPFC. We delivered blue light to vHPC terminals in mPFC in a manner governed by the real-time theta frequency-filtered (7±3 Hz), half-wave rectified vHPC LFP at various phase delays relative to ongoing vHPC theta oscillations. Closed-loop illumination delivered near-synchronously ("in-phase") with vHPC theta oscillations enhanced vHPC-mPFC theta coherence in ChR2- but not GFP-expressing mice; synchrony enhancement diminished with increasing phase delays (up to 1.5 theta cycles). In separate mice (n=17 ChR2, n=13 GFP), we tested the effects of our closed-loop paradigm on SWM, hypothesizing that in-phase stimulation will enhance SWM performance and phase-shifted stimulation will impair it. In mice trained on a delayed non-match-to-sample T-maze task with trials of 10- and 60-sec delay lengths, we delivered closed-loop illumination every other trial, either in-phase with vHPC theta oscillations or phase-shifted by 1.5 theta cycles. Closed-loop stimulation (in-phase or phase-shifted) did not impact SWM performance. Unexpectedly,

blue light illumination produced broadband reductions in mPFC power in ChR2 and GFP mice. However, ChR2 mice showed light-induced increases in mPFC theta power relative to GFP mice, particularly when light was delivered in-phase. Furthermore, light-induced changes in vHPC-mPFC theta coherence were phase shift- and delay length-dependent and more pronounced in ChR2 relative to GFP mice. Ongoing work is assessing effects of stimulation on mPFC unit phase-locking to vHPC theta oscillations. Together, our results reveal behavioral state-dependent effects of closed-loop optogenetic stimulation on long-range oscillatory synchrony.

Disclosures: A. Ntamatungiro: None. **M.V. Myroshnychenko:** None. **A.A. Duin:** None. **D. Kupferschmidt:** None. **J.A. Gordon:** None.

Poster

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Location: WCC Halls A-C

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Program #/Poster #: PSTR436.07/VV14

Topic: H.08. Learning and Memory

Support: NINDS Intramural Research Program (ZIA NS003168)

Title: Impacts of in vivo ventral hippocampal-prefrontal microcircuit plasticity on interneuron population activity and spatial cognition in mice.

Authors: *M. S. DESHPANDE[1], E. J. VAUGHAN[1], S. E. SILVERSTEIN[1], T. T. CLARITY[1], C. M. ALOIMONOS[1], J. A. GORDON[2], D. A. KUPFERSCHMIDT[1];
[1]Integrative Neurosci. Section, Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD; [2]Natl. Inst. of Mental Hlth., NIH, Bethesda, MD

Abstract: Dynamic interactions between the rodent ventral hippocampus (vHPC) and the medial prefrontal cortex (mPFC) support cognitive functions such as spatial working memory and innate spatial avoidance. *Df(16)A*$^{+/-}$ mice that model the schizophrenia-predisposing 22q11.2 microdeletion syndrome show functional dysconnectivity between these structures, disrupted spatial working memory, and reduced avoidance. Furthermore, inactivating vHPC inputs to mPFC and select mPFC interneuron (IN) classes in wildtype mice recapitulates these phenotypes. We have shown that repeated optogenetic stimulation of vHPC inputs to mPFC can persistently reshape *in vivo* functional connectivity between vHPC inputs and mPFC INs in wildtype and *Df(16)A*$^{+/-}$ mice, strengthening and weakening connections with somatostatin (SST) and vasoactive intestinal peptide (VIP)-expressing INs, respectively. How this plasticity influences spatial working memory, spatial avoidance, and task-relevant engagement of mPFC interneurons is unclear. Here we expressed the excitatory opsin ChrimsonR in bilateral vHPC neurons and Ca^{2+} indicator GCaMP6f in SST- or VIP-INs in bilateral mPFC of wildtype and *Df(16)A*$^{+/-}$ mice. Through fibers implanted in bilateral mPFC, we delivered repeated bouts of 40-Hz red light pulses to mPFC to excite vHPC inputs for 12 days over an 18-day period and

monitored postsynaptic Ca^{2+} responses on select days using fiber photometry. We then measured task-relevant Ca^{2+} activity in SST- and VIP-IN populations while mice explored an elevated plus maze (EPM) test of spatial avoidance, and during acquisition and performance of a delayed non-match-to-sample T-maze test of spatial working memory. We found that SST-IN activity increased during the sample and choice phases of the T-maze task, and diminished across the delay phase ($n=7$, $p<0.05$). In contrast, preliminary results suggest that VIP-IN activity diminished during the sample and choice phases, and progressively increased across the delay phase. Ongoing work is assessing the impact of vHPC input optogenetic stimulation and *Df(16)A*$^{+/-}$ deletion on performance and IN population-specific activity in the EPM and T-maze. These data reveal cell type-specific activity patterns during tasks of spatial cognition, and will inform how reshaping vHPC-mPFC microcircuit connectivity influences typical cognitive function and disease-relevant cognitive dysfunction.

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Poster

PSTR436. Hippocampal–Prefrontal Cortex Circuit

Location: WCC Halls A-C

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Program #/Poster #: PSTR436.08/VV15

Topic: H.08. Learning and Memory

Support: IBS-R002-A1

Title: Value-dependent reactivation of prefrontal cortical neurons during hippocampal sharp-wave ripples

Authors: *G. YOUN, M. W. JUNG;
KAIST/IBS, 291 Daehak-ro, Yuseong-gu, Daejeon, Korea, Republic of

Abstract: Hippocampal-neocortical interactions during inactive states have been implicated in memory consolidation. To investigate how the hippocampus and prefrontal cortex exchange information during inactive states and how this process is modulated by value, we trained head-fixed mice to navigate in three different virtual environments associated with three different reward probabilities. The mice showed varying running speeds and/or lick rates across the three environments, indicating that they formed spatial context-reward probability associations. Using Neuropixels probes, we simultaneously recorded neural activity from the intermediate CA1 (iCA1) and its monosynaptic target, medial prefrontal cortex (mPFC). Preliminary analysis of neural activity revealed that mPFC neurons preferentially active in the high-value context were more likely to reactivate with hippocampal sharp wave ripples during the subsequent rest period. These results suggest that the hippocampus-mPFC neural system may preferentially process valuable experiences during rest states in order to facilitate the consolidation of valuable events.

Disclosures: G. Youn: None. **M.W. Jung:** None.

Poster

PSTR436. Hippocampal–Prefrontal Cortex Circuit

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Title: Interaction between the intermediate hippocampus and medial prefrontal cortex for optimal goal-finding behavior in a virtual reality environment

Authors: S.-W. JIN, S. PARK, *S.-M. LEE, I. LEE;
Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: The neural mechanisms of how the hippocampus (HPC) and medial prefrontal cortex (mPFC) work synchronously to achieve flexible decision-making are largely unknown, especially in a goal-directed navigation task. We recorded single units between the intermediate HPC (iHPC) and mPFC while rats (n=6) performed a spatial navigation task in an immersive virtual reality (VR) environment. In our task, the body-restrained rat ran on a spherical treadmill to navigate a circular arena in the VR environment to reach a goal zone where he found a liquid reward (120 trials/day). In a given trial, the rat started from the center of the arena, facing either north or south. There were two potential goal zones: the east reward zone (ERZ) and the west reward zone (WRZ). Rats initially learned to visit only WRZ to receive rewards to criterion (> 75% correct for 2 consecutive days). Then, rats underwent reversal learning as the reward was provided only in ERZ but not WRZ. Once they completed reversal learning, a 24-tetrode hyperdrive was implanted to record from the mPFC and iHPC. After 1 week of recovery, tetrodes were lowered to the target areas while rats were re-trained in the VR environment for 9 days in preparation for the main task. Once they were ready, the main recording sessions began with the same training sequence as in pre-surgical training. Rats naturally developed a stereotyped behavior for efficient navigation in our VR environment by turning clockwise as they searched for the goal location. Therefore, the critical indicator for learning was whether the rat inhibited the temptation of entering the nearest non-reward zone and continued to travel to the farther yet more rewarding zone. Among the single units (n=1002 in iHPC and n=681 in mPFC), some cells both in the iHPC (12%, n=123/1002) and in the mPFC (11%, n=74/681) significantly changed their firing rates in those trials where rats exhibited inhibitory behavior upon facing the nearest non-reward zone compared to those trials where they failed to do so. We also measured the degree of synchronous spiking activities between the cells from the iHPC and mPFC when

rats faced the nearest non-reward zone. The proportion of the neuronal pairs exhibiting significant co-firing was greater in trials where they showed inhibitory behavior in front of the nearest non-reward zone (3.6%, n=113/3168) than in those trials where rats failed to do so (1.7%, n=21/1250) ($p<0.001$; $\chi 2$ test). Our preliminary findings suggest that the synchronous spiking between the iHPC and mPFC at the right moment of behavioral control may serve as the physiological marker to predict the efficiency of goal-directed navigation in space using allocentric visual landmarks.

Disclosures: **S. Jin:** None. **S. Park:** None. **S. Lee:** None. **I. Lee:** None.

Poster

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Topic: H.08. Learning and Memory

Support: BBSRC NLD DTP Studentship
ERC CoG MECHIDENT

Title: Context-dependent sequence learning in nonhuman primates is modulated via non-invasive transcranial ultrasound stimulation of the hippocampus and prefrontal cortex

Authors: *B. J. A. SLATER[1], D. HOWETT[2], J. NACEF[1], B. AGAYBY[1], A. EASTON, D.Phil[3], J. SALLET[4], T. D. GRIFFITHS[1], C. I. PETKOV[5,1], Y. KIKUCHI[1];
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Abstract: In natural scenarios, different rules apply under different contexts, and a given context might be associated with a specific memory sequence. A change in context often involves adapting a memory sequence. How neural systems store and retrieve the correct elements of each memory sequence depending on context remains poorly understood. Contextual information may be hippocampal dependent if context acts as a memory cue or is integrated into a context-guided memory sequence. The prefrontal cortex may be involved if the memory is stored as a schema or when sequencing rules and contexts change. The present study is adapted from a context-guided sequence learning task originally undertaken in rats (Navawongse & Eichenbaum, 2013) to work with nonhuman primates (Rhesus macaques). The task involved two spatial contexts implemented over touch screen monitors attached to the macaques' home units. The background colours on the screen established the context with which a sequence of visual objects needed to be sequenced in order (Context 1: 'A to B'; Context 2: 'C to D'). Two macaques were tested (1M, 1F) and both performed well on the task (typically > 75% correct overall). Focused transcranial ultrasound stimulation (fTUS) was applied to the hippocampus (HC) or medial

prefrontal cortex (mPFC) during the task, using a protocol known to induce post fTUS ('offline') effects that can last for over an hour. Behavioural testing sessions and fTUS were counter-balanced with sham (no fTUS) conditions, and after fTUS, each animal was returned to their home cage to work on the task for 1-2 hours. In the early stages of learning, for both macaques, stimulation of the anterior HC increased performance (91-94%) compared to sham (86-89%, $p < .001$). In the final phase of testing, context was switched in the middle of the trial and the monkey needed to correctly sequence based on the updated context. During this phase of testing, fTUS specifically to the mPFC resulted in improved performance for both monkeys (sham: 73-75%, increased with mPFC fTUS to 82-85%, $p < .001$). fTUS to the HC, however, was either ineffective (anterior HC) or inconsistent in effect across the two monkeys (posterior HC). In summary, we obtained evidence that both hippocampal and prefrontal perturbation modulates cognitive performance during specific aspects of context-guided sequence learning, remarkably with site-specific and consistent improvement in performance using fTUS.

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Poster

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Program #/Poster #: PSTR436.11/VV18

Topic: H.08. Learning and Memory

Support: R01NS118091

Title: A modulatory role of the nucleus reuniens in hippocampal-prefrontal communication

Authors: ***S. SHAO**[1], J. FERRERO[1], T. KAWANO[3], D. KHODAGHOLY[2], J. N. GELINAS[1]; [1]Neurol., [2]Electrical Engin., Columbia Univ., New York, NY; [3]Toyohashi Univ. of Technol., Toyohashi, Japan

Abstract: Memory consolidation requires functional synchrony between the hippocampus (HC) and the medial prefrontal cortex (mPFC). The nucleus reuniens (RE) has recently been reported as a structural and functional hub between HC and mPFC. The role of the RE in hippocampal-prefrontal communication during memory consolidation remains unclear. Here, we used *in vivo* electrophysiology to monitor the hippocampal-thalamic-prefrontal network in rats during non-rapid eye movement (NREM) sleep. We identified ripple-like activity in the RE in the 100-180 Hz frequency range with an average duration of 40 ms, and was associated with robust recruitment of thalamic neural spiking. RE ripple activity was phase-locked to both hippocampal sharp wave-ripples (SWR) and mPFC ripples. Hippocampal ripples are known to couple with cortical ripples for facilitating memory consolidation. Although ripples were independently detected in all three structures, we found that coupling between HC and mPFC ripples necessarily involved co-occurring RE ripples. These results suggest a modulatory role for the RE

in oscillatory synchronization between the HC and the mPFC, with potential implications for network mechanisms of memory consolidation.

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Poster

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Topic: H.08. Learning and Memory

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Title: Enhancing Object-in-Place Memory Performance Through Optogenetic Priming of the Hippocampal-Prefrontal Cortex Network

Authors: *J. BUESCHER, M.-P. CONTRERAS SANTANDER, E. ATUCHA TREVINO, M. SAUVAGE, M. PRIGGE;
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Abstract: The ability to recall the spatial location of objects is crucial for our everyday life. Such memory capability relies on the intricated communication between the hippocampus (HIP) and the medial prefrontal cortex (mPFC) and can be impaired during ageing. Despite impressive progress in understanding the role of HIP-PFC communication in object memory, approaches to enhance and improve its functionality are very limited. Therefore, we set out to explore how optogenetic activation (priming) can facilitate object-location memory. To achieve this aim, mice of the Experimental group (n=11) received a stereotactic injection of a virus with an optogenetic opsin into CA1, while implantation of an optical fiber was placed in the mPFC to stimulate the projecting terminals. *Control* animals underwent the same surgical procedure. First, we sample efficiency of our optogenetic priming protocol by numbers of cFOS and pCreb+ neurons in the PFC. For this purpose, mice received unilateral light stimulation and were perfused one-hour post-stimulation, and compared to the contralateral hemisphere.Secondly, animals were subjected to four object-in-place (OiP) sessions that were repeated every other week. Each OiP session consisted of a study and a test phase separated by an 18-min delay. In the study phase the animals encounter 4 different objects where two of them are switched locations in the test phase. In every session, 4 new objects were introduced. To prime the HIP-to-PFC pathway, the *Experimental* group received stimulation 15-min before starting the study phase in the first OiP. *Control* animals were handled the same way but did not receive any light stimulation. In the fourth OiP task, only *Control* animals received light stimulation. Results showed that priming of one hemisphere induced significantly more pCREB+ neurons relative to the DAPI count in anterior cingulate, prelimbic and infralimbic cortex (independent ttest $p<0.05$). Indicating that priming induced higher excitability in the mPFC. Behaviorally, the *Experimental* group

exhibited a significantly better memory performance in the first OiP compared to the *Controls* (one sided independent t-test $p<0.05$). Interestingly, the higher performance of the *Experimental* group compared to the controls remained until the second OiP task ($p<0.05$), when none of the groups received any stimulation. These results suggest that object location recognition memory relies on the strength of the HIP-mPFC network which can be boosted artificially. Special training or pharmacological interventions boosting this network could have implications to counteract the cognitive decline in ageing.

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Poster

PSTR436. Hippocampal–Prefrontal Cortex Circuit

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR436.13/VV20

Topic: H.08. Learning and Memory

Support: ONR N000142012578

Title: A maze paradigm to study hippocampal-frontal interactions underlying goal-oriented behavior in humans

Authors: *M. GEVA-SAGIV[1], K. KIM[2], C. LUO[2], J. J. LIN[3,4], R. O'REILLY[2], I. SAEZ[5], C. RANGANATH[2];
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Abstract: The hippocampus and its functional interactions with cortical areas are thought to support flexible behaviors, guided by previous experience. Mazes have been used as a classic approach to studying learning and memory in animal models and humans for years, since Tolman's (1948) pioneering studies of maze navigation in rats. They provide a controlled environment to study memory-based behaviors. Many models predict that hippocampal memory-related activities (for example - activities underlying high-frequency ripple oscillations) would be most informative during route planning and navigational decisions. However, the difficulty of recording electrophysiological activity directly from the human brain has limited detailed characterization of hippocampal activities underlying goal-directed behavior in humans to date. Here, we present a novel maze paradigm, implemented as an animated video game, to test brain activity during memory-guided behavior. It was tailored for use in a clinical setting, with patients, implanted with depth iEEG electrodes. To ensure goal-seeking is based on memory, rather than achieved by random exploration, we first assessed behavior with a healthy cohort (n=34 students, 18-51y). Participants used button presses to find goal locations in 24 mazes. Each maze had a unique structural layout and a colorful content theme. Each maze was repeated 3 times, enabling us to examine learning across repetitions. Learning effects were evident, based on improved recall of goal locations across repetitions (comparing accuracy between 1st and 3rd repetition $t(30)=19.66$, $p < 10^{-20}$). Finally, participants successfully recalled the goal position based on the bare maze-structure alone, suggesting they could learn the maze topology separately from content cues. Upon informed consent, patients with pharmacoresistant epilepsy, implanted with intracranial electrodes for clinical monitoring in preparation for a possible surgical cure, participated in recordings while performing the task (8 sessions, 7 patients). Patients' performance was comparable to that of the healthy cohort. Depth electrodes recorded detailed spiking activity, local field potentials, and intracranial EEG (average of 9 electrodes per patient) across multiple brain regions (including medial and frontal cortex regions). Intracranial recordings during this task will be used to characterize hippocampal and prefrontal activity during navigational decisions.

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Poster

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Topic: H.08. Learning and Memory

Support: National Science Foundation of China (No. 32200835)

Title: Representational drift in anterior cingulate neuronal ensembles during memory consolidation

Authors: *A. YANG[1], S. WANG[1], F. SU[2], W. LU[1];
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Abstract: Technological advancements have led to a general observation that cortical neuronal network activities are not stable, but susceptible to transient influence from sensory or behavioral feedback. This phenomenon, termed representational drift, plays a central role in the neocortex's continuous learning and memory-retaining function (Pikiw et al., 2022; Qin et al., 2023). There has been no examination of representational drift in the anterior cingulate cortex (ACC), a region key to long-term memory consolidation and retrieval. This study employs longitudinal in-vivo two-photon imaging after Pavlovian associative memory training to assess the degree of representational drift in the mice ACC. Network activities are evaluated by pairwise neuronal synchronization based on calcium spike time series. Calcium spikes are derived from raw calcium fluorescence traces using a deconvolution algorithm (Juczewski et al., 2020). Results demonstrate ACC representational drift (viewed by coactivity complex network structure) across 4 animals within one day up to 4 weeks post-training. The study corroborates the dynamic features of cortical neuronal ensembles during early memory consolidation and provides sampling frequency guidelines for longitudinal two-photon imaging analyses.
Citation: Pilkiw, M., Jarovi, J., & Takehara-Nishiuchi, K.. (2022). Lateral Entorhinal Cortex Suppresses Drift in Cortical Memory Representations. The Journal of Neuroscience, 42(6), 1104-1118. https://doi.org/10.1523/jneurosci.1439-21.2021
Qin, S., Farashahi, S., Lipshutz, D., Sengupta, A. M., Chklovskii, D. B., & Pehlevan, C. (2023). Coordinated drift of receptive fields in Hebbian/anti-Hebbian network models during noisy representation learning. *Nature neuroscience*, *26*(2), 339-349. https://doi.org/10.1038/s41593-022-01225-z
Juczewski, K., Koussa, J. A., Kesner, A. J., Lee, J. O., & Lovinger, D. M. (2020). Stress and behavioral correlates in the head-fixed method: stress measurements, habituation dynamics, locomotion, and motor-skill learning in mice. *Scientific reports*, *10*(1), 12245. https://doi.org/10.1038/s41598-020-69132-6

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Poster

PSTR436. Hippocampal–Prefrontal Cortex Circuit

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Topic: H.08. Learning and Memory

Support: Support contributed by “MOST 110-2326-B-007-001-MY3” (Taiwan) to CHC

Title: Acquisition of trace fear conditioning in rats without functional nucleus reuniens did not require dorsal hippocampus NMDA receptor activation

Authors: *R.-H. LIU, C.-H. CHANG;
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Abstract: The nucleus reunions (RE) plays an essential role in the cortico-thalamo-cortical circuit that connects the medial prefrontal cortex (mPFC) and the hippocampus (HPC), completing the HPC-dependent circuit that regulates the trace fear conditioning. In this procedure, a neutral conditioned stimulus (CS; tone) and an aversive unconditioned stimulus (US; foot shock) are paired but separated by a “trace” interval. Earlier, we demonstrated that RE inactivation during conditioning impaired the acquisition of trace fear, while RE inactivation during both conditioning and test led to heightened fear to tones throughout the entire test session. These findings prompted us to further investigate whether animals without functional RE acquired trace fear using the HPC-independent circuit. To first confirm the involvement of the dorsal hippocampus (DH) in trace fear acquisition, we pharmacologically blocked glutamate transmission in the DH during the conditioning phase using DL-2-Amino-5-phosphonovaleric acid (APV), an N-methyl-D-aspartic acid (NMDA) antagonist. We hypothesized that for rats without functional RE during both conditioning and test, they used the HPC-independent circuit for acquiring fear, and therefore would be insensitive to the DH manipulation. Our results showed that for the RE intact animals and compared to DH saline controls, DH NMDA blockade led to a slower increase in freezing levels during conditioning, suggesting that the within-session fear expression was impaired. Moreover, these RE intact animals displayed a significantly attenuated freezing response to the tone during the early retrieval test compared to DH saline controls, suggesting that the acquisition of trace fear in normal rats relied on DH NMDA receptor activation. On the other hand, for the rats without functional RE during both the conditioning and test, these animals showed equivalent fear expression in comparison to RE intact/DH saline controls, but were insensitive to the DH blockade of NMDA activities. Together, our results revealed that animals without functional RE acquired trace fear through the HPC-independent circuit.

Disclosures: R. Liu: None. **C. Chang:** None.

Poster

PSTR436. Hippocampal–Prefrontal Cortex Circuit

Location: WCC Halls A-C

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Program #/Poster #: PSTR436.16/VV23

Topic: H.08. Learning and Memory

Title: Top-down control of memory integration in the hippocampus

Authors: *A. F. DE SOUSA[1], A. LUCHETTI[4], S. SIMANIAN[2], D. ALMEIDA-FILHO[3], A. SILVA[2];
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Abstract: New learning can be affected by previous memories, shaping the way novel information is encoded and organized. Related memories are thought to be integrated during encoding and/or post-learning sleep via increases in the number of overlapping ensembles representing each experience. Interactions between the prefrontal cortex (PFC) and the hippocampus (HPC) are important for memory integration where the prefrontal cortex is thought to bias or inhibit memory reactivation in the HPC. However, it is currently not known how such top-down modulation is able to affect specific memories being integrated. Here we use a memory integration/linking paradigm in mice to investigate how the medial PFC is able to control the integration of similar memories encoded several days apart. We first observed that PFC activity increases during encoding of a second memory. Preventing this increase, using chemogenetics, led to memory integration and an increase in the number of overlapping memory ensembles in the dorsal CA1 but not in CA3 or DG. Whole brain analysis of cfos expression indicated that this process might be mediated via the medial entorhinal cortex (MEC). Accordingly, inhibition of PFC-MEC, but not PFC-HPC projections, was sufficient to promote memory integration. These results suggest the existence of a top-down, interregional mechanism that controls memory integration and allocation in the dCA1 via modulation of MEC activity during learning.

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Poster

PSTR437. Intrinsic Hippocampal Circuits and Inhibition

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NIH 1R01NS109362-01

Title: Long Range Projections from Lateral Entorhinal Cortex into Hippocampal area CA1 in Episodic Memory Modulation

Authors: *M. HERNANDEZ FRAUSTO[1,2], J. BASU[3];
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Abstract: Interaction between entorhinal cortex (EC) and hippocampus CA1 area (HC-CA1) promotes sequential organization that lead to the formation of episodic memories of people, places, objects, and events. Within the EC, the medial part (MEC) acts as a spatial information detector that conveys position in space, and the lateral subdivision (LEC) functions as a contextual non-spatial sensor that conveys contextual features of the environment, related to objects, novelty, and odor. The LEC sends excitatory and inhibitory projections directly to HC-CA1 that bears in a microcircuit connectivity capable to induce dendritic spiking, however we know little about the functional role of this projections in episodic memory modulation. To address the functionality of the connectivity between LEC to HC-CA1. First, with chemogenetic strategies and fiber photometry recordings in freely moving mice, I assessed the functionality of the excitatory and inhibitory projections from LEC into HC-CA1 in episodic learning freely moving behaviors. Our results suggests that the network connectivity between LEC and HC-CA1 have a differential role during encode and recall phases of episodic learning that depends of the excitatory and inhibitory network connectivity.

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Poster

PSTR437. Intrinsic Hippocampal Circuits and Inhibition

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Topic: H.08. Learning and Memory

Support: NIH BRAIN Initiative 1R01NS109994

Title: The Role of Excitatory and Inhibitory Inputs From Lateral Entorhinal Cortex to CA3 in Learning and Memory

Authors: *C. D. JOHNSON[1], V. ROBERT[2], J. BASU[3];
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Abstract: The interactions between the entorhinal cortex (EC) and hippocampus are crucial for learning and memory. Within the hippocampus, area CA3 plays an important role in episodic memory formation and recall via its ability to form and reactivate ensembles of neurons. The

circuit and cellular mechanisms that functionally drive these dynamic neural representations within CA3 and govern its role in memory encoding and recall remains unknown. Distal dendrites of CA3 pyramidal neurons (PNs) directly receive local non-spatial or contextual information from the lateral entorhinal cortex (LEC). LEC has been shown to regulate activity in hippocampal area CA1 via long-range excitatory and inhibitory projections (Melzer et al., 2012; Basu et al., 2016). However, there is a gap in functionally defining the role these cortical inputs play in hippocampal area CA3. To address this, we examined the role of LEC glutamatergic and GABAergic projections to area CA3 in episodic memory formation and recall using chemogenetic manipulations of CA3-projecting LEC neurons during freely moving behavior. We used viral tracing techniques to isolate the excitatory and inhibitory projections found in CA3 originating from LEC. To evaluate the role of LEC glutamatergic (excitatory) and GABAergic (inhibitory) projections in learning and memory, we subjected wild-type mice to contextual (novel object recognition, NOR) and spatial (Barnes Maze) tasks perturbing the activity of LEC during learning. We found that silencing either excitatory or excitatory & inhibitory projections from LEC to CA3 impaired NOR memory, consistent with previous reports (Van Cauter et al., 2013). In contrast, performance in the Barnes maze was unaffected by LEC inputs silencing. We further explored their underlying spatial navigation by rotating external cues to introduce mismatch between the proximal and distal reference frames. We observed that control mice relied more on an egocentric strategy for navigation than those that had LEC inputs silenced. This is in line with LEC neurons coding for egocentric relation to salient landmarks of the environment (Wang et al., 2018). Our results show that both excitatory and inhibitory projections from LEC to CA3 contribute to hippocampal-dependent learning and memory.

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Poster

PSTR437. Intrinsic Hippocampal Circuits and Inhibition

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Topic: H.08. Learning and Memory

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Title: Hippocampus shapes cortical sensory output and novelty coding through a direct feedback circuit

Authors: *T. BUTOLA[1], J. BASU[2], M. HERNANDEZ FRAUSTO[1], S. BLANKVOORT[3], C. G. KENTROS[4], L. PENG[1];
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Abstract: To extract behaviorally relevant information from our surroundings, our brains constantly integrate and compare incoming sensory information with those stored as memories. Cortico-hippocampal interactions could mediate such interplay between sensory processing and memory recall but this remains to be demonstrated. Recent work parsing entorhinal cortex-to-hippocampus circuitry show its role in episodic memory formation and spatial navigation. However, the organization and function of the hippocampus-to-cortex back-projection circuit remains uncharted. We combined circuit mapping, physiology and behavior with optogenetic manipulations, and computational modeling to reveal how hippocampal feedback modulates cortical sensory activity and behavioral output. Here we show a new direct hippocampal projection to entorhinal cortex layer 2/3, the very layer that projects multisensory input to the hippocampus. Our finding challenges the canonical cortico-hippocampal circuit model where hippocampal feedback only reaches entorhinal cortex layer 2/3 indirectly via layer 5. This direct hippocampal input integrates with cortical sensory inputs in layer 2/3 neurons to drive their plasticity and spike output, and provides an important novelty signal during behavior for coding objects and their locations. Through the sensory-memory feedback loop, hippocampus can update real-time cortical sensory processing, efficiently and iteratively, thereby imparting the salient context for adaptive learned behaviors with new experiences.

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Poster

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Title: Long-range GABAergic projections allow fine control of hippocampal area CA3 function by lateral entorhinal cortex

Authors: *V. ROBERT[1], K. O'NEIL[1], C. JOHNSON[1], R. DE LA TORRE[2], S. RASHID[1], J. BASU[1];

[1]New York Univ. Langone Med. Ctr., New York, NY; [2]Waisman Center, Univ. of Wisconsin-Madison, Madison, WI

Abstract: Functional interactions between the entorhinal cortex and the hippocampus are crucial for learning and memory. By integrating context-laden direct inputs from the lateral entorhinal cortex (LEC) with local feedforward dentate gyrus (DG) and feedback CA3 recurrent collateral (RC) inputs, area CA3 is poised to gate hippocampal information flow and mnemonic function. Interestingly, LEC inputs to the hippocampus comprise not only glutamatergic but also the long-range GABAergic projections which regulate dendritic excitability, synaptic plasticity and oscillatory activity in area CA1 (Melzer et al., 2012; Basu et al., 2016). However, the role of LEC glutamatergic and GABAergic inputs to area CA3 and the underlying circuit elements remain unexplored. Therefore, we manipulated LEC glutamatergic and GABAergic inputs with chemogenetics and optogenetics to examine their contributions to area CA3 function using freely moving behavior, head-fixed in vivo 2-photon imaging, and ex vivo patch-clamp electrophysiology. Silencing LEC glutamatergic inputs alone or together with LEC GABAergic inputs similarly impaired novel object recognition memory, suggesting complementary rather than opposing roles of these inputs. Silencing LEC glutamatergic inputs alone, but not together with LEC GABAergic inputs, increased the fraction of active CA3 pyramidal neuron somas and dendrites but decreased activity within each active cell, suggesting recruitment of direct excitation and feedforward inhibition. Indeed, LEC glutamatergic inputs evoked monosynaptic excitation in CA3 pyramidal neurons, as well as disynaptic inhibition which prevented somatic output. Expectably, LEC glutamatergic inputs drove spiking in CA3 SLM interneurons including putative soma-targeting subtypes, whereas LEC GABAergic inputs shunted their excitability. Further, LEC GABAergic inputs selectively boosted CA3 pyramidal neuron somatic output to combined stimulation of LEC glutamatergic and CA3 RC inputs. Accordingly, silencing LEC glutamatergic inputs alone, but not together with LEC GABAergic inputs, decreased novelty-induced remapping of CA3 place cell somas and dendrites. Altogether, our results uncover novel elements of the LEC to CA3 circuit supporting contextually-driven CA3 activity and mnemonic function through compartment- and pathway-specific disinhibition.

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Poster

PSTR437. Intrinsic Hippocampal Circuits and Inhibition

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McKnight Foudation

Title: Remapping properties of apical and basal dendrites in CA3 of navigating mice

Authors: *J. J. MOORE[1,2], D. CHKLOVSKII[2], J. BASU[1];
[1]NYU Sch. of Med., New York, NY; [2]Flatiron Inst., Simons Fndn., New York, NY

Abstract: Neurons maintain large dendritic arbors supporting non-linear input integration which can greatly boost their computational power. Input to hippocampal neurons arrives in a stratified manner onto different dendritic compartments, resulting in the remarkable property of spatial coding within an environment. Equally remarkable is neurons' ability to "re-map" or change their firing rate or location in a new environment. This happens rapidly, often within the first exposure to a new environment. Recent work suggests new place fields are formed after dendritic plateau potentials, but the dynamics of novel place field formation within dendrites and soma simultaneously has not been well characterized.
To address this issue, we used two-photon microscopy to measure calcium activity in the apical dendrites, soma, and basal dendrites of pyramidal neurons in area CA3 of mouse hippocampus during head-fixed navigation on a treadmill belt. Local textures on the belt served as spatial cues to define position. After repeated exposure to a familiar environment, the cues were switched to a novel environment to induce remapping. Regions of interest were identified and calcium signals were extracted using semi-automated methods developed by our lab. By tracking the tuning properties of soma and dendrites over several days in familiar and novel environments, we demonstrate that dendrites support flexible and stable representations of space. Specifically, apical dendrites showed higher day-to-day stability than soma or basal dendrites, and consequently provided more information to decode animal position. These are the first demonstrations of tuning, stability, and remapping in distal apical dendrites in CA3 during navigation.

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Poster

PSTR437. Intrinsic Hippocampal Circuits and Inhibition

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Topic: H.08. Learning and Memory

Support: 5 T32 NS 86750-7
5 TL1 TR 1447-8

Title: Stability and flexibility of hippocampal CA1 in an odor-cued navigational task

Authors: *M. HOPKINS[1], R. ZEMLA[2], J. BASU[3];
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Abstract: Place cells, hippocampal pyramidal neurons which show location-specific activity during animal navigation, form a spatial map of the environment and are hypothesized to be the neural substrate of episodic memory. However, place cells in low demand tasks, such as random foraging, tend to drift in their spatial tuning over days. Through chronic 2-photon calcium imaging of hippocampal area CA1, we find that the introduction of an odor-context based navigational task stabilizes place cell representations over long time scales, but that these ensembles remain flexible when task contingencies change. This supports the belief that CA1 can encode representations both stably and dynamically. We also explore mouse models that have impaired performance on this task and their underlying neural dysfunction. This work is currently ongoing.

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Poster

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Title: Anatomical and functional heterogeneity of the basolateral amygdala to ventral hippocampus circuit

Authors: *M. LI[1], Y. JIANG[1], M. C. LU[1], J. KINNEY[2], Q. SUN[1];
[1]Case Western Reserve Univ., Cleveland, OH; [2]Case Western Reserve Univ. Dept. of Neurosciences, Cleveland, OH

Abstract: The ventral hippocampus, including ventral CA1 (vCA1) and ventral CA3 (vCA3), plays an important role in social and anxiety-like behaviors. However, how the upstream brain regions innervate different sub-regions of the ventral hippocampus to participate in such behaviors properly is still incompletely understood. Although plenty of literature as well as our retrograde tracing data provided evidence that the basolateral amygdala (BLA), a region that is essential for emotion processing, projects to both vCA1 and vCA3, the detailed anatomical and functional connectivity pattern remains largely unknown. Here we performed Channelrhodopsin2 (ChR2)-based anterograde tracing and found that BLA fibers predominantly innervated basal dendrites of CA1a/b and CA3a/b in mid-ventral hippocampus, and were nearly devoid of CA1c and CA3c. Remarkably, our ChR2-assisted ex vivo patch clamp recording

revealed that BLA-CA3 synapses displayed robust temporal summation of postsynaptic potentials (PSPs) in responses to 20Hz light simulation (PSP10/PSP1 = 4.87 ± 0.67), whereas BLA-CA1 synapses did not display the facilitation (PSP10/PSP1 = 0.82 ± 0.15). To further explore whether different groups of BLA neurons innervate vCA1 and vCA3 and are responsible for the synaptic differences, we did retrograde AAV tracing in CA1 and CA3 and demonstrated that CA3-projecting BLA neurons were primarily clustered in BLA adjacent to the midline, while CA1-projecting BLA neurons were uniformly distributed in the whole BLA. By selectively targeting CA3-projecting or CA1-projecting BLA neurons using ChR2, we found that only a subset of CA1-projecting BLA neurons projected to CA3 (dual-projecting BLA neurons). Finally, our preliminary behavioral results showed that ChR2-mediated activation of BLA-CA3 projection impaired social behaviors, but had no effect on anxiety-like behaviors. Given that the previous studies showed that BLA-ventral hippocampus projection participates in both social and anxiety-like behaviors, our experiments are underway to examine the role of the subset of BLA neurons that project to CA1 only. Overall, our results provide the anatomical and functional evidence supporting the heterogeneity of BLA-ventral hippocampal connectivity, and raise the possibility that two populations of BLA neurons projecting to hippocampus differently may participate in different behaviors.

Disclosures: **M. Li:** None. **Y. Jiang:** None. **M.C. Lu:** None. **J. Kinney:** None. **Q. Sun:** None.

Poster

PSTR437. Intrinsic Hippocampal Circuits and Inhibition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR437.08/VV31

Topic: H.08. Learning and Memory

Support: NIH K01MH117444
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NIH R01MH130367

Title: Hippocampal CA3 synaptic mechanism for dorsoventral divergence in memory

Authors: M. LI, Y.-Q. JIANG, M. C. LU, ***Q. SUN**;
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Abstract: Area CA3 in the hippocampus is vital for memory formation. CA3 pyramidal neurons (PNs) receive two prominent excitatory inputs - recurrent collateral (RC) from CA3 and mossy fiber (MF) from dentate gyrus (DG) - that play opposing roles in pattern completion (memory generalization) and separation (memory discrimination), respectively. Although the dorsoventral divergence of hippocampal function has been well studied, the dorsoventral difference of CA3 synaptic connectivity remains largely unknown. Here, we report that the ratio of RC-to-MF excitatory drive onto CA3 PNs increases dramatically from dorsal CA3 (dCA3) to ventral CA3 (vCA3) by nearly 8-folds, with vCA3 PNs receiving significantly weaker MF, but stronger RC,

excitation than dCA3 PNs. In addition, we found that the weaker MF excitation in vCA3 results from a combination of the weaker unitary MF input and a smaller number MF synaptic number, compared to dCA3. The dorsal-ventral difference in RC excitation can be largely explained by the greater lengths of basal and apical dendrites in vCA3 than dCA3. Finally, using a contextual fear memory discrimination paradigm, we demonstrated that, compared to dCA3, vCA3 plays a more prominent role in recall and generalization of remote memory. Thus, our results reveal a novel CA3-based synaptic mechanism that may offer the computational advantage for ventral hippocampus to be more strongly involved in behaviors that require less precision but more generalization, such as remote memory.

Disclosures: **M. Li:** None. **Y. Jiang:** None. **M.C. Lu:** None. **Q. Sun:** None.

Poster

PSTR437. Intrinsic Hippocampal Circuits and Inhibition

Location: WCC Halls A-C

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Program #/Poster #: PSTR437.09/VV32

Topic: H.08. Learning and Memory

Support: NIH R01MH129294
NIH R01MH130367

Title: Top-down control of subcortical regions by hippocampal long-range inhibition

Authors: *J. KINNEY, M. ZHOU, D. LEE, H. WANG, M. LI, Y. JIANG, Q. SUN;
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Abstract: Inhibitory neurons are classically divided based on differences in neurochemical, morphological, and electrophysiological features and are considered *short-range* projecting neurons that suppress the activity of surrounding local neurons. Recent evidence, however, indicates that a variety of inhibitory neurons, including somatostatin-expressing (SOM+) and parvalbumin-expressing (PV+) inhibitory neurons, can send *long-range* projections to distant brain regions to coordinate brain-wide activity and contribute to behavioral tasks. We focus on area CA3 in the hippocampus, which is essential for *intra-hippocampal* information processing: linking dentate gyrus to CA1 via classic trisynaptic excitatory pathway (entorhinal cortex-->dentate gyrus-->CA3-->CA1). In comparison, little is known about the direct inhibitory connections between CA3 and *extra-hippocampal* regions. Intriguingly, here, we used cell-type and pathway-specific viral tracing to demonstrate that CA3 SOM+, but not PV+, inhibitory neurons send long-range projections to three subcortical regions: medial septum (MS), lateral hypothalamus (LH), and supramammillary nucleus (SuM). Our retrograde AAV (AAVretro)-based intersectional viral tracing indicates that the same CA3 SOM+ inhibitory neurons likely send axon collaterals to all three regions. Furthermore, our ex vivo patch-clamp recordings assisted by channelrhodopsin-2 (ChR2) demonstrated that the long-range inhibition preferentially suppresses fast-spiking (presumptive GABAergic) neurons in SuM and LH, and

preferentially suppress fast-spiking (presumptive GABAergic) and cluster firing (presumptive glutamatergic) neurons in MS. Thus, a key role of this long-range inhibitory output is to disinhibit these three subcortical regions. Moreover, we found optical stimulation of ChR2-expressing SOM+ terminals at theta frequencies are capable of entraining theta firing of the postsynaptic neurons in target subcortical regions. We propose that this long-range inhibitory projection is ideally positioned to coordinate and synchronize activity between the hippocampus and multiple subcortical regions simultaneously. We are currently using in vivo optogenetic manipulations in conjunction with behavioral assays to investigate the role of these novel long-range inhibitory neurons in behaviors.

Disclosures: **J. Kinney:** None. **M. Zhou:** None. **D. Lee:** None. **H. Wang:** None. **M. Li:** None. **Y. Jiang:** None. **Q. Sun:** None.

Poster

PSTR437. Intrinsic Hippocampal Circuits and Inhibition

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Title: A full-scale computational model of the human hippocampus CA1 area

Authors: *M. MIGLIORE[1], S. SOLINAS[2], C. A. LUPASCU[1], G. M. BOIANI[3], D. GANDOLFI[3], J. MAPELLI[3];
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Abstract: In recent years, research on computational brain models has rapidly increased, leading to large-scale implementations of brain circuits at single-cell resolution that have been proven to be instrumental for a better understanding of brain functions. The use of these models can become a disruptive technology, to significantly advance not only our understanding of the mechanisms underlying cognitive functions but also to investigate pathological conditions and discover new pharmacological treatments. Here, we describe a full-scale model of a human right hippocampus CA1 region at cellular resolution [Gandolfi et al., 2023]. Starting from high resolution microscopy images, 3D soma positioning was obtained through an image analysis

algorithm and a connectivity matrix was generated using a morpho-anatomical connection strategy [Gandolfi et al., 2022] based on axonal and dendritic probability density functions accounting for morphological properties of hippocampal neurons. The workflow, in a ready-to-use format suited to implement simulations at different scales and details, and the software to create additional network instantiations, or to generalize the process for other brain regions, are available as open access tools on the EBRAINS Knowledge Graph Platform (https://kg.ebrains.eu/). Preliminary results obtained with this model gave insight into the processes and mechanisms that can modulate and orchestrate the propagation of activity within a CA1 network. We show how the anisotropic connectivity emerging from the data-driven model building process, can generate a network global activity that can be significantly different from what expected by the widely assumed spatially uniform connectivity rule in most large-scale models.

1. Gandolfi, D., *et al.* Full-scale scaffold model of the human hippocampus CA1 area. (2023) *Nature Comput Sci* **3**, 264-276.
2. Gandolfi D, et al., (2022) A realistic morpho-anatomical connection strategy for modelling full-scale point-neuron microcircuits. Sci Rep. 12(1):13864.

Disclosures: **M. Migliore:** None. **S. Solinas:** None. **C.A. Lupascu:** None. **G.M. Boiani:** None. **D. Gandolfi:** None. **J. Mapelli:** None.

Poster

PSTR437. Intrinsic Hippocampal Circuits and Inhibition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

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Topic: H.08. Learning and Memory

Support: Royal Melbourne Hospital Neuroscience Foundation A2087

Title: Dynamic Reconfiguration of Hippocampal CA1 Pyramidal Cell-Interneuron Circuits during Spatial Learning

Authors: *D. SUN[1,2,3], R. UNNITHAN[3], C. FRENCH[2];
[2]Neural Dynamics Laboratory, Dept. of Med., [3]Dept. of Electrical and Electronic Engin., [1]The Univ. of Melbourne, Melbourne, Australia

Abstract: The formation of memory-encoding neuronal ensembles in the hippocampus is thought to be facilitated by changes in functional connections between pyramidal cells and interneurons. However, how hippocampal neural circuits reconfigure during the learning process not clear. Here, we employed in vivo calcium imaging techniques to study the activity of large hippocampal neuronal populations to elucidate this issue.To label neurons, a combination of adeno-associated viruses carrying GCaMP6 (driven by the synapsin promoter) and ChR2 (driven by the mDlx promoter) was injected into the hippocampal CA1 region in C57BL/6 mice. The synapsin promoter enables broad expression in various neuronal populations, including

pyramidal cells and interneurons. In contrast, the mDlx promoter selectively expresses in GABAergic interneurons. This dual-virus approach allows for the labelling of calcium activity in all neurons using GCaMP6, while utilizing a different fluorophore to specifically highlight the spatial footprints of interneurons. The mice (n=5) were trained to traverse a linear track, while the hippocampal calcium activity was simultaneously recorded using a miniaturized fluorescence microscope.We observed an increase in spatial sensitivity in both pyramidal cells and interneurons when the experiment proceeded, with pyramidal cells demonstrating a more robust representation. Pyramidal cells showed lower firing rates (p=0.003) and plasticity (p=0.024), but higher information content (p=0.012) on average compared to interneurons. Neuronal populations were partitioned into distinct clusters using modularity analysis. We found that pyramidal cells exhibited broader interconnectivity within their respective clusters, which underwent reconfiguration over time. In contrast, interneurons displayed stable connectivity patterns within their assigned clusters. In summary, this study advances our understanding of the neural dynamics of hippocampal pyramidal cells and interneurons during the learning process. Our findings reveal that the reconfiguration of topological connections among neuronal populations plays an important role in the formation of new memories.

Disclosures: **D. Sun:** None. **R. Unnithan:** None. **C. French:** None.

Poster

PSTR438. Place Cells: Mechanisms, Development, and Dysfunction

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR438.01/VV35

Topic: H.09. Spatial Navigation

Support: The Wellcome Trust DBT India Alliance Grant IA/S/13/2/501024

Title: Influence of environment size on CA1 spatial representations

Authors: *I. R. JAKHALEKAR[1], S. S. DESHMUKH[3,2];
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Abstract: Traditionally, rodent neural correlates of space have been studied in arenas of 1-2 sq.m. area. However, in the wild, rats have networks of burrows that run up to a few tens of meters. Apart from two recent studies, the spatial representations in spaces similar in size to rats' natural environments have not been studied. We recorded single-unit activity from dorsal CA1 in rats foraging in arenas of varying sizes and shapes ranging from 1 sq. m. to 16.5 sq. m. We observed that the number of place fields per cell increases as we go from the smallest to the largest arena while the fraction of the arena that a cell had place fields in decreases. This implies that as the area of the arena increases cells encode a smaller fraction of the arena and thus have higher spatial selectivity, but the firing is in the form of multiple distinct place fields. Our results suggest that CA1 employs an ensemble coding strategy to represent large environments.

Disclosures: I.R. Jakhalekar: None. **S.S. Deshmukh:** None.

Poster

PSTR438. Place Cells: Mechanisms, Development, and Dysfunction

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR438.02/VV36

Topic: H.09. Spatial Navigation

Support: India Alliance Grant IA/S/13/2/501024
Pratiksha trust grant PE/CHAIR-19-025.03
Shiv Nadar Institution of Eminence intramural funding

Title: Non-theta and non-spatially modulated inputs shape phase precession dynamics in place cells

Authors: *A. BISHNOI[1], S. S. DESHMUKH[2,1];
[1]Ctr. for Neurosci., Indian Inst. of Sci., Bangalore, Karnataka, India; [2]Dept. of Life Sci., Shiv Nadar Inst. of Eminence, Gautam Buddh Nagar, Uttar Pradesh, India

Abstract: Spiking activity of place cells is modulated by the spatial location of the animal and ongoing theta oscillations. Phase precessing place cells maintain a temporal code of space by firing at earlier and earlier phases of theta as the animal traverses through the neuron's place field. CA1 place cells exhibit accelerating phase precession that is banana-shaped on an average. However, individual place cells can also exhibit linear precession dynamics with a constant rate of change of phase with position. Models based on the interaction of spatial excitation and theta inhibition can successfully generate phase precession (Castro & Aguiar, 2012; Seenivasan & Narayanan, 2020; Jaramillo et al., 2014), but the simulated dynamics are typically decelerating i.e., the rate of change of phase slows down towards the end of the place field. Such decelerating phase precession has not been reported in the rat CA1, to the best of our knowledge. In this study, we asked if non-theta non-spatially (NTNS) modulated inputs can contribute to theta-modulated spiking dynamics and generate more biologically realistic phase precession. We tested this possibility by adding presynaptic Poisson noise to the inputs in a previously published model of theta phase precession (Chadwick et al., 2016). Strength of the three input sources to the model - spatially modulated input, theta oscillations, and NTNS excitation - were varied systematically, along with the asymmetry of spatial input. A total of 13328 combinations of input parameters were tested, out of which 3712 combinations generated phase locking. Phase precession was observed for 969 combinations which included both decelerating phase precession (n = 254) as seen in existing models and more biologically realistic linear (n = 556) or accelerating phase precession (n = 159). Increasing the strength of NTNS inputs reduced the tendency of the model to generate decelerating precession and generated linear or accelerating precession. Increasing the asymmetry of spatial input along with the strength of NTNS inputs increased the propensity of the simulated phase precessing neurons to accelerate. We estimated the influence of different inputs and their interactions on the shape of precession and found that

NTNS inputs contributed to biologically realistic phase precession in conjunction with spatial input amplitude. Finally, a subset of combinations (n = 259) also showed theta skipping behavior observed in CA1, CA3 and MEC, in the presence of NTNS inputs. Thus, our simple computational model can simulate a range of biologically observed theta modulation dynamics and demonstrates the relevance of NTNS inputs to these phenomena.

Disclosures: A. Bishnoi: None. **S.S. Deshmukh:** None.

Poster

PSTR438. Place Cells: Mechanisms, Development, and Dysfunction

Location: WCC Halls A-C

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Program #/Poster #: PSTR438.03/VV37

Topic: H.09. Spatial Navigation

Support: NIMH Grant R56MH125655
NIMH Grant F31MH127933

Title: Hippocampal replay events are less temporally compressed during correct trials in rats performing a spatial delayed match-to-sample task

Authors: *M. DONAHUE, L. L. COLGIN;
Univ. of Texas at Austin, Austin, TX

Abstract: The hippocampus is thought to support spatial memory via the activity of place cells, neurons with spatial receptive fields. During periods of waking rest, populations of place cells reactivate sequences of firing that occurred during earlier active behaviors, a phenomenon known as “replay”. Here, we examined replay events during rest periods from a previously published dataset (Zheng et al., Nature Communications 2021) in which rats learned a reward location on a circle track across trials of a delayed match-to-sample task. We used a Bayesian decoding algorithm to estimate angular positions on the track represented by CA1 place cell sequences during replay events. Previous research has shown that the properties of replay events are dynamic and can vary depending on environmental novelty and memory engagement (Fernandez-Ruiz et al., Science 2019; Berners-Lee et al., Neuron 2022). Further, increasing replay event durations improves memory task performance (Fernandez-Ruiz et al., Science 2019). Here, we show that replay events are longer in duration during correct trials compared to incorrect trials of a delayed match-to-sample task. We additionally show that the temporal compression of replayed paths was lower on correct trials than error trials. The rate of replay events did not differ between correct and incorrect trials. These results provide further evidence that replay properties are related to learning and suggest that changes in the temporal compression of replay may facilitate memory-guided task performance.

Disclosures: M. Donahue: None. **L.L. Colgin:** None.

Poster

PSTR438. Place Cells: Mechanisms, Development, and Dysfunction

Location: WCC Halls A-C

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Program #/Poster #: PSTR438.04/VV38

Topic: H.09. Spatial Navigation

Support: NIMH award R56MH125655
NIMH award F31MH127933

Title: Ca2 place cells that respond to social odors are preferentially reactivated during sharp-wave ripples in subsequent rest

Authors: *E. ROBSON, M. M. DONAHUE, P. G. DEMETROVICH, L. L. COLGIN;
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Abstract: The CA2 region of the hippocampus has been implicated in social memory. A recent study showed that CA2 place cell firing patterns change ("remap") during social interactions, a response that is not apparent in CA1 place cells (Alexander et al., Nature Communications 2016). Our preliminary results show that CA2 place cells remapped after a rat interacted with a familiar rat's empty home-cage containing the familiar rat's odors. On the contrary, CA2 place cells did not remap in response to interactions with an identical cage containing the rat but with clean bedding (i.e., minimal social odors). These results suggest that CA2 place cells respond to the olfactory component of social experiences. Much prior work has shown that place cell firing patterns from active exploration are reactivated in sharp-wave ripples (SWRs) during subsequent rest periods. This reactivation of place cells during SWRs is thought to contribute to memory consolidation. We recorded SWRs in CA2 during rest periods following exploration of a cage containing social odors or a cage containing a rat but minimal social odors. We found that CA2 place cells that responded to social odors preferentially increased their firing rates during subsequent SWRs more than CA2 place cells that were less responsive to social odors. In contrast, similar results were not obtained for CA2 place cells that were active during sessions with minimal social odors. These results suggest that SWR-related reactivation of CA2 place cells that code social odors may play a role in consolidation of salient aspects of social memories.

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Poster

PSTR438. Place Cells: Mechanisms, Development, and Dysfunction

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR438.05/VV39

Topic: H.09. Spatial Navigation

Title: Learning a Goal-Oriented Task Shapes the Evolution of Spatial Representation in Rat Hippocampal CA1

Authors: *E. FAILLACE[1,2], R. MITCHELL-HEGGS[1,3,2], F. GOBBO[3], J. GALLEGO[1,2], R. MORRIS[3], S. R. SCHULTZ[1,2];
[1]Bioengineering, [2]Ctr. for Neurotechnology, Imperial Col. London, London, United Kingdom; [3]Lab. for Cognitive Neuroscience, Edinburgh Neurosci., The Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: Studying spatial representation in the hippocampus is crucial for understanding spatial navigation and memory formation. Recent works have indicated that animal behaviour can have a functional effect on spatial representation. For instance, reward locations can be over-represented compared to other locations. Here, we aim to determine how learning a goal-orientated task affects the evolution of spatial representation of the task environment. In this work, we trained 5 rats in the "everyday memory task" over 21 days. Each session is composed of an exploration, sampling, and recall phase. After the initial exploration phase, we hid a food pellet in one of three sandwells, which location was learnt during the sampling phase. The rewarded well remained consistent throughout the session and was randomly repositioned for the subsequent day. GCaMP6f was expressed in CA1 pyramidal neurons. Miniscope calcium imaging recordings were performed at three learning stages representing different levels of task experience: naive (S1-2), intermediate (S8-9), and advanced (S18-21) and registered across sessions. We found that throughout the sessions, the animals significantly improved their performance, indicating an increased ability to recall the reward location. We investigated the impact of this learning on the spatial representation within the overall structural properties of the network and the dimensionality of neural dynamics. Furthermore, we characterised the spatial representation of the arena by examining changes in place cell features (e.g., field size, location, and variance) as task familiarity increased. We tracked these changes in rewarded locations versus other constant salient cues, such as the start-box and two objects at the side of the arena. This study develops our understanding of the mechanisms underlying spatial representation, specifically how individual place cells contribute to network dynamics during learning of goal-directed behaviours.

Disclosures: E. Faillace: None. **R. Mitchell-Heggs:** None. **F. Gobbo:** None. **J. Gallego:** None. **R. Morris:** None. **S.R. Schultz:** None.

Poster

PSTR438. Place Cells: Mechanisms, Development, and Dysfunction

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Program #/Poster #: PSTR438.06/VV40

Topic: H.09. Spatial Navigation

Support: Wellcome Trust Grant 207481/Z/17/Z

Title: Comparison of Spatial Action Planning for Egocentric and Allocentric Spatial Strategies in Rats performing an Episodic Memory task with Miniscope Ca^{2+}imaging

Authors: *F. GOBBO[1], R. MITCHELL-HEGGS[1,2], D. TSE[1,3], N. GARCIA-FONT[1,4], S. S. SCHULTZ[2], R. G. M. MORRIS[1,4];
[1]Univ. of Edinburgh, Edinburgh, United Kingdom; [2]Ctr. for Neurotechnology, Imperial Col. London, London, United Kingdom; [3]Dept. of Psychology, Edge Hill Univ., Ormskirk, United Kingdom; [4]Simons Initiative for the Developing Brain and Patrick Wild Ctr., Edinburgh, United Kingdom

Abstract: *Aim*: A key question in spatial navigation is if, and how, hippocampal replay encodes a memory of previously visited space or a spatial action plan that informs decision-making. Furthermore, the use of different navigational strategies may change the role of hippocampal replay. This study uses endoscopic calcium imaging in the rat hippocampus to compare the neuronal representation of space and decision making in animals learning and retrieving different reward destinations in an episodic memory task using egocentric or allocentric navigational strategies.
Experiment: 10 WT male LH Rats performed variants of the everyday memory task designed to rely on egocentric or allocentric strategies (Broadbent et al Eur.J.Neurosc 2019). Neuronal activity was recorded from CA1 neurons expressing GCaMP6f with miniature microscopes using the Inscopix system (195 cells/animal, SEM 50.7). Rats learned to retrieve food during sample trials from one of six possible sandwells whose position changed every session and were tested for recall 60 min after learning. When recalling, animals entered the arena from four possible start locations, generating distinct origin-goal trajectory combinations. 27 training sessions were performed to ensure coherent, asymptotic performance, and tests conducted to confirm that animals were using an egocentric or allocentric strategy. Consecutive sessions were recorded using Ca^{2+} imaging for each strategy (sessions 28-34). First, we compared the neural representation of the arena space between the two strategies in terms of active place cell population, neural directionality and place field symmetry. We then confirmed reactivation of neurons representing destinations, and asked if the replayed content involves the goal or the trajectory. Furthermore, we compared the reactivation along the recalled trajectory with different and egocentrically similar -but allocentrically distinct- trajectories.
Implications: In both strategies we observed prospective replay encoding the animal's future trajectory, reward destination, and alternative possibilities. Our data provides new information on whether the prospective replay involves memory replay or action planning and hints at how these may differ between egocentric and allocentric navigational strategies.

Disclosures: F. Gobbo: None. **R. Mitchell-Heggs:** None. **D. Tse:** None. **N. Garcia-Font:** None. **S.S. Schultz:** None. **R.G.M. Morris:** None.

Poster

PSTR438. Place Cells: Mechanisms, Development, and Dysfunction

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR438.07/VV41

Topic: H.09. Spatial Navigation

Support: Wellcome Trust Grant 207481/Z/17/Z

Title: Spatial recency memory: causal involvement of the hippocampus for the use of allocentric but not egocentric maps

Authors: F. GOBBO[1], R. MITCHELL-HEGGS[1,2], A. J. DUSZKIEWICZ[1,3], D. TSE[1,4], N. GARCIA-FONT[1], S. S. SCHULTZ[2], ***R. MORRIS**[1,3];
[1]The Univ. of Edinburgh, Edinburgh, United Kingdom; [2]Ctr. for Neurotechnology, Imperial Col. London, London, United Kingdom; [3]Simons Initiative for the Developing Brain and Patrick Wild Ctr., Edinburgh, United Kingdom; [4]Dept. of Psychology, Edge Hill Univ., Ormskirk, United Kingdom

Abstract: Even in familiar environments, discrete events/rewards happen that are the content of recency (or episodic) memory. Goal-oriented tasks offer an opportunity to understand how space is represented, and how this is stored and accessed during memory retrieval and planning. Spatial tasks can be solved using egocentric or allocentric coordinates (Packard & McGaugh, *Neurobiol.Learn.Mem.*1996). Using the everyday arena task paradigm (Bast et al,*J.Neurosci* 2005), we showed that hippocampal neural activity recorded before animals navigate reflects their intended destination or trajectory (Gobbo et al,*PNAS,2022*).
Here, we sought to dissociate the role of the hippocampus in allocentric vs. egocentric represention using behavioral and optogenetic techniques. Rats (n=10) were trained daily to find reward hidden in sandwells whose location changed daily, either with an allocentric protocol in which the reward was approached from different starting points in the same session, or with an egocentric protocol starting from a single start location. Learning occurred well in both protocols as shown in probe tests. Test sessions confirmed that removing extraarena cues caused performance to drop to chance for allocentric (56%) but not egocentric animals (96%). To test the requirement for hippocampal activity in the two tasks, we expressed the inhibitory opsin Jaws, or GFP in control animals (n=30). We confirmed the successful suppression of neuronal activity in a separate group of animals by means of electrophysiology. Hippocampal silencing during action planning caused a mild but significant drop in performance in allocentric but not egocentric animals. Silencing the hippocampus during the execution of the task further impaired the performance of allocentric animals. Animals performing the egocentric protocol, and controls, were unaffected by light stimulation.
Our data indicate that hippocampal activity during goal-directed navigation is required to form an action plan when allocentric representations are used, but not when navigation is based on egocentric representations. The performance of egocentric animals is likely mediated by neural activity that does not involve or need the hippocampus.

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Poster

PSTR438. Place Cells: Mechanisms, Development, and Dysfunction

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Program #/Poster #: PSTR438.08/VV42

Topic: H.09. Spatial Navigation

Support: Shanghai Pilot Program for Basic Research – FuDan University
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Lingang Laboratory (grant no. LG-QS-202203-09)

Title: Impaired spatial representation Dentate Gyrus in 5×FAD mice

Authors: *S. HAN, L. KOU, P. YUAN;
Inst. for Translational Brain Res., Fudan Univ., Shanghai, China

Abstract: One of the symptoms of Alzheimer's disease (AD) is impaired spatial memory. Ample evidence from previous research identified that a substrate for spatial memory can be the location-specific neuronal activities (place cells) in hippocampus. Place cells have been identified in subregions of hippocampus, including CA1 (Cornu Ammonis), CA3 and DG (Dentate Gyrus). A unique property of DG places cells is a stable spatial representation of the same place over days, whereas CA1 places cells dynamically remap on daily basis, suggesting that DG place cells could encode the stable components of spatial memory. However, DG place cells have not been studied in AD before and whether their impairment contribute to spatial memory dysfunction remains poorly understood. In this study, we performed single-cell calcium imaging of the granule cells in DG using a miniaturized microscope, while the mice ran through a linear track with water rewards at both ends. We imaged 5xFAD mice at the adult (6 mo) stage, as well as their age-matched litter-mate wild types. We found that 5xFAD mice showed impaired spatial representation in DG, including diminished number of place cells ($p = 0.048$) and a lower degree of spatial information ($p < 0.0001$). In addition, DG place cells in 5xFAD mice showed an increased probability of having multiple place fields. Importantly, we found that DG place cells in 5xFAD mice were less stable, as measured by inter-trial correlation of the neuronal activities. Furthermore, we developed a place cell emergence model using mutual inhibition among DG cells receiving inputs representing different spatial frequencies, and found that increased synchronized activities could result in the appearance of the specific abnormalities we observed in 5xFAD mice *in vivo*. Consistent with the model prediction, we observed more pronounced synchronized activity in 5xFAD mice. And eliminating correlated activity using a diagonal decoder increased the performance of predicting the animal's location using DG place cells in 5xFAD mice, indicating a detrimental effect of the synchronized activities for spatial representation. In conclusion, our results described a specific abnormality in the spatial representation of DG place cells in 5xFAD mice, and suggested that plaque-associated hyperactivities might give rise to this phenomenon.

Disclosures: S. Han: None. **L. Kou:** None. **P. Yuan:** None.

Poster

PSTR438. Place Cells: Mechanisms, Development, and Dysfunction

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR438.09/VV43

Topic: H.09. Spatial Navigation

Title: Impaired spatial encoding in transgenic A53T mice model of α-synucleinopathies

Authors: *M. AMIRI[1], D. SUN[2], C. FRENCH[1];
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Abstract: A key feature of hippocampal CA1 neurons is their ability to spatially tune their dynamics within the boundaries of the observable environment. Considerable studies showed Alzheimer's disease pathology (amyloid-β and tau) negatively affect spatial encoding and memory. However, very little is known about the functional effect of intraneuronal α-synuclein inclusions pathology on neural activity underlie spatial encoding in α-synucleinopathies such as Parkinson's disease and Lewy body dementia. Here, we examined the neural calcium activity of hippocampal place cells in A53T mice expressing the A53T mutant form of human alpha-synuclein and compared the results to those detected in non-transgenic wild-type (WT) littermates (n = 6 in each group).To visualise neural activity in the hippocampus, we labelled pyramidal neurons in CA1 with GCaMP6f adeno-associated virus, and a gradient refractive index (GRIN) lens was implanted to relay the signal. We recorded calcium activity from CA1 place cells using head-mounted miniaturized fluorescent microscope "miniscope", while mice ran back and forth along a linear track environment. We then evaluated the neural dynamics of CA1 place cells, calcium event rate and amplitude (ΔF/F), along with spatial tuning properties. This study is the first (to our knowledge) in vivo calcium imaging from hippocampal neural population in an α-synucleinopathies mice model. Here we report that, A53T model displayed disturbed neural calcium homeostasis as indicated by a significant increase in the calcium event amplitude (0.05926 ± 0.002948 dF/F; mean ± SEM; $p < 0.0001$; KS-test) and calcium event rate activity (0.1982 ± 0.003168 Hz; $p<0.0001$; KS-test) compared to WT littermate (0.04199 ± 0.001549 dF/F, 0.1568± 0.003246Hz). Furthermore, CA1 place cells in A53T mice were less spatially tuned, contain less spatial information, and had a sparser and less stable place field map than those in WT animals. Specifically, the stability of place field map was degraded progressively across three consecutive days exploring in a familiar environment in A53T mice, while it was remained more stable in WT genotype. Overall, our findings suggest that pathological accumulation of α-synuclein leads to significant hippocampal functional deficits in terms of intrinsic neuronal network activity related to spatial coding. These impairments are plausibly attributed to calcium dysregulation, which is a result of the loss of synaptic output capabilities associated with α-synuclein accumulation.

Disclosures: M. Amiri: None. **D. Sun:** None. **C. French:** None.

Poster

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Prince center

Title: Active experience, not time, determines within day representational drift in dorsal CA1

Authors: D. KHATIB[1], A. RATZON[1], M. SELLEVOLL[1], O. BARAK[1], G. MORRIS[2], ***D. DERDIKMAN**[1];
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Abstract: Memories of past events can be recalled long after the event, indicating stability. But new experiences are also integrated into existing memories, indicating plasticity. In the hippocampus, spatial representations are known to remain stable, but have also been shown to drift over long periods of time. We hypothesized that experience, more than the passage of time, is the driving force behind representational drift. We compared the within-day stability of place cells' representations in dorsal CA1 (dCA1) of the hippocampus of mice traversing two similar, familiar tracks for different durations. We found that the more time the animals spent actively traversing the environment, the greater the representational drift, regardless of the total elapsed time between visits. Our results suggest that spatial representation is a dynamic process, related to the ongoing experiences within a specific context, and is related to memory update rather than to passive forgetting.

Disclosures: D. Khatib: None. **A. Ratzon:** None. **M. Sellevoll:** None. **O. Barak:** None. **G. Morris:** None. **D. Derdikman:** None.

Poster

PSTR438. Place Cells: Mechanisms, Development, and Dysfunction

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Program #/Poster #: PSTR438.11/VV45

Topic: H.09. Spatial Navigation

Support: NIH Grant R01MH101297

Title: The influence of sensory experience and behavior on the representational drift in the hippocampus of mice

Authors: *J. Y. OH, J. R. CLIMER, H. DAVOUDI, D. A. DOMBECK;
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Abstract: Representational drift has been shown in many brain regions, including place cell ensembles in the primary output structure of the mouse hippocampus, CA1. The slow changes over days in the representation of space observed in this region have been proposed to play a role in distinguishing experiences over time and continual learning. However, it is also possible that representational drift occurs as a result of changes in behavior or the sensory environment that have not been accounted for in previous measurements due to the difficulty in measuring and/or controlling sensory modalities such as odors. To address these possibilities, we trained mice extensively for stereotyped behavior and also designed a multisensory virtual reality system that is capable of precisely and stably controlling the animal's visual and olfactory environment across weeks. Using longitudinal two-photon imaging of CA1 place cells in mice, we first observed representational drift in a highly familiar visual-only environment without odor control. This drift was similar in magnitude and time course to the drift observed in experiments in real environments. We are now repeating these experiments while controlling the odor landscape to determine whether this leads to a reduction in the magnitude of drift. Our preliminary results suggest that representational drift is still present in this highly controlled multisensory environment, though possibly to a lesser degree than without odor control. These findings suggest that while CA1 representational drift may largely come from internally generated processes, variability in the sensory environment may also contribute and should be considered in future experiments.

Disclosures: J.Y. Oh: None. **J.R. Climer:** None. **H. Davoudi:** None. **D.A. Dombeck:** None.

Poster

PSTR438. Place Cells: Mechanisms, Development, and Dysfunction

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Topic: H.09. Spatial Navigation

Support: Ministry of Education Tier 3 Research Fund (MOE2017-T3-1-002) and the National Medical Research Council (MOH-000962)

Title: Spatial memory in individual and populations of neurons of the non-human primate hippocampus

Authors: *H. TAN[1], J. Y. X. CHENG[2], T. P. Y. NG[1], C. OWENS[1], C. LIBEDINSKY[1,3], S.-C. YEN[1,4];
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Abstract: The non-human primate (NHP) hippocampus is known to encode location and heading, among other spatial variables. These are signalled exclusively, as well as in combination with each other, in single hippocampal neurons. With regards to location, neurons that signal occupied location (place cells), as well as viewed location (view cells), singly or jointly, have been found in some studies that did not control for the correlated sampling of the two variables. However, correlation of place and view is a common artefact arising from linear track environments or stereotyped movement patterns. Additionally, given that the visual field is highly dependent on the direction that the head is facing, head direction is also strongly correlated with viewed location. To resolve these dependencies, we sought to: 1) quantify mixed selectivity to place, view, and head direction; 2) assess the independence of spatial variables encoded by mixed selective cells, and their main and interaction effects; and 3) investigate how the signal from a population of neurons may support goal-directed navigation.
Among a preliminary sample of 32 hippocampal neurons analysed, 2 were mixed-selective to place, view, and head direction. Using a maximum likelihood approach to find the independent contributions of each variable, the cells retained mixed selectivity for all 3 variables. By conditioning the rate map of a second variable on the firing fields of a first variable, we found examples of non-linear conjunctive selectivity. Activity in the conditioned rate map within the firing field of the second variable exceeded the 95th percentile drawn from a pseudopopulation of locations of matching size outside the firing field. While cells selective to spatial variables were fewer than non-selective cells, they may not be the only contributors to accurate navigation. Decoding of population activity in a subset of 13 cells, including the 2 which were mixed-selective, showed that information gain on the goal identity was higher during the period of cue presentation, compared to during active navigation. This was true even of cells that did not show spatial selectivity.

Disclosures: H. Tan: None. **J.Y.X. Cheng:** None. **T.P.Y. Ng:** None. **C. Owens:** None. **C. Libedinsky:** None. **S. Yen:** None.

Poster

PSTR438. Place Cells: Mechanisms, Development, and Dysfunction

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Topic: H.09. Spatial Navigation

Support: National Science and Technology Innovation 2030 Major Program (2022ZD0205000)
Shanghai Municipal Science and Technology Major Project (2018SHZDZX05)

Title: Three dimensional place cells in the marmoset hippocampus and frontal cortex

Authors: *C. WEI, J. XI, C. LIU, C. XU;
Inst. of Neurosci., Shanghai, China

Abstract: One-dimensional or two-dimensional spatial modulated cells have been thoroughly examined in over various brain areas such as hippocampus, yet, three-dimensional place cells remain less unclear. Recent studies in bat and rat hippocampus[1-2] have found the 3D place cells, which were modulated by the environment and locomotion. We addressed this question in freely moving marmosets by wirelessly recording single-unit activities of the hippocampus and frontal cortex, up to 256 channels simultaneously. Place cells detected in both areas uniformly represent and cover the entire space, while being influenced by experience and conjunctively encoding with other spatial information: locomotion speed and head direction. These results demonstrate that both the hippocampus and frontal cortex process the volumetric spatial information while marmosets are continuously moving in 3D space. *Reference:[1] Yartsev, M. M., & Ulanovsky, N. (2013). Representation of three-dimensional space in the hippocampus of flying bats. Science (New York, N.Y.), 340(6130), 367-372.[2] Grieves, R. M., Jedidi-Ayoub, S., Mishchanchuk, K., Liu, A., Renaudineau, S., & Jeffery, K. J. (2020). The place-cell representation of volumetric space in rats. Nature communications, 11(1), 789.*

Disclosures: **C. Wei:** None. **J. Xi:** None. **C. Liu:** None. **C. Xu:** None.

Poster

PSTR438. Place Cells: Mechanisms, Development, and Dysfunction

Location: WCC Halls A-C

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Program #/Poster #: PSTR438.14/VV48

Topic: H.09. Spatial Navigation

Support: DFG LE2250/13-1

Title: Encoding 2D space with extrinsic and intrinsic theta sequences: A hippocampus model

Authors: *Y. YIU[1,3], C. LEIBOLD[1,2];
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Abstract: Recollection of past events relies on our ability to recall a sequence of memories. It is believed that the hippocampus supports the representation of memory sequences, as evidenced by the observation that the sequential firing of place cells at the theta timescale (6-12Hz) can encode the behavioral sequence of traversing multiple locations, known as the theta sequence. However, in a 2D space, an 1D location-sequence can assume any arbitrary shape, raising questions about how the sequences of spatial memories can be propagated by a 2D topology of place cells. We therefore theorize that theta sequences propagate along two types of 1D manifolds in a 2D space. One is the online running trajectory driven by the external sensorimotor drive of animal movement, while the other is the associative location-sequence driven by intrinsic network connectivity, inspired by the rich recurrent connectivity in the hippocampal formation.

In this study, we simulated a spiking neural network consisting of two layers of place cells in a 2D space: cornus ammonis 3 (CA3) and dentate gyrus (DG). The sequences representing the running trajectory can be extrinsically coordinated by sensorimotor drive of the animal's movement via short-term depression mechanism. This mechanism temporarily reduces the recurrent input into the place cells behind the animal, thereby propagating the spike sequences forward in the direction of travel. On top of the extrinsic sequences, intrinsically driven sequences are generated by recurrent connectivity between CA3 and DG with fixed synaptic strengths. These sequences follow the direction of the DG-CA3 projections, and thus, are independent of the running trajectory. The two-layer structure allows for simultaneous propagation of both extrinsic and intrinsic sequences. The former encodes the running trajectory, while for the latter, we demonstrated that intrinsic sequences could function as stable landmarks of neural activity for spatial memory. The spike correlations of the intrinsic sequences remain unchanged regardless of running directions, enabling reliable decoding of spatial locations based on spike timing patterns. The inclusion of intrinsic sequences thus expands the dimensions of spatial encoding, which would otherwise be limited to running trajectory alone. Furthermore, our simulation results align with the properties of the experimentally observed theta sequences in terms of directionality of spike phases and correlations. Our model offers a mechanistic explanation for how 2D space can be encoded by 1D sequences and sheds light on how intrinsic hippocampal circuits represent spatial memory.

Disclosures: **Y. Yiu:** None. **C. Leibold:** None.

Poster

PSTR438. Place Cells: Mechanisms, Development, and Dysfunction

Location: WCC Halls A-C

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Program #/Poster #: PSTR438.15/VV49

Topic: H.09. Spatial Navigation

Title: Explaining the variability of artificial place field induction in a naturalistic model of hippocampal place field formation

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[1]Neuroscience, Developmental and Regenerative Biol., [2]Dept. of Neuroscience, Developmental and Regenerative Biol., Univ. of Texas at San Antonio, San Antonio, TX

Abstract: Entorhinal grid cells have been implicated in generating hippocampal place fields. Theoretically, place fields can be formed if synaptic plasticity, gated by postsynaptic activation, strengthens co-active grid inputs and heterosynaptically weakens silent ones (Savelli & Knierim, J Neurophy 2010). Artificial place fields can indeed be induced in experimentally controlled locations following transient postsynaptic activation of hippocampal cells via juxtacellular or optogenetic stimulation (Diamantaki et al Curr Bio 2016, Cell Rep 2018, McKenzie et al Neuron 2021). We asked if naturally occurring and artificially induced place fields can be explained by the same model. We simulated 100 place cells during a previously recorded 20 min foraging

session of a rat in a 76 cm-diameter cylinder. The juxtacellular stimulation protocol was transiently applied to all cells mid-session. We excluded 24 cells that spontaneously showed high levels of pre-stimulation activity in the locations where the animal dwelled during the stimulation. We then spatially correlated these locations with the post-stimulation rate maps of the remaining 76 cells. The resulting correlation distribution was bimodal, with the high-correlation mode representing 26 cells that remapped their place fields near the stimulated locations, and the low-correlation mode representing cells that remapped elsewhere or did not remap. To further assess the net effect of the stimulation, we repeated the identical simulation without applying the stimulation protocol. We then correlated post-stimulation rate maps from the stimulated vs. unstimulated simulation. The resulting correlation distribution was also bimodal: half of the cells showed low correlation, indicating divergence between the stimulated and unstimulated simulations, whereas the other half showed higher correlation, indicating negligible divergence. To evaluate the persistence of the changes induced by the stimulation, we repeated this analysis using a later post-stimulation epoch. We found that 10 out of 38 low-correlation cells moved to the high-correlation group. Hence stimulation had a temporary effect on some of the remapping cells. Our model explains the artificial induction of place fields and its variable success in multiple experimental studies. Successful creation of a place field might depend on sufficient coverage of the stimulated locations by a combination of spatial inputs that can be strengthened to maintain the place field. If grid inputs vary from cell to cell, the same location could be viable for creating a place field in some cells but not in others.

Disclosures: S.T. Jones: None. **F. Savelli:** None.

Poster

PSTR438. Place Cells: Mechanisms, Development, and Dysfunction

Location: WCC Halls A-C

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Topic: H.09. Spatial Navigation

Support: German Research Foundation (DFG) 316803389 – SFB 1280, A14

Title: Investigating the Functional Significance of Spatial Representations in Navigation and Extinction Learning

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Abstract: Spatial navigation is crucial for the survival of animals. While there is substantial knowledge about neural representations of space, such as place cells (PC) and boundary cells (BC) in the hippocampus, their functional role in spatial navigation and learning has received limited attention. To address this open issue, we used closed-loop simulations of a spatial navigation task and extended a computational modeling toolchain for spiking neural networks.

The simulated task resembled the Morris watermaze, where an artificial agent had to find a hidden goal in an open-field environment. The model network consisted of PCs covering the environment, and BC representing the edges. The spiking activity of these cells was modeled as an inhomogeneous Poisson process and each PC or BC has its own firing field. This input was fed to 40 action selection neurons that each represents one direction of movement, distributed homogeneously across 360°. Therefore, the agent was able to move freely in any direction. If the agent enters the goal zone, learning is reinforced by potentiation of feedforward weights in a symmetric STDP learning rule with eligibility trace. As a result, the agent learned the path to the reward, i.e., it found the reward faster in later trials. (Ghazinouri et al. 2023). To differentiate between learning the most efficient path and the layout of the environment, we devised more complex tasks, including changing the starting point, moving the target (extinction learning), and implementing a T-Maze within an open field. In these tasks, we observed a nonmonotonic relationship between performance and a newly introduced variable called the overlap index. This index measures the overlap between neighboring place cells and reflects both place cell number and field size. In contrast, the Fisher information, which describes the informativeness of the place cell population about spatial location, best accounted for navigation performance in our model. We demonstrated that a symmetrical STDP learning rule performed better for simple tasks, while an asymmetrical learning rule was more effective for more sophisticated schemes. We conclude that efficiently encoding spatial information is crucial for navigation performance, as evidenced by the study's findings. Additionally, the research highlights the significance of synaptic depression in extinction learning. By investigating the effects of different learning rules and complex task variations, this study contributes to our understanding of how animals navigate their environment and adapt their behavior in response to changing circumstances.

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Poster

PSTR438. Place Cells: Mechanisms, Development, and Dysfunction

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Topic: H.09. Spatial Navigation

Support: MSCA IF (789962, European Commission)
Young Research Talents grant (#513401, Norwegian Research Council)

Title: Gain modulation of place tuning by vasoactive intestinal peptide-expressing inhibitory neurons

Authors: *N. LENKEY, A. C. GARVERT, M. NEUBRANDT, K. VERVAEKE;
Univ. of Oslo, Oslo, Norway

Abstract: Inhibitory neurons control how principal neurons (PNs) integrate excitatory synaptic inputs into output firing rates. By controlling distinct subcellular domains, inhibition enables PNs

to perform various input-output computations. Gain modulation (multiplicative/divisive modulation) is among these fundamental cortical computations enabling PNs to adapt to different sensory input strengths and maintaining input tuning selectivity. While in sensory cortices, inhibitory neurons that express vasoactive intestinal peptide (VIP) control the gain of visual and auditory responses by disinhibiting PN dendrites (1, 2), the role of VIP cells in modulating spatial tuning remains unexplored. To address this, we studied the modulation of place tuning by VIP cells in area CA1 of the hippocampus and in the retrosplenial cortex (RSC), a major hippocampal target, where place-tuned neurons have recently been discovered (3).We used two-photon microscopy and Ca2+ indicators in head-restrained mice performing a goal-oriented spatial task, where mice run on a linear track enriched by tactile cues. First, we recorded the activity of VIP cells in RSC using VIP-Cre transgenic mice and Cre-dependent GCamp6s virus. Our results show that locomotion either activates or inhibits VIP cells, confirming previous work in the hippocampus and other brain areas. Next, to test how VIP cells affect place tuning of PNs, we crossed VIP-Cre mice with Thy1-GCaMP6s mice and used optogenetics utilizing red-shifted opsins (Cre-dependent ChrimsonR and ArchT virus) to up- or downregulate VIP cell activity while simultaneously imaging the activity of PNs. In RSC, we found a robust modulation of place tuning. Optogenetic activation of VIP cells strongly increases place cell responses. In contrast, inhibition of VIP cells decreases place cell responses without altering spatial selectivity, suggesting a disinhibitory role for VIP cells in this cortical circuit. Further analysis of the tuning curves confirms that VIP cells exert a largely multiplicative/divisive gain modulation on PN activity. Finally, we used Bayesian decoding models to show that spatial information significantly decreases when VIP cells are inhibited and increases when VIP cells are activated in RSC. Ongoing experiments explore whether VIP cells play a similar role in the hippocampus. Altogether, these data demonstrate the ubiquitous role of VIP cells in controlling gain in cortical circuits and highlight a key contribution to spatial coding in the cortex.
References: (1) Pi HJ et al., Nature, 2013, 28;503(7477):521-4; (2) Zhang S et al., Science, 2014, 8;345(6197):660-5; (3) Mao D et al., Nat Commun., 2017, 15;8(1):243.

Disclosures: **N. Lenkey:** None. **A.C. Garvert:** None. **M. Neubrandt:** None. **K. Vervaeke:** None.

Poster

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Topic: H.09. Spatial Navigation

Support: BB/V001728/1

Title: Regulation of Place Cell Formation and Stability by OLM Inhibitory Interneurons in the Hippocampus

Authors: *M. UDAKIS, H. WEI ZHU, J. R. MELLOR;
Bristol Univ., Bristol, United Kingdom

Abstract: The hippocampus plays a crucial role in the processing of spatial information, underpinned by the formation of place cells that encode representations of space. The formation of place cells in the CA1 region depends on the synaptic plasticity of excitatory inputs from CA3, triggered by their coincident activity with entorhinal cortex layer III inputs via the temporoammonic (TA) pathway, leading to sustained dendritic depolarisations in the form of plateau potentials. However, the regulatory mechanisms underlying this plasticity process and its impact on place cells' stability is unclear. OLM interneurons selectively target distal dendritic regions where TA inputs are received, and their inhibitory synapses have been shown to undergo synaptic plasticity. Thus, we propose that OLM inhibitory interneurons by modulation of the impact of TA pathway inputs at both short and long timescales may play a role in regulating place cell formation and stability. To test this hypothesis, we employ a combination of in vitro and in vivo techniques, including optogenetics, to manipulate OLM interneuron activity and investigate their role in these processes. Using whole-cell patch clamp in mouse brain slices, we demonstrate that coincident activation of CA3 and TA inputs induces long-term potentiation (LTP) in CA3 but not in TA inputs, mimicking the plasticity observed during place cell formation. We find that OLM interneuron activation during plasticity induction inhibits CA3 LTP. Additional findings suggest this effect is mediated by OLM interneurons reducing dendritic non-linearities generated by stimulation of the TA pathway. To assess the impact of OLM activity on place cell stability, we utilise in vivo calcium imaging in mice to record CA1 place cell and OLM interneuron activity in familiar and novel environments. Miniscope experiments reveal that place cell representations remain stable across multiple sessions within a familiar environment but with a significant and well characterised drift, while global remapping occurs when exposed to novel environments. OLM interneuron activity was highly correlated with mouse velocity during exploration. To investigate the involvement of OLM interneurons in place cell remapping, we selectively activate these interneurons using optogenetics during environment exposures. We find enhancing OLM interneuron activity at specific regions of a familiar environment suppresses the expression of place cells without inducing remapping. Ongoing experiments aim to examine whether OLM interneuron activity is differentially regulated by exposure to novel environments and the impact this has on place cell remapping.

Disclosures: M. Udakis: None. **H. Wei Zhu:** None. **J.R. Mellor:** None.

Poster

PSTR438. Place Cells: Mechanisms, Development, and Dysfunction

Location: WCC Halls A-C

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Program #/Poster #: PSTR438.19/VV53

Topic: H.09. Spatial Navigation

Title: A Dual-input Firing Rate Model for CA1 Place Cell Phase Precession and Theta Sequences

Authors: Y. LU[1], A. FERNANDEZ-RUIZ[2], *J. RINZEL[3];
[1]Courant Inst. of Mathematical Sci., New York Univ., New York, NY; [2]Neurobio. and Behavior, Cornell Univ., Ithaca, NY; [3]New York Univ. Ctr. for Neural Sci., New York, NY

Abstract: Hippocampal place cells, that fire when an animal enters a specific location in the environment, are believed to be crucial for spatial memory and navigation. During locomotion, hippocampal network dynamics are dominated by the theta rhythm. Place cell firing exhibits phase precession - its firing shifts to earlier phases of theta oscillation as an animal traverse the place field of that cell. Despite numerous theories attempting to explain the generation of phase precession, the underlying mechanism remains elusive. In this study, we investigate a dual-input model (Fernández-Ruiz et al, 2017) as a potential mechanism for CA1 place cell phase precession; it argues that the interplay of the strength and phases of location/time-dependent inputs from CA3 and entorhinal (mEC) areas accounts for the phase advance of spikes in CA1 place cells with respect to the theta rhythm. We applied a mean-field firing rate model to implement this idea. Our model considers an ensemble of CA1 place cells with identical place fields. We model the mean-membrane potentials in three compartments - soma, proximal dendrites and distal dendrites, the firing rate at soma and the mean synaptic output over the cells. With increasing CA3 input innervating the proximal dendrites and decreasing mEC input innervating the distal dendrites, the place cell firing rate in our simulation shows a phase precession in a range consistent with experimental observations. Moreover, we demonstrated that the rate coding predominantly relies on CA3 inputs (Zutshi et al, 2022). Additionally, we studied the role of local inhibition in CA1 and reproduce the alteration of rate and phase shifts observed with optogenetic silencing of CA1 PV+ cells (Royer et al, 2022). Our computational model provides supporting evidence for the dual-input hypothesis. Further, we extended the dual-input model to account for theta sequence generation: place-specific cell ensembles represent spatial trajectories in a compressed manner within a single theta cycle. With the extended model, we replicated phase precession of the one-ensemble model and incorporated a unique place field for each ensemble. We propose a potential mechanism for theta sequence generation by coordinating these place cell ensembles with local inhibition in CA1.

Disclosures: Y. Lu: None. **A. Fernandez-Ruiz:** None. **J. Rinzel:** None.

Poster

PSTR438. Place Cells: Mechanisms, Development, and Dysfunction

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Program #/Poster #: PSTR438.20/VV54

Topic: H.09. Spatial Navigation

Title: Changes in ionic driving force at excitatory and inhibitory synapses in hippocampal place fields are counterbalanced by NMDARs.

Authors: *S. GRITZ[1], S. SENTHILKUMAR[1], A. D. MILSTEIN[2];
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Abstract: Place cells in hippocampal area CA1 exhibit spatially selective firing during spatial navigation. Underlying the increase in firing is a ~10 mV subthreshold membrane potential depolarization that in large part reflects an increase in the strength of excitatory synapses active within a cell's place field. Evidence suggests this increase in excitation arises during learning via long-term potentiation of AMPAR-mediated synaptic currents. However, as a place cell depolarizes, AMPARs lose ionic driving force as they approach their equilibrium potential (0 mV), while inhibitory GABARs increase driving force with increasing distance from their equilibrium potential (-70 mV). This predicts that even if AMPAR conductance increases in-field, and GABAR conductance stays equal at positions out-of-field and in-field, GABAR currents will increase in-field, while AMPAR currents will be blunted. However, recent in vivo experiments showed that optogenetic depolarization of place cells does not change the ratio of excitation to inhibition within a place field (Valero et al., Science, 2022). In that study, a simple model indicated that in order to explain the experimental data, synaptic inhibitory conductance would have to decrease dramatically from out-of-field positions to in-field. However, this model did not account for the presence of voltage-dependent NMDAR-type glutamate receptors at excitatory synapses in CA1 place cells. Here we examine the effects of NMDARs on the ratio of excitation to inhibition in hippocampal place cells and discuss its implications for the role of spatially biased synaptic inhibition in place field expression.

Disclosures: S. Gritz: None. **S. Senthilkumar:** None. **A.D. Milstein:** None.

Poster

PSTR439. Grid Cells and Spatially Modulated Cells

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR439.01/VV55

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Support: ERC Synergy Grant 951319
Solberg Research Fellowship
Centre of Neural Computation 223262
Centre for Algorithms in the Cortex 332640
Kavli Foundation
Ministry of Science and Education, Norway

Title: Efficient sampling of nearby space by entorhinal-hippocampal sweeps

Authors: *R. J. GARDNER, A. VOLLAN, M.-B. MOSER, E. MOSER;
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Abstract: Networks of entorhinal grid cells and hippocampal place cells generate anticipatory "sweep" trajectories during alert states, once per theta cycle (Vollan et al., same session). Each

sweep consists of a sequence of network activity representing an outgoing path, originating from the animal's present location and terminating in nearby space. The functional role of sweeps and their relationship to the external sensory environment have not been determined, however.
We used Neuropixels probes to record large-scale neural population activity in medial entorhinal cortex and hippocampus in freely moving rats. We then applied a latent-variable model (Wu et al. 2017) to the neural activity to infer the trajectories of sweeps. Like earlier studies (Johnson and Redish 2007, Kay et al., 2020), we found that sweeps alternately pointed to the animal's left and right side. However, while sweeps were formerly interpreted as expressions of navigational trajectories, we found that sweeps commonly point to unnavigable locations, outside opaque walls of an open-field arena and beyond edges of elevated running tracks. As well as spanning such unvisited locations in familiar environments, sweeps extended into unfamiliar space, raising questions about the function of sweeps during the first moments of exploration in a novel environment.
We hypothesized that sweeps, instead of representing possible behavioral paths, might serve to efficiently scan the entire environment. We therefore measured how quickly sweeps covered the binned 2D environment, and we compared this with the coverage rate of simulated sweep angles determined by various rules. We found that empirical sweeps covered space faster than all simulated strategies.
A biologically plausible strategy for maximizing environmental coverage is to maximize sampling of nearby space on a short time scale. We therefore asked what pattern of sampling directions would be chosen by an ideal agent tasked with sampling nearby space with optimal efficiency. Remarkably, the simulated agent chose alternating directions which closely resembled empirical sweep angles. Furthermore, in both the empirical data and in the simulation, the consistency of alternation was positively correlated with locomotion speed.
In conclusion, we find that sweep directions are suggestive of a neural algorithm for efficiently sampling nearby space.

Disclosures: **R.J. Gardner:** None. **A. Vollan:** None. **M. Moser:** None. **E. Moser:** None.

Poster

PSTR439. Grid Cells and Spatially Modulated Cells

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Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR439.02/VV56

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Centre of Neural Computation 223262
Centre for Algorithms in the Cortex 332640
Trond Mohn Foundation TMS2021TMT04
Kavli Foundation
Ministry of Science and Education, Norway

Eccellenza Grant PCEGP3_194220
ERC Starting Grant 850769

Title: Ultraslow periodic sequences of neural activity in the medial entorhinal cortex

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Abstract: Brain function emerges from the dynamic coordination of interconnected neurons, often in the form of neuronal oscillations. While such oscillations are mostly studied at subsecond time scales, we reported previously[1,2] that neurons in medial entorhinal cortex (MEC) can organize their activity into minute-scale oscillations that manifest as periodic sequences of activity across neurons in the population. These ultraslow periodic sequences, recorded with calcium indicators while mice ran in darkness under a benchtop two-photon microscope, entrained nearly the entire entorhinal cell population and transcended epochs of locomotion and immobility. It remained to be determined, however, whether the periodic sequences took the form of travelling waves, mirroring properties of rhythms at subsecond time scales. Moreover, the functional role of the ultraslow periodic sequences was not established, and it remained an open question whether the sequences were expressed in spiking activity or if, instead, they reflected some unknown dynamics unique to calcium imaging. Here we show that the periodic sequences do not share features of traveling waves, with the activity of participating cells not moving smoothly across anatomical space. By creating a simple model, we further illustrate the potential role of the periodic sequences in facilitating certain patterns of neuronal activation in downstream structures, such as ramping or stochastic activity, that unfold at behavioural time scales. Finally, by performing large scale Neuropixels recordings in head-fixed mice during self-paced running in darkness, we found that ultraslow oscillations and periodic sequences are present also in spike activity from electrophysiology data. The organization of MEC activity into ultraslow sequences may facilitate the encoding of experience at behavioral time scales.
References:
1. S. Gonzalo Cogno, H. Obenhaus, R. Jacobsen, F. Donato, M.-B. Moser, E. Moser, Minute-scale oscillatory sequences in medial entorhinal cortex. Program No. 408.02. 2022 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2022.
2. Gonzalo Cogno S., Obenhaus H.A., Jacobsen R.I., Donato F., Moser M-B., Moser E.I. (2022). Minute-scale oscillatory sequences in the medial entorhinal cortex. bioRxiv. doi: https://doi.org/10.1101/2022.05.02.490273.

Disclosures: S. Gonzalo Cogno: None. **A. Lautrup:** None. **H.A. Obenhaus:** None. **R.I. Jacobsen:** None. **S. Andersson:** None. **C. Clopath:** None. **F. Donato:** None. **M. Moser:** None. **E.I. Moser:** None.

Poster

PSTR439. Grid Cells and Spatially Modulated Cells

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Program #/Poster #: PSTR439.03/VV57

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Support: ERC Synergy Grant (Kiloneurons): 951319
Centre of Neural Computation 223262
Centre for Algorithms in the Cortex 332640
Kavli Foundation
Ministry of Science and Education, Norway

Title: A medial entorhinal microcircuit rhythmically scans nearby space

Authors: *A. VOLLAN, R. J. GARDNER, E. I. MOSER, M.-B. MOSER;
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Abstract: The navigational abilities of mammals rely on spatial maps represented by position-tuned neurons in the hippocampal region. Constructing and using spatial maps likely require a representation of locations in the animal's surroundings. While it is simple to read out an animal's self-location from place- and grid cell activity, it is unclear how these cells represent locations in the space around the animal. Here we used Neuropixels probes to record neural activity from 100s-1000s of cells in the parahippocampus of freely moving or resting rats to determine how cells in the brain's navigation circuit map nearby space.

We first describe a novel "internal" direction (ID) signal, expressed by a dedicated set of theta-rhythmic directional cells in parasubiculum (ID cells). During locomotion, ID typically pointed to either side of the animal (~45° relative to head direction) and flickered rhythmically from right to left on alternate theta cycles.

We reasoned that ID would be well suited to steer spatial representations in particular directions in nearby space. By decoding position from the joint activity of co-recorded grid cells, we found that grid cells express "sweeps" - rapid trajectories traveling outwards from the animal's location once per theta cycle, as previously reported in place cells (Johnson & Redish 2007, Kay et al 2020). Remarkably, the direction of sweeps closely matched ID. Individual grid modules expressed co-aligned sweeps with lengths proportional to the grid module spacing. Place cells also expressed ID-aligned sweeps but were delayed by ~20ms relative to grid cell sweeps. ID and sweeps persisted and remained coupled in the absence of sensory inputs during REM sleep. During slow-wave sleep, ID-aligned sweep-like trajectories could be identified but their rhythmic nature was lost.

Lastly, we searched for a microcircuit that can generate sweeps from directional input. To this end, we identified putative monosynaptic connections between ID cells, conjunctive grid-ID cells, and pure grid cells using temporal cross-correlation. We found that ID cells projected to grid-ID cells with similar preferred directions. Grid-ID cells projected to pure grid cells with a slightly shifted grid phase. The direction of translation between the two grids closely matched the preferred direction of the presynaptic grid-ID cell. The observed tuning relationships are in line with continuous attractor network models of grid cells where directional input translates a bump of activity on the toroidal grid cell manifold. Based on our findings, we hypothesize that sweeps facilitate navigation by scanning the animal's surroundings (see Gardner et al, same session).

Disclosures: **A. Vollan:** None. **R.J. Gardner:** None. **E.I. Moser:** None. **M. Moser:** None.

Poster

PSTR439. Grid Cells and Spatially Modulated Cells

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR439.04/VV58

Topic: H.09. Spatial Navigation

Support: Research Council of Norway (iMOD, NFR grant #325114
Department of Mathematical Sciences, NTNU
Ministry of Science and Education, Norway

Title: A topological approach for characterizing functional neural ensembles

Authors: ***E. HERMANSEN**[1], M. VAUPEL[1], D. KLINDT[2], B. DUNN[3];
[1]Norwegian Univ. of Sci. and Technol., Trondheim, Norway; [2]Stanford Univ., Stanford, CA;
[3]NTNU, Dept. of Mathematics, Trondheim, Norway

Abstract: Computations of the brain are thought to be performed in groups of neurons. However, defining such groups in large-scale recordings is challenging, in particular if the represented variable is not known. Here, the two main approaches in population analyses - studying neural correlations and population coding - are combined in a framework for finding neural ensembles and characterizing their function topologically. This exploits the complementary information found in the correlation structure and the neural state space and we reveal the topology in recordings of functional networks such as grid, head direction, place and orientationtuned cells, and expose a toroidal topology of ensemble activity during head-fixed wheel running in darkness [Obenhaus et al. 2022, Campbell et al, 2021]. Furthermore, we decode the internal dynamics and compare it to the external variable. The findings pave the way for studying mixed population recordings where the specificity of the cells is difficult to measure or unknown, either in simplistic experiments with high experimental control or during natural behaviors in heterogeneous environments.

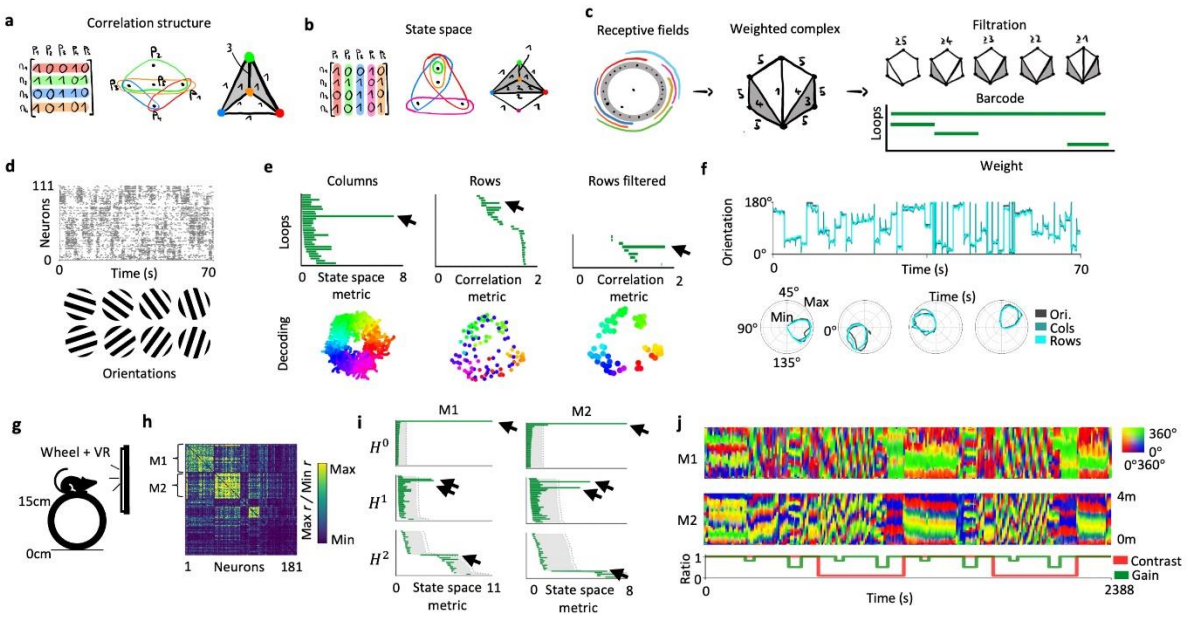

Figure 1: Topological analysis of neural ensemble representation. a/b. The correlation structure/state space (right) group the states/neurons (left, middle) containing the same neurons/states. c. Barcode (right) of the complex (middle) constructed from activity sampled from a circular representation (left). d. Spike trains (top) from V1 cells in a macaque monkey during presentation of drifting gratings (bottom) [Graf et al, 2011]. e. Barcodes and 3D projections of the columns, rows and filtered rows (single fields in h) of the activity in d, colored by the identified loops. f. Dynamics (top) and single-cell tuning (bottom) of recorded and decoded orientations. g. MEC recordings of head-fixed mice navigating a repeated VR-track. h. Clustered crosscorrelations, revealing ensembles M1 and M2. i. Barcodes suggesting toroidal topology. j. VR-positions (top) during gain/contrast manipulations (bottom), color-coded by internal states for M1 and M2.

Disclosures: **E. Hermansen:** None. **M. Vaupel:** None. **D. Klindt:** None. **B. Dunn:** None.

Poster

PSTR439. Grid Cells and Spatially Modulated Cells

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Support: ERC Synergy Grant 951319
Centre of Neural Computation 223262
Centre for Algorithms in the Cortex 332640

Kavli Foundation
Ministry of Science and Education, Norway

Title: Event structure sculpts lateral entorhinal dynamics

Authors: *B. R. KANTER, C. M. LYKKEN, M.-B. MOSER, E. I. MOSER;
NTNU, Trondheim, Norway

Abstract: Our experience of the world is a continuous stream of events, things occurring at a particular time and place. How this flow of experience is parsed and organized impacts our perception and memory of such events, yet the neural mechanisms underlying this process are largely unknown. The lateral entorhinal cortex may play a key role due its convergence of multisensory inputs, its encoding of events and their temporal relationships, and its connections with the hippocampal memory system. Here, we simultaneously recorded hundreds to thousands of neurons from lateral entorhinal cortex (LEC), medial entorhinal cortex (MEC), and area CA1 of the hippocampus in freely behaving adult male rats as we manipulated event structure. In the absence of defined events, population activity in LEC, but not MEC or CA1, continuously drifted along a one-dimensional manifold without reversing direction. This drift was caused by slow dynamics in the firing rates of LEC neurons over the course of minutes. After learning a task with a single recurring event, LEC dynamics were constrained to a stable state space trajectory defined by the event, but the drift was maintained along an orthogonal axis. In a more naturalistic task with hierarchical event structure, stable trajectories for each level of the hierarchy were simultaneously encoded along separate coding dimensions. Finally, event boundaries caused discrete jumps in state space that were mechanistically distinct from drift. Together, these results provide a dynamical systems explanation of how events are encoded within a temporal context.

Disclosures: **B.R. Kanter:** None. **C.M. Lykken:** None. **M. Moser:** None. **E.I. Moser:** None.

Poster

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Centre of Neural Computation 223262
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Ministry of Science and Education, Norway

Title: Development of toroidal topology in the grid cells network

Authors: *M. GUARDAMAGNA[1], E. HERMANSEN[1], J. CARPENTER[1], C. M. LYKKEN[1], B. DUNN[2], M.-B. MOSER[1], E. I. MOSER[1];
[1]Norwegian Univ. of Sci. and Technology, Kavli Inst. for Systems Neurosci., Trondheim, Norway; [2]Dept. of Mathematics, Norwegian Univ. of Sci. and Technol., Trondheim, Norway

Abstract: Grid cells are a fundamental anchor for the brain's spatial navigation system and provide a metric for the brain's spatial map. It has been recently discovered that at the network level these neurons organize on a toroidal manifold, irrespective of sensory inputs or behavioral states (Gardner et al., 2022), in agreement with continuous attractor theories for the formation and operation of grid cells. However, the developmental origin of the toroidal topology remains to be determined. Does it precede or follow the tuning of individual grid cells? Is the organization of the network learned from experience or is it experience-independent? We sought to study the developmental timeline of the torus, starting from the earliest indications of grid cell selectivity, and working our way back to postnatal day 10 (P10) - before pups can actively explore or open their eyes and before space is likely to be relevant for the animals. In the first set of experiments, we recorded the activity of thousands of medial entorhinal cells (LII and LIII) from P15, when rat pups start to explore outside of the nest (approximately two days after eye opening). Each experimental session consisted of a pre-sleep trial, followed by the exploration of a large arena (1x1m). Using cross-correlations between the firing activity of cell pairs to identify clusters and characterize the topological structure with persistent cohomology (Hermansen et al., 2022), we were able to find evidence of toroidal manifolds from P15, before any stable periodic tuning of individual grid cells. The torus exhibited adult-like characteristics from the first exposure to the experimental room, irrespective of the behavioral state (sleep or arena exploration). From P16-17 onward, we observed the gradual emergence of individual grid cells in the cell population from which the toroidal manifold was initially detected. Multiple grid modules could be identified in the population activity as early as P15. In a second round of experiments, we characterized the topology of medial entorhinal neurons at even earlier developmental stages. Starting from P10 - an age when eyes and ear canals are closed, and pups do not move much - we recorded up to 1000 cells in LII and LIII of the medial entorhinal cortex, while pups slept in a heat-controlled pot. We were able to consistently uncover clear toroidal topology, with stable tuning, as early as P11 (preceding many major developmental milestones) and we are currently exploring whether similar structure is present at P10. Our results reveal that spatial mapping has strong experience-independent components, rooted in the early postnatal organization of medial entorhinal cortex circuitry.

Disclosures: M. Guardamagna: None. **E. Hermansen:** None. **J. Carpenter:** None. **C.M. Lykken:** None. **B. Dunn:** None. **M. Moser:** None. **E.I. Moser:** None.

Poster

PSTR439. Grid Cells and Spatially Modulated Cells

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Topic: H.09. Spatial Navigation

Support: ERC Synergy Gran
RCN FRIPRO Grant
Centre of Neural Computation
Centre for Algorithms in the Cortex
Kavli Foundation
Ministry of Science and Education, Norway

Title: grid cells have a uniform phase distributions - in physical space and in the internal population state space

Authors: *V. A. NORMAND[1], E. HERMANSEN[2], T. WAAGA[3], R. GARDNER[4], B. DUNN[5], Y. ROUDI[4], E. I. MOSER[6], M.-B. MOSER[7];
[1]Kavli Inst. for Syst. Neurosci., Trondheim, Norway; [2]Dept. of Mathematical Sci., Norwegian Univ. of Sci. and Technol., Trondheim, Norway; [3]Kavli Inst. For Systems Neurosci. / CNC, Kavli Inst. For Systems Neurosci. / CNC, Trondheim, Norway; [4]Kavli Inst. For Systems Neurosci., Trondheim, Norway; [5]NTNU, Dept. of Mathematics, Trondheim, Norway; [6]Kavli Inst. Systems Neurosci., [7]Kavli Inst. Systems Neurosci, Trondheim, Norway

Abstract: Grid cells in the medial entorhinal cortex have spatial firing fields patterned in a hexagonal grid. These cells are organized into modules, defined by a common grid spacing and orientation. Within each module, the firing patterns of the cells are spatially offset from one another, i.e., every grid cell has a distinct phase. However, the extent of phase variation within grid modules is unknown. While some computational models of grid cells, such as continuous attractor networks (CANs), assume a uniform phase distribution, others, including feed-forward models, can function without this requirement and exhibit clustering of grid phases. Here, we analysed the phase dispersion of several hundreds of grid cells recorded simultaneously from a range of grid modules while rats were foraging in a square environment of up to 4x4 meters. We assessed the similarity of observed phase distributions to random uniform and non-uniform distributions using statistical tools including Ripley's L-function, SVMs, persistence homology and likelihood ratios. Accounting for the number of recorded neurons and phase estimation uncertainties, our analyses revealed that the phase distributions of most modules did not significantly differ from uniform distributions. Yet, some extreme cases such as very small spacing cells in a large environment showed significantly clustered phase patterns. Additionally, by projecting phases onto a torus using topological data analysis, we found that the spatial phase distributions matched that of the torus. These observations are in line with a key assumption of CAN models, but also suggest that for grid spacings that are small compared to size of the environment, other processes, similar to those of feedforward models, might be at play.

Disclosures: V.A. Normand: None. **E. Hermansen:** None. **T. Waaga:** None. **R. Gardner:** None. **B. Dunn:** None. **Y. Roudi:** None. **E.I. Moser:** None. **M. Moser:** None.

Poster

PSTR439. Grid Cells and Spatially Modulated Cells

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Topic: H.09. Spatial Navigation

Support: ERC Synergy Grant 951319
Ministry of Science and Education, Norway
Centre of Neural Computation 223262
Centre for Algorithms in the Cortex 332640
Kavli Foundation

Title: Independent realignment of grid cell modules during hippocampal remapping

Authors: *C. M. LYKKEN, B. R. KANTER, A. NAGELHUS, M. GUARDAMAGNA, E. I. MOSER, M.-B. MOSER;
NTNU, Trondheim, Norway

Abstract: Grid cells in the medial entorhinal cortex (MEC) create a universal metric of space that may be a basis for environment-specific maps and memories in the hippocampus. Grid cells are organized into discrete modules: All cells within a module operate coherently, but it is thought that different modules could, under some circumstances, operate independently of each other. This independence may underlie the generation of unique hippocampal maps for each environment. However, experimental evidence for discordant responses among grid modules is limited, and it has never been shown in conjunction with place cell remapping. To test whether grid modules realign independently during place cell remapping between distinct environments, we chronically implanted Neuropixels probes in MEC and hippocampus in rats to simultaneously record multiple grid modules and place cells. We found that place cells exhibited robust and stable global remapping between rooms. There were only slight differences in rotation between grid modules, and their rotation was largely coherent with border and head direction cells. Despite this, each grid module shifted independently between rooms. The difference in the translation of grid modules was large, and the distance between them was similar to what is expected by chance. Further, this differential translation of grid modules was sufficient to produce remapping in a grid-to-place cell model. These findings are the first experimental evidence demonstrating that functionally independent grid cell modules may underlie hippocampal remapping.

Disclosures: C.M. Lykken: None. **B.R. Kanter:** None. **A. Nagelhus:** None. **M. Guardamagna:** None. **E.I. Moser:** None. **M. Moser:** None.

Poster

PSTR439. Grid Cells and Spatially Modulated Cells

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Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR439.09/VV63

Topic: H.09. Spatial Navigation

Support: NIH NINDS R00NS116129

Title: Investigating the septo-entorhinal cortex circuitry supporting spatial coding by grid cells.

Authors: *J. HERNÁNDEZ, B. GUTIERREZ-GUZMAN, H. DANNENBERG;
George Mason Univ., Fairfax, VA

Abstract: The septo-hippocampal circuitry, including the medial septum (MS), the hippocampus (HPC), and medial entorhinal cortex (MEC), provides a neural substrate for episodic memory and spatial navigation. Grid cells in the MEC, a functionally defined cell type that tessellating the environment by firing at multiple locations in space that fall on the vertices of equilateral triangles, have been hypothesized to serve path integration and memory-guided navigation. However, the circuit mechanisms that generate spatial periodicity in grid cell firing remain elusive. Previous experimental data using pharmacological inactivation of the medial septum (MS) demonstrate that a combination of theta (6 - 10 Hz)-rhythmic GABAergic, glutamatergic, or cholinergic input from septo-hippocampal projection neurons is essential for spatial periodicity in grid cell firing. However, how the three main cell types in the MS act synergistically to enable spatial periodicity in grid cell firing remains elusive. Furthermore, little is known about the time course of reduction in grid cell firing after inactivation of MS inputs. We here use temporally precise optogenetic inactivation of choline acetyltransferase (ChAT)+ cholinergic MS neurons, parvalbumin (PV)+ GABAergic MS neurons, and the whole MS in freely behaving mice to test the hypothesis that cholinergic modulation of weak theta-rhythmic input can rescue neural dynamics in the MEC and spatial periodicity in grid cell firing. Our preliminary results show that cell-type-specific silencing of individual populations of cholinergic or GABAergic MS neurons does not disrupt spatial periodicity in grid cell firing as observed with pharmacological inactivation of the whole MS. Our results will contribute to understanding the contribution of individual cell types in the MS and their interactions to spatial coding by grid cells in the MEC and to understanding the temporal dynamics in grid cell firing as a function of medial septal inputs.

Disclosures: J. Hernández: None. **B. Gutierrez-Guzman:** None. **H. Dannenberg:** None.

Poster

PSTR439. Grid Cells and Spatially Modulated Cells

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR439.10/VV64

Topic: H.09. Spatial Navigation

Support: NIH NINDS R00NS116129
NIH NINDS R01NS39600

Title: A neural sequence code of 2D trajectories explains spatial periodicity in grid cell firing

Authors: R. R.G.[1], G. A. ASCOLI[2], ***H. DANNENBERG**[2];
[1]Mathematical Sci., [2]Bioengineering, George Mason Univ., Fairfax, VA

Abstract: Grid cells, first discovered in the medial entorhinal cortex of freely foraging rats, fire at multiple locations in space that fall on a hexagonal lattice. The spatial periodicity in grid cell firing has been interpreted as a neural metric for space providing animals with a global coordinate system in navigating physical and mental spaces. However, recent experimental data have challenged this view of a global metric in favor of a more local computational function of grid cells. Moreover, the specific computational problem being solved by grid cells and how local computations result in the experimentally observed global firing pattern has remained largely elusive. We here provide mathematical proof that spatial periodicity in grid cell firing is implied by four simple axioms about a neural sequence code of trajectories in 2D space and that the hexagonal firing pattern of grid cells emerges as the most parsimonious solution to such a sequence code. We thereby provide a teleological cause for the existence of grid cells and reveal the underlying nature of the global geometrical organization in grid maps as a direct consequence of a simple local sequence code using a minimal number of neurons. Our results show that sequence coding of trajectories by grid cells provides a mechanistic explanation for how grid cells serve path integration. Moreover, a theory of sequence coding of trajectories by grid cells predicts and provides an intuitive explanation for many previously puzzling experimental observations of grid cell properties such as the rotation of grid maps against the walls of rectangular environments, the fragmentation of grid maps in 1D space, and rescaling of grid maps in response to changes in the environment. A neural sequence code of trajectories unifies two major theories in neuroscience, namely that cell sequences provide a neural syntax and that grid cells serve path integration, into one simple and intuitive theoretical framework.

Disclosures: R. R.g.: None. **G.A. Ascoli:** None. **H. Dannenberg:** None.

Poster

PSTR439. Grid Cells and Spatially Modulated Cells

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR439.11/WW1

Topic: H.09. Spatial Navigation

Support: NIH NINDS R00NS116129

Title: Cholinergic dynamics supporting exploratory behavior, learning, and recall of hippocampus-dependent memories

Authors: *F. FAROKHI MOGHADAM, B. GUTIERREZ GUZMAN, X. ZHENG, H. DANNENBERG;
George Mason Univ., Fairfax, VA

Abstract: In the hippocampal formation, cholinergic modulation supports the formation of spatial memories and cognitive functions that enable memory-guided navigation. The major

source of cholinergic innervation to the hippocampus is originated from the medial septum/diagonal band of Broca (MSDB). In particular, cholinergic dynamics have been hypothesized to facilitate changes in brain states supporting either learning or recall of spatial memories. However, recent experimental data from mice support an alternative hypothesis, namely that cholinergic dynamics are a function of movement activity. We therefore recorded and quantified cholinergic dynamics in freely behaving mice and tested the alternative hypotheses that cholinergic dynamics in the septo-hippocampal circuitry are primarily explained either by i) the speed of animal movement, ii) behavioral activities such as grooming or rearing, iii) hippocampus-dependent learning or recall of spatial memories, or a combination of these factors. We used a fiber photometry approach to monitor cholinergic dynamics on a sub-second timescale in freely behaving mice. In detail, we targeted Cre-dependent expression of a genetically encoded Calcium sensor (GCaMP) to choline acetyltransferase (ChAT)-positive septo-hippocampal projection neurons in ChAT-Cre mice. We monitored population activity of cholinergic projection neurons via a chronically implanted optical fiber targeting the MSDB. To quantify cholinergic dynamics as a function of hippocampus-dependent learning and recall, we recorded cholinergic activity in mice performing an object location memory task. Mice were video-tracked, and pose and exploratory behavioral activities of mice during the task were analyzed using deep learning approaches. Consistent with previous data, our results show that cholinergic activity increases as a function of movement speed and transiently increases during rearing. Preliminary data show a trend of higher cholinergic activity when the mouse is exploring the object at the novel location after correcting for the effects of movement speed and rearing. This finding is consistent with the hypothesis that acetylcholine supports the encoding of novel information. Quantifying temporal dynamics in cholinergic modulation will allow us to test long-standing hypotheses of cholinergic modulation of memory function. Furthermore, understanding the neurophysiology of cholinergic dynamics will help us understand and develop novel hypotheses on how changes in temporal dynamics in cholinergic modulation contribute to disease pathology in neurological disorders with cholinergic dysfunction.

Disclosures: F. Farokhi Moghadam: None. **B. Gutierrez Guzman:** None. **X. Zheng:** None. **H. Dannenberg:** None.

Poster

PSTR439. Grid Cells and Spatially Modulated Cells

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR439.12/WW2

Topic: H.09. Spatial Navigation

Support: NIH/NINDS Intramural Research Program

Title: A spatially consistent map in the medial entorhinal cortex is necessary for spatial memory

Authors: Y. MA[1], ***T. MALONE**[2], N.-W. TIEN[4], G. WANG[3], K. ZHANG[3], M. V. MYROSHNYCHENKO[5], J. TYAN[3], J. A. GORDON[6], D. A. KUPFERSCHMIDT[7], Y. GU[8];

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Abstract: The medial entorhinal cortex (MEC) is crucial for spatial memory. It is also a major component of the hippocampal-entorhinal circuit that is hypothesized to form a “cognitive map” for memory-guided navigation. However, little is known about how the cognitive map in the MEC develops during learning and influences memory. By imaging MEC calcium dynamics while mice learned to pursue water rewards in a novel virtual environment for ten days, we discovered that during successful learning (good performers), the overall dynamics of the MEC map gradually became more spatially consistent and then stabilized. In contrast, mice with unsuccessful learning (poor performers) showed an MEC map with low spatial consistency that did not improve during learning. The largest difference in spatial activity consistency between the good and poor performers existed at cues before the reward and immediately before the reward, suggesting that modulating the MEC map consistency in these areas could affect reward-predictive behavior after spatial memory has already been established. To test this hypothesis, we optogenetically modulated the spatial consistency of the MEC map while mice pursued water rewards in a well-learned environment. In the good performers, stimulating the MEC at locations randomly distributed along the track prior to the reward impaired reward-predictive behavior, whereas mimicking the consistent activity at cues prior to the reward did not alter the behavior. In the poor performers, consistent optogenetic stimulation immediately before the reward improved reward-predictive behavior. This effect remained true when consistent stimulations just before the reward and at cues prior to the reward were combined, but not when the stimulation was only applied to the cue locations. Together, these results indicate that specifically reducing the spatial consistency of the MEC map interfered with spatial memory. In addition, consistent activity before the reward directly drives reward-predictive behavior, whereas consistent activity at cues is important for the map and supports reward-predictive behavior. In summary, our findings reveal the establishment of a spatially consistent cognitive map in the MEC during successful learning and indicate that the map is not simply passively shaped by upstream inputs, but that spatial memory is determined by the MEC map.

Disclosures: Y. Ma: None. **T. Malone:** None. **N. Tien:** None. **G. Wang:** None. **K. Zhang:** None. **M.V. Myroshnychenko:** None. **J. Tyan:** None. **J.A. Gordon:** None. **D.A. Kupferschmidt:** None. **Y. Gu:** None.

Poster

PSTR439. Grid Cells and Spatially Modulated Cells

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR439.13/WW3

Topic: H.09. Spatial Navigation

Support: NIH/NINDS Intramural Research Program

Title: Auditory spatial information shapes the MEC cognitive map during navigation.

Authors: *D. NGUYEN, G. WANG, Y. GU;
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Abstract: Navigation within laboratory settings is mainly studied through visual modality. But navigation in the natural world often requires the employment of multisensory information, for example: auditory information helps pry animals detect predator's movement so that they can escape from danger; olfactory information leads animals to food sources. Here we focus on auditory spatial information: how they are encoded in the brain during navigation and how this encoding is compared to that of visual information, which dominates the navigation of many animals. We investigated these questions in the microcircuit of the medial entorhinal cortex (MEC), a brain area that is heavily involved in spatial navigation. Calcium dynamics of the MEC were measured using two-photon imaging when mice navigated virtual linear tracks, where auditory and visual spatial cues could be flexibly controlled. We established an auditory virtual reality (AVR), in which mice only relied on auditory cues to determine water reward locations. During AVR navigation, MEC specifically encoded the most task-relevant auditory cues that were associated with reward location. This encoding developed with experience and was specifically associated with the successful determination of the reward. We further combined AVR with visual VR and found that in environments with only auditory or visual cues, which delivered equivalent spatial information, the MEC recruited separate cell populations to form different cognitive maps. Finally, when auditory and visual cues were both present in the same environment, the MEC used separate cell populations to encode auditory and visual cues. In summary, our study demonstrates that auditory spatial information shapes the MEC map, which is different from that of visual spatial information, indicating the MEC's capability to distinguish spatial cues in different sensory modalities.

Disclosures: D. Nguyen: None. **G. Wang:** None. **Y. Gu:** None.

Poster

PSTR439. Grid Cells and Spatially Modulated Cells

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR439.14/WW4

Topic: H.09. Spatial Navigation

Support: KVPY, Department of Science and Technology, Government of India
UGC Senior Research Fellowship, Government of India

Title: Stochastic resonance enhances signal propagation and velocity integration in a model grid cell network.

Authors: *D. GUPTA, I. SHAIKH, C. ASSISI;
IISER, Pune, India

Abstract: Grid cells in the MEC possess spatially periodic receptive fields that form a hexagonal lattice that tiles space. They maintain a constant orientation and phase relative to one another and are prime candidates for a neural coordinate system to map trajectories through space. Grid cell receptive fields remain stable despite sensory input noise, fluctuations in neuronal excitability, and the stochasticity of ion channel dynamics. In this study we use a biophysically realistic continuous attractor network model of grid cells to examine how it overcomes the deleterious effects of noise and may even use noise to form a robust spatial representation.
The network was built by connecting a simple network motif consisting of conductance based models of excitatory stellate cells and inhibitory interneurons. This motif behaved as a bistable system - an external perturbation was required to switch the activity of neurons. When we drove the bistable system using a 10 Hz oscillatory drive, akin to the theta drive from the medial septum, we found that specific levels of noise were required to cause periodic switching between the states of the network. Thus, the presence of noise could improve the system's response to weak or subthreshold oscillatory inputs, a phenomenon known as stochastic resonance. To construct a one dimensional attractor network, we connected the motifs into a ring. We introduced an asymmetry in the network such that the activity propagated in one direction when the stellate cells were stimulated. As before, interneurons received an oscillatory drive, and an additive noisy current. We found that the activity reliably propagated along the ring only at specific noise levels. We extended this network to a multi-bump attractor network to reproduce periodic grid fields. We found, this network could integrate 1D velocity inputs. However, the accuracy of the decoded trajectory peaked at non-zero levels of noise.
Our results suggest that the MEC network might rely on endogenously generated noise to uncover weak signals and maintain robust representations of spatial trajectories using stochastic resonance.

Disclosures: D. Gupta: None. **I. Shaikh:** None. **C. Assisi:** None.

Poster

PSTR439. Grid Cells and Spatially Modulated Cells

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR439.15/WW5

Topic: H.09. Spatial Navigation

Support: UGC Senior Research Fellowship, Government of India

Title: Discrete modules emerge in a biophysically realistic grid cell network

Authors: I. SHAIKH, **S. NABAR**, *C. ASSISI;
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Abstract: Continuous attractor network (CAN) models with radially symmetric local excitatory connectivity and long-range inhibition generate hexagonal firing patterns that resemble the receptive fields of grid cells in the medial entorhinal cortex (MEC). Local excitation promotes the formation of activity clusters, while long-range inhibition prevents the spread of excitation beyond a specific range, resulting in a hexagonal grid-like structure. The distance between the vertices of this grid is determined by the extent of the inhibitory surround. In the MEC, grid cell spacing is not uniform along the dorsoventral (DV) axis, ranging from approximately 40 cm at the dorsal end to nearly 100 cm at the ventral end. Moreover, grid spacing changes in discrete steps forming distinct grid cell modules along the DV axis. Grid scales exhibit a geometric progression, with the ratio between successive scales falling between 1.4 and 1.7. CANs have effectively simulated this step-like progression and further refined the sequence of scale ratios. By gradually altering the spatial extent of inhibition along the DV axis in CANs, discrete jumps in spatial frequency occur, closely matching experimentally observed values. CANs typically consist of rate-based neurons that operate as linear dynamical systems with interactions following simple sigmoidal or rectification nonlinearities. In this study, we investigate the emergence of grid modules within a one-dimensional biophysically realistic network model composed of conductance-based stellate cells and inhibitory interneurons. All interactions between stellate cells are mediated by inhibitory interneurons. The interneurons mutually inhibit each other. Our findings recapitulate some of the results seen in rate-based CAN models - as the spatial extent of inhibitory interactions is continuously varied, grid cells segregate into discrete modules. The spatial scale associated with each module exhibits the same geometric progression observed in experimental studies. Gradients along the dorsoventral axis in the MEC are not restricted to the spatial extent of inhibition. Systematic changes in the frequency of subthreshold oscillation and resonance, input resistance, membrane time constants and synaptic integration properties of stellate cells are seen. These gradients are mainly attributed to changes in HCN channel conductance and a potassium leak current. Unlike rate-based CAN models, our model allows us to manipulate each of these parameters and explore their impact on the observed modularity in the network.

Disclosures: **I. Shaikh:** None. **S. Nabar:** None. **C. Assisi:** None.

Poster

PSTR439. Grid Cells and Spatially Modulated Cells

Location: WCC Halls A-C

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Program #/Poster #: PSTR439.16/WW6

Topic: H.09. Spatial Navigation

Support: UGC - Senior Research Fellowship, Government of India

Title: Subthreshold dynamics shape grid cell activity in a biophysically detailed continuous attractor model

Authors: *I. SHAIKH, C. ASSISI;
IISER, Pune, India

Abstract: Grid cells in the Medial Entorhinal Cortex (MEC) fire when the animal is at the vertices of spatially periodic hexagonal lattices, which tile the two-dimensional range of the animal. Continuous Attractor Network (CAN) models explain how this pattern can emerge due to network interactions. Aside from a few exceptions, most models use rate-based neurons which are usually linear dynamical systems with interactions that follow a simple sigmoidal or rectification nonlinearity. Spiking CAN models typically consist of integrate-and-fire neurons. However, in contrast to these models, stellate cells in the MEC, putative grid cells, display a rich dynamical repertoire showing post-inhibitory rebound, depolarization sag, subthreshold oscillations, and resonant responses. Furthermore, several of these properties vary systematically along the dorsoventral axis of the MEC. It is unclear how these properties can contribute to the emergence of grid cell receptive fields.
We developed a CAN model of a grid cell network consisting of conductance-based stellate cells and interneurons. In addition to currents that contribute to spike generation, stellate cells have hyperpolarization-activated depolarizing currents (HCN) and a persistent sodium current. Inhibitory Interneurons receive a sinusoidal drive to model the theta drive from the medial septum. Stellate cells interacted only via an inhibitory intermediary, while inhibitory interneurons mutually inhibited each other and the stellate cells. We found that a center-surround connectivity led to periodic spatial receptive fields in one dimension and hexagonal fields in two dimensions. To track the movement of an animal, we introduced directional asymmetries.Our model was able to integrate complex trajectories accurately over several seconds and gradually drifted when external inputs were absent. Stellate cells in our model exhibit a depolarizing ramp, similar to that observed in grid cells when an animal first enters a receptive field. In the absence of lateral excitation from stellates, this ramp can be explained by inhibitory modulation in our model. We found that the first spike generated by a grid cell when the animal enters its grid field is a rebound spike, which occurs at a preferred phase of the theta oscillation. HCN knockout experiments show a clear expansion of grid scale and grid field sizes. Our model network replicates this observation. As expected from experiments, theta phase precession persists in absence of HCN. To our knowledge, this is the first biophysically detailed grid cell attractor model that uses realistic membrane currents to reproduce dynamics observed in experiments.

Disclosures: I. Shaikh: None. **C. Assisi:** None.

Poster

PSTR439. Grid Cells and Spatially Modulated Cells

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR439.17/WW7

Topic: H.09. Spatial Navigation

Title: Local activity principle as a normative theory on the emergence of grid cells

Authors: *M. S. GUINTO, J. YOSHIMOTO;
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Abstract: Grid cells, which were first discovered in the entorhinal cortex of mammals, are normatively thought of as instrumental in spatial navigation because of their periodic firing characteristics as the animal traverses its surroundings. In recent years, converging evidence from etiological and teleological investigations of hexagonal grid cells indicate that they might arise from optimally efficient coding schemes, with several metabolic, sparsity, and nonnegativity constraints supporting the formation of regular lattices that approximate what has been observed in animal experiments. Here we introduce a normative theory that suggests how grid-like representations can also be seen as a consequence of the local activity principle, a circuit-theoretic concept introduced by Leon Chua in 1998. Past studies on the local activity principle, for which explicit mathematical criteria have been laid out, have shown deep connections with Turing's work on morphogenesis and provided a formal, quantitative characterization of Prirogine's "instability of the homogeneous." We demonstrate how the so-called "edge of chaos," a subset within the locally active regime, in state space (as opposed to anatomical space) contributes to the emergence of grid representations. By focusing on the importance of confined spheres of influence for efficient computation, this work supplements existing approaches that address the challenge of understanding the ontology and possible universality of grid cells in the cortex.

Disclosures: M.S. Guinto: None. **J. Yoshimoto:** None.

Poster

PSTR439. Grid Cells and Spatially Modulated Cells

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Topic: H.09. Spatial Navigation

Support: JSPS KAKENHI 23H02595
JSPS KAKENHI 20J01255
JSPS KAKENHI 23K14231

Title: Grid code for future spatial information in the entorhinal cortex

Authors: *A. OUCHI, S. FUJISAWA;
RIKEN, Saitama, Japan

Abstract: Although grid cells in the entorhinal cortex play fundamental roles in spatial navigation, how the entorhinal grid system support for planning a future traveling path is still to be elucidated. Here, we investigated the neuronal representation of current and future spatial information in the entorhinal cortex and hippocampal CA1, performing large-scale extracellular recordings from these brain areas of rats moving toward a destination in the square arena. In the entorhinal cortex, we found a subset of cells that have a grid representation of the space shifted

toward the traveling direction; firing rate maps of these cells did not show gridness based on current animal locations, but they displayed grid presentation when rate maps are estimated based on future animal locations. Furthermore, these travel-direction-shifted grid cells were firmly phase modulated by theta oscillations (5-12Hz), and the phase preferences of these cells differed from traditional grid cells in layers 2, 3, and 5. We hypothesize that these travel-direction-shifted grid cells underlie the mechanisms of spatial navigation for future travels in the network of the entorhinal cortical and hippocampus.

Disclosures: A. Ouchi: None. **S. Fujisawa:** None.

Poster

PSTR439. Grid Cells and Spatially Modulated Cells

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Topic: H.09. Spatial Navigation

Support: DFG Individual Research Grant (AL 1730/3-1)
DFG Collaborative Research Centres (SFB-1436, Project-ID 425899996)

Title: Grid cells activity in a homing task requiring path integration

Authors: *J. PENG[1,2], B. THROM[1,2], T.-Y. YEN[1,2], M. NAJAFIAN JAZI[1,2], H. MONYER[1,2], A. KEVIN[1,2];
[1]Dept. of Clin. Neurobio., Heidelberg Univ., Heidelberg, Germany; [2]German Cancer Res. Ctr. (DKFZ), Heidelberg, Germany

Abstract: Converging evidence from experiments in both rodents and humans suggests that grid cells contribute to path-integration-dependent navigation. How grid cell activity might contribute to path-integration-dependent behavior has remained to be determined as grid cell activity has been studied mostly during random foraging or other behavioral paradigms with limited navigational demands. Here we recorded grid cell activity in freely moving mice performing a path integration task (AutoPI task) in which the mouse searched for a lever on an arena and returned to the home base to collect a reward. Surprisingly, grid cells did not have a stable periodic firing pattern during the task. Using a combination of single-cell analysis and grid-module-level decoding techniques, we found that error accumulated in the phase and orientation of the grid representation when mice navigated in darkness. Moreover, we often observed a translation of the grid pattern within trials, whereby the grid representation went from being anchored to the room reference frame to being anchored to a task-relevant, moving object. Despite this re-anchoring which caused discontinuity in integration of self-motion information by grid cells, we found that error accumulation in the orientation of the grid cell pattern predicted the homing direction of the mouse. Our findings provide novel insights into the function of grid cells in path-integration-dependent behavior.

Disclosures: **J. Peng:** None. **B. Throm:** None. **T. Yen:** None. **M. Najafian Jazi:** None. **H. Monyer:** None. **A. Kevin:** None.

Poster

PSTR439. Grid Cells and Spatially Modulated Cells

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR439.20/WW10

Topic: H.09. Spatial Navigation

Support: NIH RF1NS121919

Title: Robust Variability of Grid Cell Properties Within Individual Grid Modules Enhances Encoding of Local Space

Authors: ***W. REDMAN**[1], S. ACOSTA[2], X. WEI[3], M. GOARD[4];
[1]Univ. of California Santa Barbara, Santa Barbara, CA; [2]Univ. of California Santa Barbara Neurosci., Univ. of California Santa Barbara Neurosci., Santa Barbara, CA; [3]The Univ. of Texas at Austin, Austin, TX; [4]Univ. of California, Santa Barbara, Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: Grid cells in rodent medial entorhinal cortex are organized into discrete modules, characterized by similar grid spacing and orientation. This modularity has fundamentally shaped the way in which the functional role of these neurons has been interpreted in spatial navigation. In particular, because it has been assumed that the grid properties within module are identical, integration across multiple modules is necessary for accurate localization in space. However, the validity of this assumption has not been systematically tested. By analyzing recently recorded grid cell data, we find small yet robust variability in grid spacing and orientation within individual modules, on the order of centimeters and degrees, respectively. Computational modeling of synthetic grid populations with similar levels of heterogeneity reveals that single modules can encode information of local space with much higher accuracy. This challenges a long-held notion about the nature of the grid code and the necessity of multiple modules for spatial localization.

Disclosures: **W. Redman:** None. **S. Acosta:** None. **X. Wei:** None. **M. Goard:** None.

Poster

PSTR439. Grid Cells and Spatially Modulated Cells

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR439.21/WW11

Topic: H.09. Spatial Navigation

Title: Navigation in Abstract Cognitive Spaces Through Extraction of Low-DimensionalVelocity Signals from Non-Spatial Data for Grid Cell Integration

Authors: *A. IYER[1], S. CHANDRA[4], S. SHARMA[2], I. R. FIETE[3];
[1]MIT, Cambridge, MA; [2]Brain and Cognitive Sci., MIT, CAMBRIDGE, MA; [3]Ctr. for Learning and Memory, MIT, Cambridge, MA; [4]Massachussetts Inst. of Technol., Cambridge, MA

Abstract: Grid cells in the medial entorhinal cortex exhibit striking spatial representations during navigation. Recent studies have shown that grid cells are not limited to spatial navigation but also represent abstract cognitive spaces. Such spaces include a viewed cartoon image that is deformed along two abstract feature dimensions or an environment in which auditory pitch varies upon a lever press. Here, we hypothesize that the brain is able to quickly form representations of these abstract spaces by learning to extract a low-dimensional velocity signal during navigation through the space, and then piping this signal into grid cells, whose internal structure is then mapped onto the space through the operation of velocity (path) integration. We developed a computational model that takes in abstract time-varying inputs of distinct modalities and of vastly different statistics and dimensions, and extracts from these inputs low-dimensional velocity signals. The model does so by predicting the next state given past states (state transitions) and by applying a geometric consistency constraint in which movement along closed loops should result in the same state (loop closure), which is itself computed by the output grid cells. The model provides a computational hypothesis for how animals extract abstract velocities in diverse non-spatial contexts, leading to potential insights into how grid cells encode abstract cognitive spaces.

Disclosures: A. Iyer: None. **S. Chandra:** None. **S. Sharma:** None. **I.R. Fiete:** None.

Poster

PSTR440. Feedback, Reinforcement, and Reward

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Program #/Poster #: PSTR440.01/WW12

Topic: H.10. Human Learning and Cognition

Support: NSF CAREER Award BCS1943767

Title: Temporal regularities guide feature-based learning in complex reward environments

Authors: *A. YAZDANPANAH, M. C. WANG, M. P. BENZ, A. SOLTANI;
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Abstract: In naturalistic reward environments, people are constantly faced with the task of learning the values of multi-feature objects from limited feedback, a challenge referred to as the curse of dimensionality. To mitigate the curse of dimensionality, humans adopt different

strategies including feature-based learning which involves learning the value of informative features. Attention can bias this process by selecting a subset of those features for more efficient reward learning. However, it is currently unknown how attention is guided by certain characteristics of the environment to influence learning and decision making. One possibility is that the spatial and/or temporal patterns or regularities of features in the environment could deploy attention to guide learning similar to how perception is influenced by visual statistical learning (i.e., subtle statistical relationships among visual objects in space or time). To test whether regularities in the environment could guide attention for feature-based learning, we conducted an experiment in which human participants (N=57) learned about reward probabilities associated with 16 different visual stimuli, each with two features (shape and pattern), through reward feedback that followed selection between a pair of stimuli on each trial. To introduce temporal regularities of features that could guide attention, we interleaved choice/learning trials with bouts of trials in which participants were presented with a sequence of visual stimuli and were required to identify the next stimulus in the sequence. We implemented temporal regularities in only one of the two features (manipulated feature) of stimuli presented during the pattern-detection task. Importantly, both features were equally informative about the reward outcome and there was no overlap between the set of stimuli used in the learning and temporal pattern-detection tasks. We used a combination of model-free analyses and fit of choice data based on multiple reinforcement learning models to study the effect of our manipulation on feature-based learning. We found that participant's choice behavior and value estimations were both biased towards the manipulated feature (i.e., feature with regularity during the pattern-detection task). In addition, participants demonstrated a stronger tendency to learn about the manipulated feature of the unchosen option, which resulted in higher learning rates for the manipulated feature. Overall, our results show that the existing patterns of features in the environment could deploy attention to mitigate the curse of dimensionality by biasing reward-based learning toward such features.

Disclosures: **A. Yazdanpanah:** None. **M.C. Wang:** None. **M.P. Benz:** None. **A. Soltani:** None.

Poster

PSTR440. Feedback, Reinforcement, and Reward

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Program #/Poster #: PSTR440.02/WW13

Topic: H.10. Human Learning and Cognition

Support: Duke Health Scholars Award to R. A. Adcock
Duke Institute for Brain Sciences Germinator Award to A. H. Sinclair

Title: Neural Correlates of Motivational States that Bias Reinforcement Learning and Memory Formation

Authors: *A. H. SINCLAIR, Y. WANG, R. ADCOCK;
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Abstract: Motivation influences goals, decisions, and memory formation. *Imperative* motivation links urgent goals to actions, narrowing the focus of attention and memory. Conversely, *interrogative* motivation integrates goals over time and space, supporting rich memory encoding for flexible future use. Here, we investigated whether these motivational states engage distinct neural systems to influence learning and memory. In an fMRI study with human participants (N=44, ages 18-35, 25 women), we manipulated motivational states by randomly assigning participants to read one of two cover stories before a reinforcement learning task. The Imperative group imagined *executing* a museum heist, whereas the Interrogative group imagined *planning* a future heist. During the subsequent reinforcement learning task, participants repeatedly chose among four doors representing different museum rooms (choice phase). Choosing a door revealed a trial-unique painting and an associated reward value (feedback phase); rewards were later converted to bonus payments. Importantly, only the cover stories differed between groups; the reinforcement learning task was identical, and all participants had the same expectations about how and when bonus payments would be awarded. The next day, we conducted a surprise memory test on the paintings. Replicating our prior behavioral findings, we found that Imperative motivation enhanced short-term reward learning, increasing exploitative choices, points earned, and optimal choices. Conversely, Interrogative motivation enhanced long-term memory formation, increasing next-day recognition memory and recall of painting-value associations. fMRI data was preprocessed with fmriprep and analyzed with FSL. During the choice phase, striatal activation was greater in the Imperative group relative to the Interrogative group. During the feedback phase, activation in the hippocampus, ventral visual stream, and default mode network predicted subsequent memory. However, different motivational states were associated with distinct routes to memory formation. In line with theoretical predictions, amygdala activation drove memory in the Imperative group, but not in the Interrogative group. Overall, we demonstrate that motivational states impact whether or not memories are encoded successfully, the nature of memory representations (rich and associative vs. sparse and decontextualized), and the neural mechanisms that contribute to memory formation. Our findings offer broader implications for enhancing education, behavior change, clinical interventions, and communication.

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Poster

PSTR440. Feedback, Reinforcement, and Reward

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Program #/Poster #: PSTR440.03/WW14

Topic: H.10. Human Learning and Cognition

Support: NSF GRFP
BBRF Young Investigator Grant

Title: Information-seeking around aversive outcome anticipation

Authors: *I. AITSAHALIA[1,4], P. SEPULVEDA[2,4], S. DONG[5], R. CHEN[5], Y. CHAI[5], N. H. NAQVI[3], K. IIGAYA[2,4,3];
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Abstract: Anticipating negative or uncertain outcomes can be a major source of anxiety and fear, especially among populations with psychiatric disorders. This aversive anticipation, called dread, can be formalized through anticipatory utility computation, a behavioral economic theory which provides a normative framework for analyzing the value from the anticipation of a future reward or punishment, in addition to the experience of reward or punishment itself (Berns et al., 2006; Loewenstein, 1987). While research has shown that advanced information of pleasant outcomes tends to boost positive anticipation (Iigaya et al., 2016; 2020), and therefore most people choose to receive information in behavioral paradigms, many studies have suggested heterogeneous impacts of advanced information about future aversive experiences on anticipatory feelings. For example, a classic study showed that patients waiting for medical operations experience greater fear if they receive more information about the operation in advance (Janis, 1958). Other researchers, however, suggest a large individual variation on the preference for advanced information.We extended a behavioral task (Bromberg-Martin & Hikosaka, 2009) to probe information-seeking behaviors in a dreadful context (Iigaya et al., 2016; 2020), while we recorded a variety of biophysical modalities, including heart-rate, skin conductance response, and pupil dilation, to validate this task. At the start of each trial, participants are presented with the probability of receiving punishment and the duration of the delay until they receive the outcome. They then choose between an "information" target to find out about the outcome, or a "no-information" target to simply wait for the outcome without knowledge. After the delay period the outcome is played for the participant: either punishment (a loud scream) or a neutral outcome, according to the probabilities presented. The aversive nature of the outcome was supported by participants' self-reports and physiological measures (increase in pupil area and skin conductance after the presentation of the scream). We found a wide range of information-seeking preferences, which were modulated by the delay and probability of the negative outcomes. Advance information about prospective outcomes impacted participants' experience, as reflected by a higher pupil sizes at the moment non-aversive outcomes were revealed. Our study suggests that information modulates the experience of dread, an important step into understanding critical disorders such as post-traumatic stress or anxiety disorders.

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Poster

PSTR440. Feedback, Reinforcement, and Reward

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR440.04/WW15

Topic: H.10. Human Learning and Cognition

Support: NIH Grant P20GM103430

Title: Real-time visual attention training through closed-loop eeg neurofeedback

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Abstract: Attention is the ability to process important information while dismissing distractions during a task. Visual attention involves finding a target among competing stimuli. Sustained attention, a cognitive skill, is the maintaining of focus over time. Our overall hypothesis is that a single neurofeedback training session can enhance this ability. In this study, we developed an innovative Python-based Brain-Computer Interface (BCI) that incorporates a Multi-Layer Perceptron (MLP) algorithm to decode brainwave patterns during a visual attention training task. This study processed scalp electroencephalography (EEG) signals in real-time via 8-channel and 16-channel wet-electrode systems. Our overall experiment involves three phases: open-loop pre-evaluation, neurofeedback training unique to the closed-loop, and open-loop post-evaluation. The first and third phases as open-loop forms have similar procedures. During the open-loop form, we primed participants to discriminate a sequence of composite images. Each image was a fair superimposition of a scene and a face image. The participants were asked to respond (with a push button) to the intended subcategory (e.g., indoor scenes) while withholding their responses for the irrelevant subcategories (e.g., outdoor scenes). We made use of data from four participants to develop the MLP model. This open-loop classification model demonstrated a commendable average classification accuracy of 91%. During the closed-loop form as a neurofeedback training phase, the composition and transparency of the sequence of blended images are adjusted using the open-loop model in the pre-evaluation phase and based on the participant's decoded attentional level toward a specific image category. We hope to expand the recruitment of human subjects for the closed-loop form and neurofeedback training. The results from such a neurofeedback platform will have a significant impact on developing low-cost and portable attention training systems for patients with attention deficits.

Disclosures: **M. norouzi:** None. **K. Wellington:** None. **B. Garneau:** None. **R. Abiri:** None.

Poster

PSTR440. Feedback, Reinforcement, and Reward

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR440.05/WW16

Topic: H.10. Human Learning and Cognition

Title: Generalization in reinforcement learning and the role of sleep

Authors: *A. JASKIR, M. J. FRANK;
Cognitive & Psychological Science, Carney Inst. for Brain Sci., Brown Univ., Providence, RI

Abstract: When trained on specialized tasks, cutting-edge algorithms in deep reinforcement learning (i.e. learning through trial-and-error) can outperform human experts, but humans remain unsurpassed in quickly transferring learning between tasks with shared structure. For example, a musician trained on guitar can generalize a scale from one part of a fretboard to another and can apply this knowledge to speed learning to play a cello, despite differences in the desired song to play or the movements to achieve that song. Drawing upon recent work interfacing computer science and cognitive neuroscience (Lehnert et. al., 2020), we hypothesized that humans form "reward-predictive" state abstractions (RPAs) that support such transfer. RPAs are achieved by clustering situations which share analogous action-reward sequences (e.g., all fret positions on a guitar are reducible to twelve unique notes). Furthermore, we propose that sleep plays a constructive role in these abstractions in biological agents, whereby extensive rest allows for reprocessing and discovery of event similarities; this hypothesis draws upon the Complementary Learning Systems, a computational theory of memory consolidation in the hippocampus and cortex (McClelland et al, 1995; Singh et al, 2022). We developed a novel sequential decision-making task to test for RPA transfer. Specifically, participants in the Learning Block were asked to learn through trial-and-error which sequences of key presses lead to reward; the sequences share a hidden rule (uninstructed to participants) where pairs of keys can be arbitrarily exchanged in any sequence without affecting the resulting reward order and therefore are compressible. In the Generalization block, the sequence changed but the underlying hidden structure remained. Performance in the Generalization block would be enhanced if participants learn RPAs. Collecting participant data through the online platform Prolific, we demonstrate that human behavior reflects performance enhancements distinctive to RPAs. We further characterize the effects of sleep on this learning process and motivate the neural implications of these findings. This work provides insight into the flexibility of human cognition and the underexplored role of sleep in reward learning.

Disclosures: **A. Jaskir:** None. **M.J. Frank:** None.

Poster

PSTR440. Feedback, Reinforcement, and Reward

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR440.06/WW17

Topic: H.10. Human Learning and Cognition

Support: Brainstorm Program at the Robert J. & Nancy D. Carney Institute for Brain Science

Title: Long-term credit assignment in humans critically depends on sequential structuring of events

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Abstract: To refine our future behavior, it is essential to learn whether past actions or events led to good or bad outcomes and assign credit accordingly. For instance, if you are feeling worse than usual today, identifying which actions or events (such as not getting enough sleep or skipping breakfast) may have contributed to that state can improve choices in the future. However, assigning credit is a notoriously difficult problem, especially when we want to consider the longer-term consequences of our actions. In the present study, we explored human credit assignment strategies through a novel behavioral paradigm where online participants (n=218) had to either observe or select activities with different short- and long-term pain-related consequences for an avatar and subsequently predict the impact of those activities on that avatar's pain level. Behavioral results suggest that when activities are randomized over time, participants tend to learn short-term consequences, but fail to learn long-term ones. However, learning of long-term consequences can be improved if activities are blocked in time, such that each activity is repeated multiple times in succession. Furthermore, when participants had the freedom to select activities, we identified a relationship between activity selection patterns and learning, where increased repetition in activity selection was related to a learned preference for activities that reduce long-term, but not short-term, pain. In order to understand the potential computations underlying participant behavior, we compared several computational models and found that standard model-free algorithms (i.e., temporal difference learning) fail to properly learn long-term consequences of activities in our task, whereas Bayesian models that take into account the causal structure of the environment effectively learn both short- and long-term consequences regardless of whether activities are repeated or not. Thus, neither model adequately aligned with participant behavior across different activity repetition patterns, suggesting the necessity to develop more refined models that account for constraints on human credit assignment. Overall, our results demonstrate that credit assignment critically depends on the order in which actions are selected, with repetitions aiding the learning of long-term consequences. This raises intriguing questions about whether individuals might deliberately repeat actions (i.e., perseveration) to enhance their ability to assess long-term consequences, warranting further exploration into how action selection and subsequent credit assignment may correlate with or predict real-world behavior.

Disclosures: S. Bruinsma: None. **F.H. Petzschner:** None. **M.R. Nassar:** None.

Poster

PSTR440. Feedback, Reinforcement, and Reward

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR440.07/WW18

Topic: H.10. Human Learning and Cognition

Title: Neural representations of Bayesian learning process in dynamic Environment

Authors: *Y. ZHANG, X. WAN;
Beijing Normal Univ., Beijing, China

Abstract: In a dynamic environment, maintaining accurate beliefs requires the learning rate to beadjusted based on experiences. This means that while stable beliefs should be maintained even inthe presence of noisy data, they must remain flexible during periods of uncertainty or change.However, the underlying mechanism by which the brain implements such adaptive learningprocesses is not fully understood. To investigate this question, we conducted an experimentrequiring participants to predict the current value of a number based on previous feedbackinformation drawn from five normal distributions with different means but the same variances.Participants had to assess whether the fluctuations in the numbers were caused by noise or changesin the true value for accurate predictions. Combining computational modeling with MEG(Magnetoencephalography), we studied the neural processes involved in this task and found that aBayesian inference model best described and predicted participant behavior. The Bayesianinference-based learning process can be divided into two components: the stable update (model-freecomponent) and the dynamic adjustment based on the probability of environmental change (model-based component). During the feedback phase, an unsigned prediction error (UPE) was encoded inthe putative ACC (anterior cingulate cortex) Theta band (4-7 Hz) signal, which is believed to berelated to the model-based component of the learning process. In contrast, signed prediction error(SPE) was represented by Beta band (15-30 Hz) signals in the putative primary motor cortex (PMC)region during both the feedback and motor preparation and execution phases. While the beta bandsignals during motor preparation and execution were mutually decodable, the signal during thefeedback phase could not be decoded from the other two phases. This suggests that during thefeedback phase, the beta band signal in PMC may differ from the motion preparation and executionphases and may involve the stable update (model-free component) process of Bayesian updating.

Disclosures: Y. Zhang: None. **X. Wan:** None.

Poster

PSTR440. Feedback, Reinforcement, and Reward

Location: WCC Halls A-C

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Program #/Poster #: PSTR440.08/WW19

Topic: H.10. Human Learning and Cognition

Support: Brain & Behavior Research Foundation Grant 28187
National Institute of Mental Health R01 MH131532-01

Title: Attentional bias toward rewarding vs contextual cues in trauma exposed individuals

Authors: *C. SHARP[1], P. RJABTSENKOV[1], C. MARINO[1], Z. ALI[1], S. BAVDEKAR[1], E. PINEDA[1], A. LAZAROV[2], B. SUAREZ-JIMENEZ[1];
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Abstract: Decreased pursuit and anticipation of rewards are common symptoms of trauma-related psychopathology. However, little is known regarding how trauma exposure may affect

attention allocation to conditioned reward cues. We used eye-tracking to examine how trauma exposure affects attention to rewarding versus contextual cues from a virtual reality (VR) reward conditioning task. Trauma exposed (TE) and non-trauma-exposed (HC) participants completed an eye-tracking task before and after the VR reward conditioning task. During the eye-tracking task, participants freely viewed matrices of pictures of the conditioned reward stimuli (MN) and the surrounding environment (CX) from the VR task. We compared participants' total dwell time and number of first fixations on each cue type as measures of attentional bias. While not statistically significant, our preliminary data show that HCs' percentage of first fixations on CX images increased from pre-task to post-task while in TEs this decreased from pre-task to post-task. However, while HCs' percentage of total dwell time on CX images was higher than that of TEs at both pre-task and post-task, percentage of total dwell time on CX images did not increase from pre-task to post-task in either HCs or TEs.The directionality of our preliminary findings suggests that trauma exposure may bias attention toward valenced cues and away from contextual cues during one's initial scanning of an environment. This attentional bias toward valenced cues may serve as a signature for trauma exposure that could be targeted for treatment.

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Poster

PSTR440. Feedback, Reinforcement, and Reward

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Program #/Poster #: PSTR440.09/WW20

Topic: H.10. Human Learning and Cognition

Support: James S McDonnell Foundation
NSF GRFP

Title: Striatal involvement in reward processing in the human infant brain

Authors: *J. E. TRACH[1], T. S. YATES[1], D. CHOI[1], L. BEHM[1], C. T. ELLIS[4], S. D. MCDOUGLE[1,2], N. B. TURK-BROWNE[3,2];
[1]Dept. of Psychology, [2]Wu Tsai Inst., [3]Yale Univ., New Haven, CT; [4]Dept. of Psychology, Stanford Univ., Stanford, CA

Abstract: The ability to learn from positive and negative feedback is an essential cognitive capacity throughout life but perhaps especially when first learning to make sense of the world during early development. Behavioral work suggests that even very young human infants can use reward feedback to learn and guide their behavior to maximize the reward (i.e., positive feedback) they receive from their environment. However, it is unknown how the infant brain learns from and processes rewards. Prior research in adults and adolescents suggests that regions of the striatum may be involved. At the same time, human infants are extremely altricial and it remains unclear whether this reward processing system is present or mature early in

development. Furthermore, methodological challenges have limited the study of subcortical structures in infants, which cannot be resolved with traditional infant neuroimaging techniques such as EEG and NIRS. Recent advances have made it possible to conduct fMRI studies in awake and behaving infants. We thus used fMRI to measure whole-brain activity during an infant-friendly reinforcement learning paradigm. Infants were presented with two solid color shapes, one associated with a high reward probability (80%) and the other with a low reward probability (20%). One shape was randomly selected on each trial, and then converted to an outline that revealed either a rewarding stimulus (dynamic smiley face) or no outcome. Infants who had a minimum of 20 trials during which they were looking at the screen and not moving their head excessively were included in further analyses. Data collection is ongoing and so results are preliminary. We have so far extracted fMRI activity from three ROIs in the basal ganglia (bilateral accumbens, caudate, and putamen) during the reward period. This analysis revealed preliminary evidence of increased activity to rewarded versus nonrewarded trials in bilateral caudate and a trend in the same direction in bilateral accumbens. This aligns with past research in adults and provides initial support for the involvement of the striatum in reward processing during infancy. In planned analyses, we will leverage the computational framework of reinforcement learning to relate reward prediction error (the learning signal in reinforcement learning) to striatal activity and examine value representations in other regions of the brain, namely the medial prefrontal cortex. Overall, this work marks a promising step in our understanding of reinforcement learning in the infant brain.

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Poster

PSTR440. Feedback, Reinforcement, and Reward

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Program #/Poster #: PSTR440.10/WW21

Topic: H.10. Human Learning and Cognition

Support: NIDA/NIH (ZIA DA000642)
R01DC015426

Title: Midbrain signaling of identity prediction errors depends on orbitofrontal cortex networks

Authors: *Q. LIU[1], Y. ZHAO[1], S. ATTANTI[2], J. L. VOSS[3], G. SCHOENBAUM[1], T. KAHNT[1];
[1]Natl. Inst. of Drug Abuse, Baltimore, MD; [2]Mayo Clin. Alix Sch. of Med., Scottsdale, AZ; [3]Dept. of Neurol., Chicago, IL

Abstract: Previous work has shown that the lateral orbitofrontal cortex (OFC) represents expectations about the identity of rewards and that the dopaminergic midbrain responds to reward identity prediction errors (iPE, i.e., value-matched violations of reward identity

expectations). We hypothesized that the lateral OFC directly contributes to the computation of iPEs in the midbrain by signaling reward expectations. To test this, we used network-targeted transcranial magnetic stimulation (TMS) to modulate activity in the lateral OFC network. Healthy human subjects (N=31, 11 males) performed a trans-reinforcer reversal learning task during functional magnetic resonance imaging (fMRI) in two sessions (order counter-balanced); once after sham stimulation and once after continuous theta burst stimulation (cTBS). Stimulation coordinates in the lateral prefrontal cortex (LPFC) were individually selected based on maximal resting-state fMRI connectivity with seed regions in the lateral OFC. The task required subjects to learn associations between visual cues and equally-valued food odor rewards. Unpredictably for the subject, these associations were reversed multiple times throughout the task, eliciting iPEs. Functional connectivity between the lateral OFC and the rest of the brain was significantly reduced after cTBS relative to sham in the first block of the experiment, validating our network-targeted stimulation procedure. Relative to sham, cTBS impaired behavioral performance in the first block of the task, and disrupted representations of expected reward identity in the lateral OFC. Importantly, fMRI responses to iPEs in the midbrain were significantly modulated by OFC-network targeted cTBS relative to sham. These results suggest that neural representations of expected outcome identity in the lateral OFC directly contribute to signaling of iPEs in the midbrain, presumably by providing the predictions necessary for computing the error signal. Taken together, our findings support a model in which midbrain iPEs are generated by comparing incoming sensory information with identity expectations represented in the OFC.

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Poster

PSTR440. Feedback, Reinforcement, and Reward

Location: WCC Halls A-C

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Program #/Poster #: PSTR440.11/WW22

Topic: H.10. Human Learning and Cognition

Support: BECA DOCTORADO NACIONAL ANID FOLIO 21230767

Title: Differential neuronal activity in the mPFC and Nucleus Accumbens during habituation to rewards

Authors: *I. ALLIENDE[1], J. VALDES[2];
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Abstract: The reward system plays a crucial role in processing stimuli with motivational valence and goal-directed behaviors. Dopamine (DA) is one of the main neuromodulators associated with this system, being released from the ventral tegmental area (VTA) to limbic areas such as the nucleus accumbens (NAc) and cortical areas such as the medial prefrontal cortex (mPFC).

Microdialysis studies have demonstrated that novel and unpredictable motivational stimuli increase DA release in the mPFC and NAc when a highly hedonic reward such as chocolate is consumed. However, upon subsequent exposure to the stimulus, the levels of DA released in the NAc decrease compared to the first exposure. This phenomenon is called "habituation" and has been described specifically for the NAc-shell, an area highly responsive to rewarding stimuli, while the mPFC and NAc-core do not show this habituation. Other studies in animals where the mPFC is injured have shown that this structure, especially in its infralimbic portion (IL), is necessary for the occurrence of the habituation phenomenon. Also studies in addicted animals show that the habituation of NAcc to natural rewards under drug abuse is suppressed, instead the mPFC shows a decrease in DA release.
Together, these findings suggest a possible mechanism where the IL-mPFC would have an inhibitory controller role over the NAc-shell that is altered under conditions of addiction. However, the available results are studied by microdialysis, which shows changes in DA release, but there are no studies that account for changes in the neuronal activity of the circuit involved, which help us understand this phenomenon.
In my PhD thesis I employed in vivo free-motion electrophysiology to record the activity of neurons in the IL-mPFC and NAc-shell during a habituation protocol to natural rewards.Furthermore, I am currently conducting experiments on animals addicted to amphetamines to compare both conditions. The results reveal that the habituation is associated with changes in neuronal activity patterns, increasing the IL-mPFC activity and decreasing the NAc activity. We observed that in IL pyramidal cells, the repeated administration of chocolate doesn't generate significant changes in the number of excited cells, but it does generate an increase in the frequency of excited cells. On the other hand, in the MSN putative NAc cells, the same protocol generates a decrease in excitatory activity in relation to the number and frequency. These results emphasize the presumed inhibitory role of IL on NAcc in the occurrence of the habituation phenomenon, providing key information to elucidate the changes in neuronal activity that occur in response to rewards.

Disclosures: I. Alliende: None. **J. Valdes:** None.

Poster

PSTR441. Language Processing and Production

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR441.01/WW23

Topic: H.11. Language

Support: The Swiss National Science Foundation (SNSF)

Title: Functional language mapping with stereo-EEG

Authors: *L. FANDA[1], J. MONNEY[1], F. ARTONI[1], P. MEGEVAND[2];
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Abstract: In epilepsy surgery, functional language mapping (FLM) plays a key role in individualizing cortical resections and limiting their potential negative consequences on language functions. Despite the growing popularity of stereo-EEG, FLM with stereo-EEG electrodes is not a standardized procedure. Here, we systematically assessed cortical responses to simple language tasks in patients undergoing stereo-EEG monitoring.Three basic tasks were used to examine language-related cortical activity: picture naming, auditory naming, and sentence completion. Thus far, 7 patients participated in the study at Geneva University Hospitals' Epilepsy Monitoring Unit. Preprocessing included applying a high-pass filter at 1Hz, a line noise comb notch filter, and rejecting epochs with significant residual artifacts. For each presentation modality, baseline-normalized, event-related spectral perturbation (ERSP) and event-related potentials (ERPs) were computed. These measures were time-locked to the delivery of the stimulus and time-warped to account for varying stimulus durations and realign response events. A significance threshold of 95% was applied to each frequency with respect to its relative baseline (pre-stimulus). The ERP and ERSP preliminary analysis revealed significant functional response on 7.5% of channels per patient, i.e., 9, 5, and 6 channels on average respectively for visual naming, auditory naming, and sentence completion. We detected 4 typical response patterns with respect to baseline: stimulus/response evoked ERPs combined with ultra-HF (150-300 Hz) power decrease, broadband frequency (2-300 Hz) power increase with decay towards the end of the stimulus presentation, HF (50-150 Hz) power increase combined with LF (2-20 Hz) power decrease during stimulus presentation, and anticipatory pre and post HF power increase. These distinct patterns suggest the involvement of several distinct cortical processes. Our functional mapping protocol allows for assessing language function by analyzing cortical responses to visual and auditory inputs. Further work will probe the correspondence between stereo-EEG, fMRI and direct electrical stimulation-based language mapping, and establish the clinical relevance of the various response patterns.

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Poster

PSTR441. Language Processing and Production

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Program #/Poster #: PSTR441.02/WW24

Topic: H.11. Language

Support: National Science Foundation of China 31730038

Title: Mapping cortical representations of experiential and distributional semantics for concrete and abstract concepts

Authors: *C. LIU, G. XUE;
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Abstract: Recent studies have differentiated two types of semantic information, including the experiential semantics (i.e., information about perceptual, affective, spatio-temporal, and other features of human experience) or distributional semantics (i.e., information about statistical co-occurrence of words), yet the neural representations of these information are unclear. In particular, it is still debated whether the two kinds of semantics are complementary, or the experiential semantics can completely cover the other one. We hypothesized that the concreteness of the stimuli plays a key role and may solve this contradiction, such that concrete concepts involve more perceptual experiences and are better captured by experiential semantics, whereas abstract concepts are learned through language and are better represented by distributional semantics. To test this hypothesis, we collected functional MRI data from 24 participants. Participants judged the familiarity of 420 words (roughly half concrete and half abstract), each for four times. We created the neural representational similarity matrix of the words in each of 200 subregions across the whole brain by calculating the Pearson correlation between the activation patterns (i.e., Neural RSM). Similarly, the experiential and distributional semantic representational similarity matrices (i.e., semantic RSM) were created with the human-rated features of words or word vectors from a word2vector model, respectively. Spearman correlations were conducted between the neural RSMs and semantic RSMs. Partial correlation analysis revealed that when all words are included, both experiential and distributional semantics had unique contributions to the neural RSM and they played a complementary role. The semantic representations were found in a widespread, left-lateralized network. Supporting our hypothesis, experiential and distributional semantics made more contribution to the neural representations of concrete and abstract words, respectively. These results suggest that semantic representations in human brains include both feature-based experiential semantics and association-based distributional semantics that contribute differentially to concrete and abstract words.

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Poster

PSTR441. Language Processing and Production

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Program #/Poster #: PSTR441.03/WW25

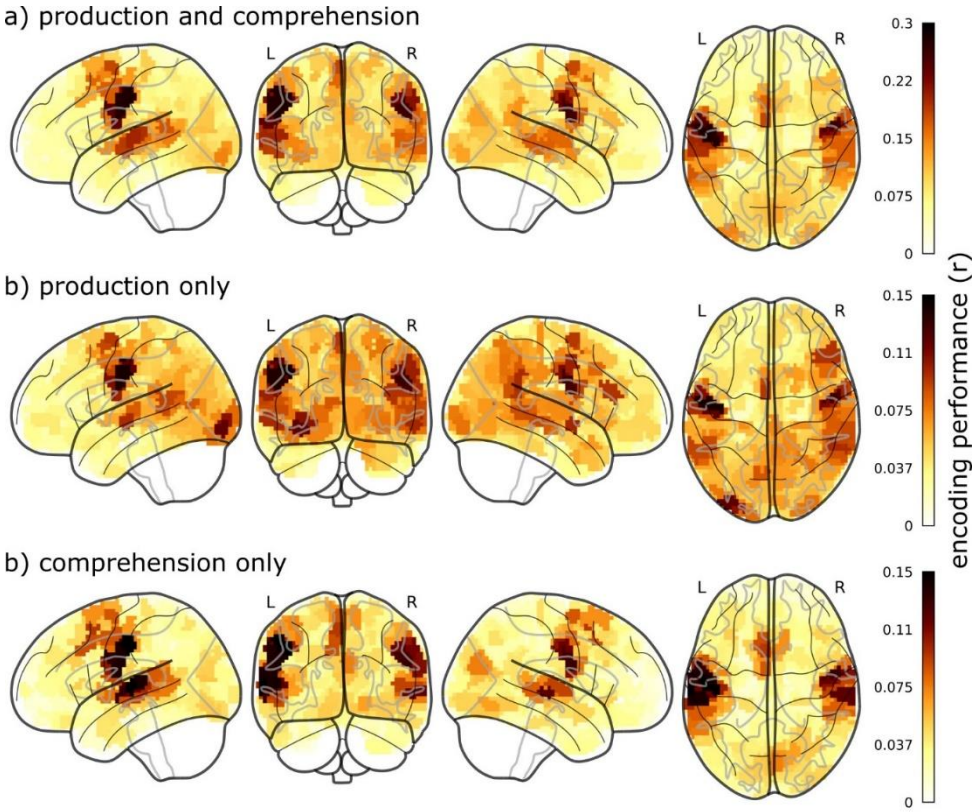
Topic: H.11. Language

Support: NIH Grant DP1HD091948
NIH Grant R21MH127284

Title: Mapping cortical language representation during real-time natural dialogues

Authors: *Z. ZADA, S. A. NASTASE, S. SPEER, L. MWILAMBWE-TSHILOBO, L. TSOI, S. BURNS, U. HASSON, D. TAMIR;
Princeton Univ., Princeton, NJ

Abstract: How is language encoded in the brain during everyday conversations? Typical studies of the neural basis of language present subjects with predetermined, isolated words or sentences, and do not consider the role of spontaneous language production or interactive conversational dynamics. Here, we aim to address both gaps and map brain areas involved in both speech production and comprehension during natural dialogue. We developed a hyperscanning paradigm to collect fMRI data in 30 dyads (60 human subjects) as they freely discussed 10 topics across 5 runs. Topics were presented as a starting point, but each dyad was free to pursue the discussion in different ways. fMRI data were preprocessed using fMRIPrep and spatially downsampled to 1,000 parcels. To characterize the linguistic content encoded in each parcel, we estimated parcel-wise encoding models to predict held-out BOLD signals during speech production or comprehension from word embeddings extracted from the GPT-2 language model. To account for turn-taking during natural conversations, we split the regressors into different subsets for speaking and listening, and fit both submodels jointly using banded ridge regression; this allows the model to learn different weights for each process, and allows us to quantify the relative contribution of each submodel. Then, we correlated the actual and predicted BOLD activity for left-out runs in each parcel, quantifying the extent of linguistic content in the signal. We found strong encoding performance bilaterally in the somatomotor, premotor, and superior temporal cortex (Fig. 1). When evaluated separately, speech production more strongly recruited the somatomotor cortex, while speech comprehension more strongly recruited the superior temporal cortex. These brain maps concur with existing research on the functional role of language areas, but extend them to a naturalistic setting and with an explicit language model. Our findings lay the foundation for assessing brain-to-brian coupling between speakers and listeners in the embedding space.



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Poster

PSTR441. Language Processing and Production

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Program #/Poster #: PSTR441.04/WW26

Topic: H.11. Language

Support: NIDCD grant R01DC014960

Title: Right-hemisphere functional connectivity increases in proportion to lesion size after left-hemisphere stroke

Authors: *N. VLADYKO[1], A. T. DEMARCO[1], P. E. TURKELTAUB[1,2];
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Abstract: Aphasia is an acquired language disorder that often results from a stroke to left-hemisphere (LH) language areas. Stroke may alter brain activity and functional connectivity (FC) in lesioned and spared areas after a LH stroke. Changes in connectivity may be important for aphasia outcomes at the chronic stage of recovery. However, the findings on the influence of the LH stroke on the RH FC diverge: some find an increase in contralesional FC at the chronic stage, while others report a decrease or no difference compared to controls. Our study examined the relationship between lesion size and contralesional FC. We hypothesized that larger LH lesions would produce greater increases in RH FC relative to small lesions. In this study, 78 chronic LH stroke survivors (35 F, mean age = 60.91; mean days post-stroke = 1592; median lesion size = 80.125 cc) and 72 demographically matched controls (36 F; mean age = 61.1) were included. All participants completed a 14.5-minute movie-watching fMRI scan. After standard pre-processing, FC was calculated for all 246 Brainnetome parcels for each participant. Correlations between lesion size and RH edges revealed that larger LH lesions corresponded to greater FC of many RH edges. Next, we median-split the stroke group based on lesion size (small lesion: n=37, mean lesion size = 31.23 cc; large lesion: n=38, mean lesion size = 177.48 cc), and compared each group to controls using edgewise t-tests. Both groups showed increased RH intrahemispheric FC relative to controls, however, the increase was greater for the large lesion group compared to the small lesion group. Both groups showed reduced interhemispheric FC compared to controls. These findings contribute to the understanding of functional reorganization in the RH after LH stroke and reveal an increase in the RH FC at the chronic stage. The next steps will be to determine if the spatial pattern of RH FC increases relates to lesion location and if FC changes contribute to behavioral aphasia outcomes.

Disclosures: **N. Vladyko:** None. **A.T. Demarco:** None. **P.E. Turkeltaub:** None.

Poster

PSTR441. Language Processing and Production

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR441.05/WW27

Topic: H.11. Language

Support: NSF Grant 1R01DC020088-001
Burroughs-Wellcome Foundation

Title: Scaling laws for language encoding models in fMRI

Authors: *R. J. ANTONELLO, A. VAIDYA, A. G. HUTH;
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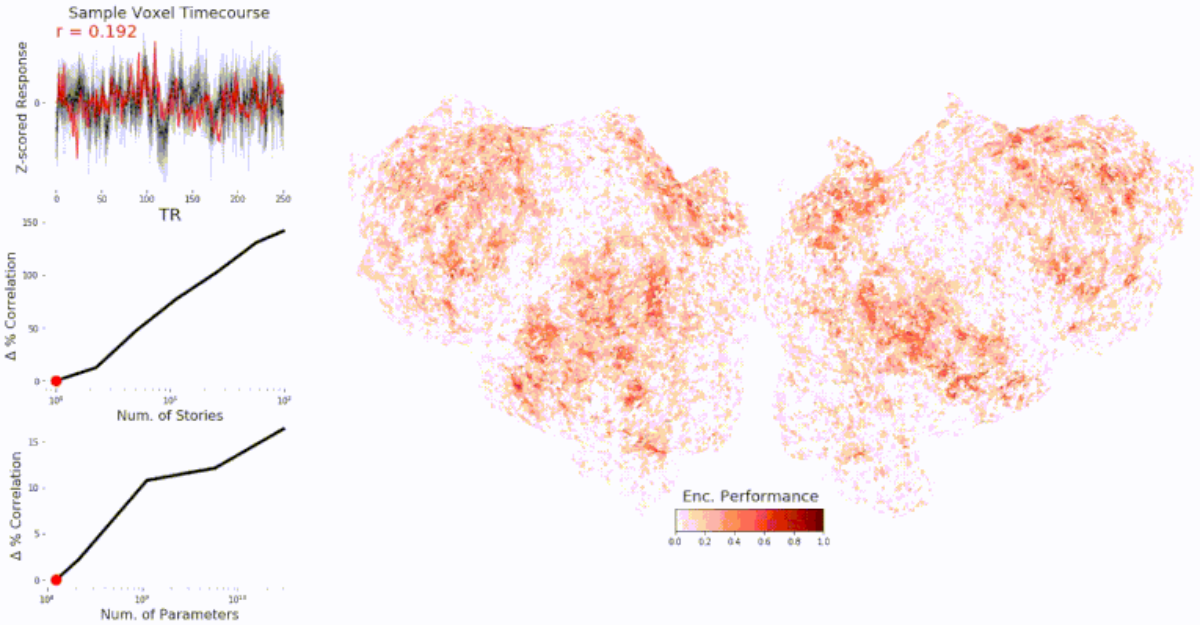
Abstract: Representations from transformer-based unidirectional language models are known to be effective at predicting brain responses to natural language. However, most studies comparing language models to brains have used GPT-2 or similarly sized language models. Here we tested whether larger open-source models such as those from the OPT and LLaMA families are better at predicting brain responses recorded using fMRI. Mirroring scaling results from other contexts, we found that brain prediction performance scales log-linearly with model size from 125M to 30B parameter models, with ~15% increased encoding performance as measured by correlation with a held-out test set across 3 subjects. Similar log-linear behavior was observed when scaling the size of the fMRI training set. We also characterized scaling for acoustic encoding models that use HuBERT, WavLM, and Whisper, and we found comparable improvements with model size. A noise ceiling analysis of these large, high-performance encoding models showed that performance is nearing the theoretical maximum for brain areas such as the precuneus and higher auditory cortex. These results suggest that increasing scale in both models and data will yield incredibly effective models of language processing in the brain, enabling better scientific understanding as well as applications such as decoding.



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Poster

PSTR441. Language Processing and Production

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR441.06/Web Only

Topic: H.11. Language

Support: Kennedy Krieger Institute

Title: Differentiating reading gains in children with attention deficit hyperactivity disorder and children with reading difficulties using fMRI data

Authors: *T. HOROWITZ-KRAUS[1], M. KHASHAB[2], S. GANAIEM[3], R. FARAH[2], J. FOTANG[4], K. ROSCH[4];
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Abstract: Reading is defined as the ability to decode printed text to sounds in the spoken language and comprehend it semantically. This ability also relies on different cognitive sub-processes and executive functions (EF), which contribute to the reading process. Dyslexia, or Reading Difficulties (RD), is a neurodevelopmental disorder affecting 15% of children

worldwide. This disorder is highly comorbid with attention-deficit/hyperactivity disorder (ADHD). Children with RD+ADHD demonstrated greater reading and EF challenges than those with RD-only. The goal of the current study is to determine the effect of EF-based reading intervention on behavioral and neurobiological correlates for EF among 8-12 y.o. English-speaking children with RD+ADHD (n=18), RD-only (n=18) and typically developing children (n=18). Behavioral and neurobiological data were collected from all participants before and after eight weeks of training with the EF-based reading computerized program. Functional MRI data were collected using 5 minutes of resting state condition, pre and post-intervention.Separate 3 Group (RD+ADHD, RD-only, typical readers) x 2 Test (Pre, Post-intervention) repeated measures ANOVA were conducted for reading and EF measures. Functional connectivity matrices within and between EF networks, including the cingulo-opercular (CO), fronto-parietal (FP), ventral and dorsal attention networks (VAN, DAN) were defined and compared between the participants and conditions. Prediction models connecting behavioral and neurobiological changes were conducted as well using regression analysis.Training had different effects among the three groups. While typical readers showed the highest scores in reading and EF measures, children with RD+ADHD showed significantly greater gains in comparison to children with RD-only in reading (reading fluency, rate and comprehension) and EF measures (switching, inhibition and working memory). Furthermore, greater changes in functional connectivity following the intervention, mainly between FP and DAN, were found among children with RD+ADHD and RD-only, with a significant decrease in functional connectivity of FP-DAN found in the RD+ADHD group. Changes in FP-DAN significantly predicted changes in naming abilities following training across all groups.The current study's results strengthen the role of EF in the reading process. It also supports the differences, both behaviorally and neurobiologically, in EF among children with RD-only and those with RD+ADHD. In an attempt to get closer to a precision-education approach, a differential intervention for these groups may be indicated.

Disclosures: **T. Horowitz-Kraus:** None. **M. Khashab:** None. **S. Ganaiem:** None. **R. Farah:** None. **J. Fotang:** None. **K. Rosch:** None.

Poster

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Program #/Poster #: PSTR441.07/WW28

Topic: H.11. Language

Support: Health Resources and Services Administration T32HP10260
NIMH R01MH126531

Title: Maternal COVID-19 Infection Impacts the Intergenerational Transmission of Cognitive Functioning in the Postpartum Period: Results From the COVID-19 Mother-Baby Outcomes Initiative

Authors: *J. M. WARMINGHAM, V. CHAVES, A. LAVALLEE, M. HUSSAIN, G. KURMAN, M. KYLE, D. DUMITRIU;
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Abstract: Children's cognitive and language development in the first six months of life depends on parental support and interaction. Maternal COVID-19 infection during the perinatal period has the potential to alter maternal cognitive and executive functioning, which may influence infant cognitive and language development among children born during the pandemic. **Aims**: Identify the influence of COVID-19 infection on a mother's performance in verbal IQ, executive functioning, and cognitive domains, and test associations between maternal cognitive functioning and infant communication and cognitive skills. **Methods**: Participants were mother-infant dyads (n=283) enrolled in the COVID-19 Mother Baby Outcomes (COMBO) Initiative. Of those enrolled, 32.3% (n=91) of mothers had COVID-19 prior to delivery. At 6 months postpartum, mothers and infants participated in a virtual research visit (via Zoom). Mothers completed performance-based assessments of executive functioning (Trail Making Test A & B), verbal IQ (American National Adult Reading Test), and cognitive functioning (MoCA-blind). With the instruction of a research assistant via zoom, mothers guided infants through the Developmental Assessment of Young Children (DAYC-2) for 6-month-olds. **Results**: Maternal COVID-19 infection was associated with lower maternal cognitive functioning (*B*=-.129, *p*=.019) and lower executive functioning (*B*=.156, *p*=.018) when controlling for sociodemographic factors, including education. Among mothers without COVID-19 infection, higher maternal verbal IQ was associated with higher scores on infant scores on expressive language (*r*=.21, *p*=.005), communication (*r*=.15, *p*=.047), and infant cognition (*r*=.24, *p*=.002). In the COVID-positive group, maternal verbal IQ was not associated with infant development (*p*s>.05). In models adjusted for sociodemographic factors and child age, higher maternal verbal IQ was associated with higher scores in the domains of infant cognition (*B*=.19, p=.005), expressive language (*B*=.198, *p*=.005), and communication (*B*=.15, *p*=.034). Neither maternal executive functioning nor cognitive functioning (MoCA score) were associated with infant development scores (*p*s>.05). In adjusted models predicting infant development, there were no significant interactions between maternal COVID-19 infection and maternal cognitive functioning (*p*s>.05). **Summary:** COVID-19 infection is associated with lower postpartum maternal executive and cognitive functioning and attenuation of intergenerational transmission of cognitive functioning. Maternal verbal IQ was most strongly associated with infant development outcomes at 6 months old.

Disclosures: J.M. Warmingham: None. **V. Chaves:** None. **A. Lavallee:** None. **M. Hussain:** None. **G. Kurman:** None. **M. Kyle:** None. **D. Dumitriu:** None.

Poster

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Program #/Poster #: PSTR441.08/WW29

Topic: H.10. Human Learning and Cognition

Support: NIMH R01MH126531

Title: Longitudinal Associations Between Maternal Cognition and Infant Cognition in a Subsample of the COVID-19 Mother Baby Outcomes (COMBO) Initiative

Authors: *V. CHAVES, M. KYLE, J. WARMINGHAM, A. LAVALLEE, I. AHMED, S. HYMAN, G. ATWOOD, G. KURMAN, D. DUMITRIU;
Columbia Med. Ctr., New York, NY

Abstract: Background: Maternal verbal cognition and caregiving behavior is associated with many facets of infant development in the first two years of life. However, evidence of longitudinal associations between maternal cognition and caregiving behavior with infant development is scarce, particularly among infants born during the pandemic. **Aim of the study:** Determine the potential predictive power of maternal verbal cognition and maternal sensitivity on communication, fine and gross motor, problem solving and personal social development at 18 months old, as well as the impact of SARS-CoV-2 infection on maternal verbal cognition. **Methods:** The sample is composed of mother-infant dyads (n=146) enrolled in the COVID-19 Mother-Baby Outcomes (COMBO) Initiative. Of those, 44.5% (n=65) had a history of SARS-Cov-2 infection perinatally. Maternal sensitivity was coded from a diaper change paradigm recorded via video visit (Zoom) at 4-months postpartum. Maternal cognition was measured at 9-months postpartum with the American National Adult Reading Test (AMNART) via video visit (Zoom). Finally, infant development was measured at 18-months via maternal report using the Ages and Stages Questionnaire, 3rd edition (ASQ-3). **Results:** Maternal COVID-19 infection was associated with lower maternal verbal cognition (r=0.22, p=0.049) but not infant development outcomes. Maternal verbal cognition was not associated with infant cognitive development. However, verbal cognition was a significant predictor of infant's communication (r=0.32, p=0.009) and fine motor (r=0.31, p=0.01) scores. Greater maternal sensitivity was associated with lower infant fine motor scores (r= -0.51, p=0.01). When controlling for covariates, such as baby gender, medical coverage, and ethnicity, maternal sensitivity was not associated with infant development. Higher maternal verbal cognition was associated with higher fine motor scores (B=0.38, p=0.035) but not other child development outcomes at 18 months. **Conclusion:** We assessed the longitudinal associations between maternal cognition and sensitivity on infant's cognitive development in a subset of mother-infant dyads enrolled into the COMBO Initiative. Surprisingly, coded sensitivity at 4 months was related to lower fine motor scores at 18-months in unadjusted but not adjusted models. Notably, maternal COVID-19 infection was associated with lower scores on maternal verbal fluency. Maternal verbal cognition was a significant predictor of infant's communication in unadjusted models and fine motor in the adjusted model, suggesting that maternal verbal abilities are important to promote development in the first 2 years of life.

Disclosures: V. Chaves: None. **M. Kyle:** None. **J. Warmingham:** None. **A. Lavallee:** None. **I. Ahmed:** None. **S. Hyman:** None. **G. Atwood:** None. **G. Kurman:** None. **D. Dumitriu:** None.

Poster

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Program #/Poster #: PSTR441.09/WW30

Topic: H.11. Language

Support: R01DC014960
K99DC018828

Title: The effect of white matter-hyperintensities on aphasia outcome in post stroke patients

Authors: *N. NAJIB[1], D. MORALES-SANZ[3], A. DEMARCO[3], P. E. TURKELTAUB[2]; [1]Neurol., [2]Neurol. Dept, Georgetown Univ., Washington, DC; [3]Georgetown university, Washington, DC

Abstract: Small vessel ischemic disease (SVID) is a multifactorial finding that can manifest as white matter hyperintensities (leukoaraiosis) in brain MRIs. Following stroke, several studies suggest that SVID may be a contributing factor to aphasia severity, in addition to other predictors like age, time since stroke, and lesion size. One challenge in studying the importance of SVID in post stroke aphasia is that there are several qualitative rating scales available to measure the severity of leukoaraiosis. Yet, it is unknown whether different rating scales perform similarly or not. Here, we attempt to replicate prior findings that presence and severity of leukoaraiosis contributes independently to post-stroke aphasia, comparing common leukoaraiosis rating scales. Participants included 70 patients (29 F, 41 M) with chronic left hemisphere stroke, who underwent a neuroimaging battery as part of ongoing research at Cognitive Recovery Lab, including high resolution T1 and T2-FLAIR-weighted scans. In order to measure the leukoaraiosis, scans were visually inspected and the right brain hemisphere only was scored on three different qualitative scales: 1- Fazekas Deep White matter hyperintensities (DWMH, Fazekas et al. 1987) ranged from 0 to 3, 2- Fazekas Periventricular Hyperintensities (PVH, Zimmerman et al. 1986) ranged from 0 to 3, 3- Manolio ranged from 0 to 9 (Manolio et al. 1994). The Western Aphasia Battery-Revised (WAB) was used to quantify aphasia severity. We constructed regression models predicting each WAB measure (WAB Aphasia Quotient (AQ)and the four subscores) based on each of three different scales for SVID. All models included lesion size (mean:107.2 cc, SE: 93.4), age (mean: 61.4 years, SE: 11.3) and months since stroke (mean: 57.6, SE: 64.7) as additional predictors. There were negative effects of leukoaraiosis on different measures of the WAB-AQ, with a reduction of 21 points for patients with grade 3 of Fazekas scales (both DWMH and PVH) compared to patients with grade 1 Fazekas scales). There was no effect of Manolio scale scores on WAB scores. The results for sub-scores were similar in terms of statistically significant effects, but different in magnitude. Lesion volume was inversely related to all WAB scores. Months since stroke was positively related to some WAB scores. Severity of aphasia after stroke relates to severity of both deep and periventricular leukoaraiosis based on the Fazekas scales but not Manolio scale. Objective quantitative measures of leukoaraiosis should be investigated, as they may provide greater fidelity than rating scales and could reveal more specific relationships between white matter disease severity and aphasia outcomes.

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Poster

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Program #/Poster #: PSTR441.10/WW31

Topic: H.11. Language

Title: Cross-linguistic differences in neural encoding and processing of stop consonants: the impact of language experience on attention allocation

Authors: *A. OLIVEIRA[1], Y. KOTHARI[1], T. BLODER[2], Y. SHINOHARA[3], V. SHAFER[1], P. CUNHA[1];
[1]The Grad. Center, CUNY, New York, NY; [2]Catholic Univ. Eichstätt-Ingolstadt, Eichstätt, Germany; [3]Waseda Univ., Tokyo, Japan

Abstract: This study aimed to investigate the impact of language experience on the neural encoding and processing of stop consonant speech sounds. While previous research indicated minimal differences in P1 and N1 amplitude and latencies, recent studies have suggested that bilingual or nonnative experience may induce an Nd effect (Datta et al., 2020). The Nd effect refers to heightened negativity in the Auditory Event-Related Potential (AEP) when attending to a stimulus compared to ignoring it. Yet, it remains unclear whether the Nd effect is influenced by bilingualism itself or the nonnative nature of the target speech sounds. Thus, the present study examined cross-linguistic disparities in AEPs to three types of stop consonants: short lag [pa], pre-voiced [ba], and long lag aspirated [p^ha]. Participants included English monolinguals, Hindi, Spanish, and Portuguese bilingual listeners. All language groups shared the [pa] phoneme, while Hindi and English included [p^ha], and Portuguese, Spanish, and Hindi included [ba]. Electroencephalogram (EEG) data were collected from 42 participants (approximately 10 per group) using 17 scalp electrodes, with a sampling rate of 1000 Hz and a low-pass filter set at 100 Hz. Participants ignored the speech stimuli and watched a muted movie. The data were cleaned and re-referenced to Oz. Results revealed greater negativity in P1 and N1 amplitudes for bilingual participants compared to monolinguals (by approximately 2 μV). Moreover, P1 latencies were approximately 20 ms earlier for bilinguals compared to monolinguals. These findings suggest that bilingual listeners allocate attention differently when processing speech stimuli.

Disclosures: A. Oliveira: None. **Y. Kothari:** None. **T. Bloder:** None. **Y. Shinohara:** None. **V. Shafer:** None. **P. Cunha:** None.

Poster

PSTR441. Language Processing and Production

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Program #/Poster #: PSTR441.11/WW32

Topic: H.11. Language

Support: JSPS KAKENHI 21H00525

Title: Effect of the temporal processing on the time course of effective connectivity for understanding implicit intention of a speaker in discourse.

Authors: *S. TOKIMOTO[1], N. TOKIMOTO[2];
[1]Mejiro Univ., Tokyo, Japan; [2]Shobi Univ., Saitama, Japan

Abstract: In everyday speech, speakers often communicate their intentions implicitly by indirect expressions (e.g., interrogatives to request something, "Can you reach the salt?"). The speaker's implicit intention is assumed to be derived by pragmatic inference, but the details of this inference are unknown. To discuss the neural mechanisms of the inference, we analyzed the EEG associated with the understanding of a speaker's implicit intentions in discourse, paying attention to temporal processing. Experimental discourses with three speakers were manipulated by two factors: (1) the context to derive a speaker's intention from to be explicit or implicit. With the implicit context, higher-order inference is assumed; and (2) a speaker's intention referring to past or future behavior. EEGs of 24 Japanese native speakers were recorded during the auditory comprehension of the discourse by 64 electrodes on the scalp. We placed 34 regions of interest (ROIs) in the brain, referring to recent fMRI studies on understanding indirect utterances and episodic memory retrieval. After the source localization of the EEG, we examined information flow between the 34 ROIs by calculating partial directed coherence (PDC) for the theta, alpha, beta, and gamma bands every 200 ms after the onset of a critical word, at which the speaker's intention (Yes or No) was recognized. To examine the effect of context implicitness on the inference, we calculated the difference in PDC between explicit and implicit conditions for past and future discourse, respectively. As a result, a significant directed network and its time course for four EEG bands were depicted as a manifestation of the contextual effect. The significant PDC differences corresponding to the contextual implicitness were both positive and negative, indicating that higher-order inference should be understood not only as an increase in information flow, but also as a decrease. Significant PDC differences were observed between multiple ROIs up to 900 ms after the critical word in past discourse, but up to 500 ms in future discourse, suggesting that the time course of the two processing were different with each other. Furthermore, the ROIs that functioned as hubs in past discourse were right inferior frontal gyrus (IFG, BA 47), left IFG (BA 10), left caudate, right insula (BA 13), and left medial prefrontal cortex whereas in future discourse they were left cingulate gyrus (BA 31), left IFG (BA 47), left middle temporal gyrus (BA 21), left superior frontal gyrus (BA 10), left fusiform gyrus (BA 20), and right precuneus (BA 19), suggesting that apparent formal similarity in inference differs significantly in terms of the network of neural activity.

Disclosures: S. Tokimoto: None. **N. Tokimoto:** None.

Poster

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Program #/Poster #: PSTR441.12/WW33

Topic: H.11. Language

Title: Investigating the neural architecture of speech processing pathways

Authors: *P. NIKOLOV[1], J. LIN[1], C. CORNELL[1], K. YOON[1], S. KIM[1], P. COX[2], S. DAMERA[3], J. RAUSCHECKER[1], M. RIESENHUBER[1];
[1]Georgetown Univ. Med. Ctr. Interdisciplinary Program In Neurosci., Washington, DC; [2]Neurosci., Lehigh Univ., Bethlehem, PA; [3]Children's Natl., Washington, DC

Abstract: Introduction:In current “dual-stream” models of language processing in the brain, speech perception and speech production are thought to be subserved by the anteroventral and dorsal streams, respectively. Computational models of speech processing (“internal forward and inverse models”) propose dynamic interactions of neural representations involved in speech perception and speech production. Yet, despite their importance for speech processing and acquisition, these interactions are still poorly understood. Motivated by a computational model (internal model) framework, we investigated neural signal dynamics and selectivity in an EEG experiment (N=20) involving the use of stimuli optimized to differentially activate perceptual and articulatory neural representations.
Methods:Our preliminary analyses are based on a sample of 20 healthy L1-English speaking adults who completed speech perception and production tasks while their neural activity was recorded using a 64 channel Biosemi Active2 EEG system. A novel aspect of our experiment was the design of stimulus sets optimized to differentiate perceptual (‘percDiff’) and articulatory (‘prodDiff) neural representations. Overt production onset times were determined by three expert manual raters. EEG data were preprocessed using EEGLAB. Using FieldTrip, cluster-based permutation statistics were run using a cluster-identification threshold of 0.01. Overt production was analyzed from 200 to 120ms prior to acoustic production onset, and perception data were analyzed from 0 to 300ms post-stimulus onset.
Results and Discussion: Perception task: Preliminary results reveal three distinct and significant clusters when listening to percDiff word pairs; around 150ms over central channels we found a cluster ($p = 0.009$) that showed larger differences between the percDiff but no significant difference for prodDiff word pairs. Later, at 210 and at 240ms over central/parietal channels we found two distinct clusters, ($p=0.028$ and $p=0.015$, respectively) that did show a significant difference between prodDiff as well as percDiff pairs. For the prodDiff case, we found one significant cluster ($p=0.015$) between 210-240ms that showed significant difference between prodDiff word pairs overt frontal channels.These results are compatible with model predictions of an initial perceptual processing of auditory signals, followed by sensorimotor transformations via the parieto-frontal dorsal stream.Overt production task: Ongoing analyses are investigating signal flow across the hypothesized two processing streams and the transformation between perceptual and articulatory representations.

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Poster

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Program #/Poster #: PSTR441.13/WW34

Topic: H.11. Language

Title: Effects of Second Language Learning with a Cartoon Cloze Test Examined by EEG Functional Connectivity Analysis

Authors: *N. NAGAIGUCHI[1], S. YAZAWA[3], K. HIRAKI[3], S. SHIMADA[2];
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Abstract: The Cloze Test is a type of fill-in-the-blank test in which blanks are placed in sentences and is an efficient method for assessing language proficiency. The test is expected to improve language proficiency by guessing a word from the preceding and following sentences. Nowadays, e-learning (studying with tablets, smartphones, etc.) and fun materials like manga, are integrated in the field of education. In this study, we examine the effects of the second language learning training using the Cartoon Cloze Test app by measuring the changes in brain activity. 24 healthy males and females (mean age 23.5 ± SD 4.29) whose second language was English participated in the experiment. The participants' brain activity was measured using electroencephalography (EEG) while solving English tasks before and after a one-month training period (pre- and post-test). The training consisted of English composition and the Cartoon Cloze Test in which blanks were provided in the cartoon dialogues. In the pre- and post-tests, the participants had to answer two types of Cloze Tests: the multiple-choice test, in which they had to select the answer from a list of choices, and the fill-in-the-blank test, in which they had to enter the appropriate word. Functional connectivity analysis was conducted based on the EEG data measured during the pre- and post-tests. The calculated connectivity was compared between the pre- and post-tests. In addition to the tests, a test measuring the individual's overall English proficiency was administered before and after the training. The test scores measuring overall English proficiency and the fill-in-the-blank test showed a statistically significant improvement in the post-test. In the post-test, the EEG analysis showed a significantly higher connectivity in the left frontal lobe for both types of the Cloze Tests. Moreover, there was a significantly higher connectivity between the left frontal and right temporal lobes only for the fill-in-the-blank test. The left frontal lobe is related to analyzing vocabulary, while the right temporal lobe is related to analyzing grammar. Unlike the multiple-choice test, the fill-in-the-blank test requires participants to guess the appropriate word for the blank from the context and to change the word form according to subject, tense, etc. by themselves. Therefore, the training may have improved the bilateral connectivity related to analyzing vocabulary and grammar. These findings suggest that training with the Cartoon Cloze Test enhances functional connectivity in the brain regions related to syntactic parsing and semantic decision, and improves proficiency in the fill-in-the-blank test and overall English test.

Disclosures: **N. Nagaiguchi:** None. **S. Yazawa:** None. **K. Hiraki:** None. **S. Shimada:** None.

Poster

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Program #/Poster #: PSTR441.14/WW35

Topic: H.11. Language

Support: NIH DP5 OD031843
Army xTech BOLT prize competition (Army Medical Research & Development Command)

Title: Dynamics of discourse comprehension: real-time neurobiological insights from fused fMRI/EEG analysis

Authors: *M. HONG, K. ABOUD;
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Abstract: Approximately 43 million U.S. adults struggle with basic text comprehension. Despite a wealth of research exploring the neural underpinnings of reading disorders in children, reading comprehension in adults remains under-studied. This study fills the gap, applying high-resolution fused MRI-EEG analysis to identify neural mechanisms underlying reading comprehension in adults with varying range of reading abilities. We examined typical adults as they read medical passages in the MRI, and in a separate session, while EEG data was collected. Joint Independent Component Analysis (ICA) revealed comprehension variations associated with differences in network exchanges related to word reading and oral language between groups with lower and higher reading comprehension abilities, with early and late signals involved. These exchanges interacted with executive function regions, aligning with the Simple View of Reading model. This study underscores the complexity of neural network interactions in adult reading comprehension and suggests potential avenues for developing targeted interventions catered to adults with varying levels of reading comprehension ability. This investigation offers crucial contributions to our understanding in the field of neuroscience and brain-based reading comprehension interventions.

Disclosures: M. Hong: None. **K. Aboud:** None.

Poster

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Topic: H.11. Language

Support: NIH Grant DP1HD091948
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NIH Grant R01NS109367-01

Title: Unraveling the Neural Basis of Everyday Conversations through Deep Speech-to-Text Models

Authors: A. GOLDSTEIN[1,2], ***L. NIEKERKEN**[3,4], H. WANG[3], B. AUBREY[3], Z. ZADA[3], T. SHEFFER[2], S. A. NASTASE[3], A. SINGH[3], H. GAZULA[5], M. SCHAIN[2], A. RAO[3], G. CHOE[3], C. KIM[3], W. DOYLE[6], D. FRIEDMAN[6], S. DEVORE[6], P. DUGAN[6], A. HASSIDIM[2], M. BRENNER[2,7], Y. MATIAS[2], O. DEVINSKY[6], A. FLINKER[6], U. HASSON[3];
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Abstract: Humans effortlessly transduce the continuous acoustics of speech into rich linguistic meaning during everyday conversations. In this study, we leverage 100 hours (half a million words) of spontaneous open-ended conversations and concurrent high-quality electrocorticography recordings to decipher the neural basis of real-world speech production and comprehension. By capturing unconstrained conversations in real-world settings, we encompassed the richness and diversity of human speech production and comprehension. Employing Whisper, a deep multimodal speech-to-language model, we developed electrode-wise encoding models capable of accurately predicting neural responses to both acoustic and semantic aspects of speech, with single-electrode correlations up to 0.5 in precentral gyrus and superior temporal gyrus and 0.35 in inferior temporal gyrus. We uncover a distributed cortical hierarchy in speech and language processing, with sensory and motor regions involved in speech processing and language areas engaged in semantic and contextual processing. Moreover, we identify distinct regions responsible for speech production and comprehension. Our findings demonstrate mixed selectivity in most electrodes, indicating overlapping processing of speech and linguistic content, as well as speech production and comprehension. Notably, our encoding model allowed us to track processes of speech planning for the first time. We observed a temporal gradient of information processing, where encoding in high-level brain areas such as inferior temporal gyrus ($M = -480$ ms, $SD = 284$ ms) peaked before motor areas ($M = -305$ ms, $SD = 391$ ms, $t(80) = -1.95$, $p < 0.05$) and auditory areas ($M = -250$ ms, $SD = 307$ ms, $t(48) = -2.72$, $p < 0.005$) during production. This study offers a comprehensive account of the unfolding neural responses during fully natural, unbounded daily conversations. By leveraging a multimodal deep learning approach, we highlight the power of deep learning in unraveling the neural mechanisms underlying natural language processing in the human brain. Our research provides valuable insights into the unique complexity and dynamics of human speech processing, contributing to our understanding of language in real-world contexts.

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Poster

PSTR441. Language Processing and Production

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR441.16/WW37

Topic: H.11. Language

Support: Becas Nacionales para Estudios de Posgrado (Conacyt)

Title: The effect of prosody on figurative language comprehension: the case of albures

Authors: *E. RUIZ ALANIS, A. REYES-AGUILAR;
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Abstract: A set of elements that permit the non-literal interpretation of a speech act are necessary for understanding figurative speech. *Albur* (*albures* in plural) is a word play with scatological or sexual connotations that is typical of Mexican Spanish. Prosody is a key element of *albures* because it makes it easier to interpret them in a non-literal manner.To better understand the influence of prosody in the comprehension of *albures*, we performed a behavioral test to validate a set of stimuli (*albures*) for their further use in an MRI protocol to understand the neurocognitive basis of albur comprehension. Participants watched a video with either 37 or 38 stimuli, and were instructed to hit the space bar each time they recognized an *albur*. Because of the distinctive intonation of albures, we observed that the stimuli with the highest rate of detection contained the most salient prosodic cues. However, only slightly, the detection was also influenced by facial and hand gestures. Additionally, we found that the stimuli can be split into two categories: *albures* that depended on morphological reanalysis, which were the ones with the highest detection rate, and *albures* that relied on polysemy.

Disclosures: E. Ruiz Alanis: None. **A. Reyes-Aguilar:** None.

Poster

PSTR441. Language Processing and Production

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR441.17/WW38

Topic: H.11. Language

Support: Institutional Funds Penn State College of Medicine

Title: Differing contributions of acoustic parameters to sound-symbolic associations for shape- and size-optimized pseudoword sets

Authors: *S. NAYAK[1], S. LACEY[1], L. NYGAARD[2], K. SATHIAN[1];
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Abstract: Sound-symbolism is the idea that there is a non-arbitrary relationship between the meaning of a word and its sound. It has been most commonly studied using pseudowords signifying shape or size. Previously, we analyzed the contributions of the acoustic parameters of a set of 537 consonant-vowel-consonant-vowel (CVCV) pseudowords, created from phonemes with established sound-symbolic shape associations and thus optimized for the shape domain, to their ratings on a rounded/pointed scale (Lacey et al., Cognitive Science, 2020). Here, we extended this analysis to a new set of 638 CVCV pseudowords optimized for sound-symbolic size associations. This new set was created using the same phonemes as the prior set but included three additional vowels with established size associations; each pseudoword in the new set contained one of these vowels in the first, second, or both vowel positions. Participants listened to the pseudowords and rated them on scales capturing the categorical opposites of the shape (rounded, N=30; pointed, N=27) or size (small, N=28; big, N=29) domains. We measured both vocal (mean pitch, pitch standard deviation, pulse number, fraction of unvoiced frames [FUF], jitter, shimmer, mean autocorrelation, mean harmonics-to-noise ratio [HNR], and duration) and spectro-temporal (speech envelope, spectral tilt, and fast Fourier transform [FFT]) acoustic parameters. Vocal parameters were compared to ratings using correlations (Bonferroni-corrected for 9 tests), and spectro-temporal parameters were compared to ratings by computing correlations (Bonferroni-corrected for 3 tests) between the respective representational dissimilarity matrices (RDMs). For the shape-optimized set, all vocal parameters, except for mean pitch, contributed significantly to their shape ratings, but none contributed to their size ratings. For the size-optimized set, all vocal parameters, except for mean pitch, again significantly contributed to shape ratings; but, importantly, duration, FUF, jitter, mean autocorrelation, and mean HNR were significantly correlated with size ratings. All three spectro-temporal parameters contributed significantly to both shape and size ratings in both pseudoword sets. These results indicate the differing contributions of acoustic parameters to sound-symbolic associations of pseudowords for the size and shape domains.

Disclosures: **S. Nayak:** None. **S. Lacey:** None. **L. Nygaard:** None. **K. Sathian:** None.

Poster

PSTR441. Language Processing and Production

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR441.18/WW39

Topic: H.11. Language

Support: Institutional funds from Penn State College of Medicine

Title: Acoustic stimulus parameters underlying sound symbolism in words and pseudowords

Authors: *J. DORSI[1], S. LACEY[2], L. NYGAARD[3], K. SATHIAN[2];
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Abstract: Sound symbolism, the idea that words sound like their meanings (e.g., "knife" *sounds* pointy; "ball" *sounds* round), has mainly been studied in the context of pseudoword ratings for dimensions such as roundedness or pointedness. However, sound symbolism in real words is still not well understood. Here, 24 participants (20 female) rated the *sounds* of real words and pseudowords on a "very round" to "very pointy" scale. We used 160 real words (nouns selected from the list of Sidhu et al., Psychonomic Bulletin & Review, 2021, ranging from very round [e.g., "olive"] to very pointy [e.g., "fork"]) and two classes of non-words: 320 consonant-vowel-consonant-vowel (CVCV) pseudowords created from phonemes with established sound-symbolic associations for shape, and 160 'derived' pseudowords created by replacing some of the phonemes of the real words to make highly word-like pseudowords (e.g. “usive” from “olive” & “foft” from “fork”). We tested the correlations between participants’ round/pointy ratings and twelve different acoustic stimulus parameters: three spectro-temporal (speech envelope, fast Fourier transform [FFT], and spectral tilt); and nine related to voicing (duration, mean pitch, pitch standard deviation, pulse number, fraction of unvoiced frames [FUF], jitter, shimmer, mean autocorrelation, and harmonics-to-noise ratio [HNR]). For the CVCV pseudowords, we found strong and significant correlations between representational dissimilarity matrices (RDMs) of ratings and the spectro-temporal properties of FFT and speech envelope, replicating prior findings (Lacey et al., Cognitive Science, 2020), and a weaker but still significant correlation with spectral tilt. For the real words and the derived pseudowords, the correlations between RDMs of rating and all three spectro-temporal parameters were small, challenging the import of these parameters on shape ratings for these stimuli. For the CVCV pseudowords, we replicated the significant correlations reported by Lacey et al. (2020) for pulse number, FUF, jitter, shimmer, mean autocorrelation, and HNR. The derived pseudowords had largely similar correlations with ratings for these vocal parameters. Importantly, these vocal parameters also correlated significantly with ratings of the real words. These results suggest that physical properties of the speech signal influence how round/pointy real words sound, offering key support for sound symbolism in real language.

Disclosures: J. Dorsi: None. **S. Lacey:** None. **L. Nygaard:** None. **K. Sathian:** None.

Poster

PSTR441. Language Processing and Production

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR441.19/WW40

Topic: H.11. Language

Support: Institutional funds Penn State College of Medicine

Title: Acoustic parameters underlying sound-symbolism in English nouns: a machine learning approach.

Authors: *V. KUMAR[1], J. DORSI[3], S. LACEY[2], L. NYGAARD[4], K. SATHIAN[5];
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Abstract: Sound symbolism refers to the non-arbitrary association between the sound of a word and its meaning (e.g., pseudowords like "lomo" and "teke" sound rounded and pointed, respectively). Previously, using the machine learning "K-nearest neighbors" (KNN) algorithm, we found a combination of acoustic parameters that strongly predicted how rounded/pointed a set of 537 consonant-vowel-consonant-vowel (CVCV) pseudowords sounded. However, it remains unclear whether these acoustic properties also mediate sound-symbolic associations found in natural language (e.g. for real words such as "balloon" and "spike"). Unlike pseudowords, measuring sound symbolism in real words is confounded by semantic knowledge; listeners might indicate that "balloon" *sounds* round because they know it refers to a round object. To understand what acoustic features effectuate sound-symbolic associations in real words, we selected 160 nouns (from the list of Sidhu et al., Psychonomic Bulletin & Review, 2021) that ranged in ratings of meaning from very round (e.g. "olive") to very pointy (e.g., "fork"). Next, we created a set of 160 pseudowords by altering the selected real words to make word-like pseudowords ("derived pseudowords"; e.g. "usive" & "foft"). Finally, we chose 320 CVCV pseudowords from our prior work. We asked 24 participants (20 female) to rate the sounds of all our stimuli on a "very rounded" to "very pointy" scale. We used the KNN algorithm to identify the optimal combination of parameters that predicted sound ratings for each stimulus type. The parameters were the same acoustic properties examined in our prior work: three spectro-temporal (fast Fourier transform [FFT], spectral tilt, speech envelope) and nine related to voicing (harmonics-to-noise ratio [HNR], pulse number, fraction of unvoiced frames [FUF], mean autocorrelation [MA], shimmer, jitter, mean pitch, pitch standard deviation [PSD], and duration). The best parameter combination differed by stimulus type, but with some notable similarities, e.g. FFT, FUF, and HNR were part of the optimal model for all three stimulus types. Finally, we used the optimal KNN model for the derived pseudowords (comprising FFT, FUF, MA, & HNR) to generate predicted sound ratings for the real words. We found that these predicted sound ratings correlated with a measure of the roundedness/pointedness of each word's *meaning* (Sidhu et al., 2021). Using parameters important for predicting the ratings of *pseudoword sounds* makes this analysis unconfounded by any semantic bias. This correlation suggests that words do sound like their meanings, supporting the central claim of sound symbolism.

Disclosures: V. Kumar: None. **J. Dorsi:** None. **S. Lacey:** None. **L. Nygaard:** None. **K. Sathian:** None.

Poster

PSTR441. Language Processing and Production

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR441.20/WW41

Topic: H.06. Social Cognition

Title: Natural and artificial metacognition in humans and Large Language Models

Authors: *S. YOSHIZAWA[1], K. MOGI[2];
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Abstract: In recent years, various AI systems, generative AIs in particular, have made significant progresses. Comparison with humans is important in assessing the ability of AI (Turing 1950), enhancing alignment of AI with humans (Yudkowsky 2016), and facilitating human collective intelligence (Woolley et al. 2010). One of the Large Language Models, Chat Generative Pre-Trained Transformer (ChatGPT, OpenAI 2023) has been examined regarding its cognitive capabilities. In false-belief-tasks (Baron-Cohen 1992) related to the theory of mind (Baron-Cohen et al. 1995), ChatGPT performs robustly in comparison with humans in in-context learning. (Kosinski 2023, Moghaddam 2023), while its vulnerabilities have been reported (Dsiri 2022). ChatGPT is based on the Transformer architecture (Vaswani et al. 2017), a variation of artificial neural networks. Metacognition in neural networks has been discussed in a "what they know and what they do not know" context (Clark and Karmiloff-Smith 1993, Fleming 2021). Confidence judgments on a particular knowledge is a typical example of metacognition. (Kepecs and Mainen 2012). Here we examined the generic metacognitive abilities of Large Language Models, by analyzing the confidence judgments of ChatGPT. We used the retrospective confidence judgments (RCJs) (Hart 1965) to make comparisons between typical human subjects and ChatGPT. We designed the task with different degrees of uncertainty based on the “Uncertainty Response (UR)” paradigm (Smith and Washburn 2005), which has been used to measure metacognition in animals with quantitative evaluation of the subjects' confidence rates under variable contexts such as “post-decision wager” paradigms. (Persaud 2007). As ChatGPT is context-sensitive and order-sensitive, a robustness check was conducted to evaluate the degree of genericity and universality of the responses of the generative AI to prompts. Based on the results, we analyze the metacognitive capabilities of the currently available Large Language Models such as ChatGPT. We make comparisons with human metacognitive abilities subserved by neural networks including medial and lateral prefrontal cortex, precuneus, and insula (Vaccaro and Fleming 2018). Finally, we discuss the role of language in artificial and natural intelligence, considering the related brain circuits involved in the latter (Fernandez-Duque et al. 2000).

Disclosures: S. Yoshizawa: None. **K. Mogi:** None.

Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR442.01/WW42

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: A complete pipeline for high-plex spatial proteomic profiling and analysis of neural cell phenotypes on a Spatial Molecular Imager and a Spatial Informatics Platform.

Authors: *S. BONNETT, A. ROSENBLOOM, M. CONNER, T. PHAN-EVERSON, Z. LEWIS, G. ONG, Y. LIANG, E. BROWN, L. PAN, A. WARDHANI, M. KORUKONDA, C. BROWN, D. DUNAWAY, E. ZHAO, D. MCGUIRE, S. WOO, B. FILANOSKI, R. MEREDITH, K. CHANTRANUVATANA, B. BIRDITT, H. YI, E. PIAZZA, J. REEVES, J. LYSSAND, V. DEVGAN, M. RHODES, G. GEISS, J. BEECHEM;
NanoString Technologies, Inc., Seattle, WA

Abstract: The brain is complex and heterogeneous where cell function and cell-to-cell communication are critical for rapid and accurate performance. The ability to explore protein-driven activities at high resolution within the spatial context of their immediate environment is critical to gain comprehensive pictures of brain development, activity, aging, disease or dysfunction, and inflammatory responses. Many existing approaches for high-plex single-cell spatial proteomics face issues around simplicity, speed, scalability, and big data analysis. Here, we present an integrated workflow that addresses key concerns around high-plex proteomics. The CosMx™ Spatial Molecular Imager (SMI) and AtoMx™ Spatial Informatics Platform comprise an end-to-end workflow that efficiently handles highly multiplex protein analysis at plex sizes exceeding 68 targets. The CosMx protein assays use oligonucleotide-conjugated antibodies, that are detected using universal, multi-analyte CosMx readout reagents. The CosMx Mouse Neural Cell Typing and Alzheimer's Pathology panel is optimized to comprehensively profile neural cell lineages across the brain as well as the progression of Alzheimer's disease (AD). Furthermore, 80% of the antibodies making up both panels are cross-reactive with human tissue antigens. The AtoMx spatial informatics platform provides full analysis support, including whole-slide image viewer, and methods for performing built-in or fully customizable analyses for cell typing, ligand-receptor analysis, neighborhood analysis and spatial differential expression. The CosMx protein assay reagents were validated on the FFPE adult mouse brain, mouse embryo, and Alzheimer's positive human brain. We used the CosMx Mouse Neural Cell Typing and Alzheimer's Pathology panel with the CosMx SMI to identify multiple neuronal subtypes, different reactive states of astrocytes and microglia, cell degeneration and proliferation. In a single-cell exploration of mitochondria, we noted distinct patterning of key immune targets based on their immediate microenvironment. Additionally, evaluated the co-expression patterns and activation states of microglia within 200 µm from the amyloid plaque and tau tangles. CosMx SMI is a high-plex spatial multi-omics platform that enables the detection of > 68 proteins at subcellular resolution. In combination with the high-plex CosMx Mouse Neural Cell Typing and Alzheimer's Pathology panel, we present a flexible and scalable informatics platform, a robust solution for comprehensive neural and disease phenotyping that captures the complexity of neuronal and glial cellular activity with full spatial context.

Disclosures: S. Bonnett: A. Employment/Salary (full or part-time):; NanoString. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString, NanoString. **A. Rosenbloom:** A. Employment/Salary (full or part-time):; NanoString. E. Ownership Interest (stock, stock options,

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Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR442.02/WW43

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: High-plex in situ proteogenomic profiling of human brain sections with spatial molecular imaging

Authors: *A. HECK, K. YOUNG, A. ROSENBLOOM, A. WARDHANI, M. WALTER, G. ONG, R. KHAFIZOV, M. KORUKONDA, Z. LEWIS, T. PHAN-EVERSON, S. BONNETT, C. WILLIAMS, P. DANAHER, R. LIU, J. CEJA NAVARRO, C. KANG, J. REEVES, G. GEISS, D. W. RUFF, J. M. BEECHEM;
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Abstract: Spatially resolved transcriptomics and proteomics work in concert to create a full biological picture, including cell function, cell activity and cell-to-cell communication. The ability to profile numerous RNAs and proteins on the same tissue section in spatial context is especially advantageous for dissecting neurobiological mechanisms that are largely dependent upon spatial relationships.
Here, we demonstrate the “proteogenomic” capability of the CosMx™ Spatial Molecular Imager (SMI), a single-cell spatial biology platform that leverages the cyclic in situ hybridization chemistry to enable high-plex detection of RNAs and proteins at subcellular resolution. In this proteogenomic assay, 40+ proteins and 6,000 RNAs are targeted on the same FFPE human brain section, delivering highly quantitative results. The 40+ target proteins focus on neural cell typing

and neurodegenerative disease pathology (including amyloid precursor protein, amyloid beta, and phosphorylated tau variants) and are labeled with oligonucleotide-conjugated antibodies. These antibodies are detected via several rounds of reporter-probe binding and fluorescence imaging on the SMI instrument. The same brain section undergoes hybridization with probes targeting 6,000 RNAs that cover broad biological areas and emphasize neuroscience. The >4,900 neuroscience-related genes targeted cover >80 pathways and enable robust neuronal and glial cell typing as well as exploration of key ligand-receptor interactions. As RNA and protein SMI assays utilize the same reporter chemistry, RNA detection occurs on the SMI instrument using the same readout reagents. The SMI proteogenomic approach uses high-plex protein data from a large area of tissue to identify smaller regions of interest on the same slide for 6,000-plex RNA profiling, which offers a more comprehensive view of the cell functions and states observed in the protein assay.
Furthermore, this proteogenomic approach enables enhanced cell segmentation accuracy. The abundant cellular information from the protein data can be used alongside a new cell segmentation framework that leverages data from morphology staining (i.e. GFAP, IBA1, NeuN or OLIG2, and S6) and the 40+ different proteins measured by the SMI platform. Thus, by combining the high-plex neuro protein and RNA assays, we achieve unparalleled cell segmentation, enabling better assignment of transcripts to cells and opening numerous avenues for biological inquiry. We ultimately demonstrate the use of CosMx SMI to create a spatial cell atlas of the brain, define neighborhoods, and probe numerous pathways and cellular phenotypes.

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Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR442.03/WW44

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Spatially Resolved Single-Cell High Plex Neural Proteomic Analysis Over Extended Human Brain FFPE Tissue Samples

Authors: T. RANE[1], T. PHAN-EVERSON[1], G. ONG[1], Z. LEWIS[1], ***A. ROSENBLOOM**[2], S. A. BONNETT[1], E. PIAZZA[1], P. DANAHER[1], A. WARDHANI[1], M. KORUKONDA[1], C. BROWN[1], C. KANG[1], J. LYSSAND[1], J. JUNG[1], G. GEISS[1], D. W. RUFF[1], D. DUNAWAY[1], J. M. BEECHEM[1];

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Abstract: The human brain features long-ranging complexities where critical cell function and cell-to-cell communication take place over relatively large interstitial distances and far from the cell somas. The ability to explore protein-driven activities at high resolution, within the spatial context across a large environment and passing through multiple microenvironments, allows a comprehensive picture of brain biology to emerge and is applicable to a myriad of open questions regarding brain development, activity, aging, and neurodegeneration. The CosMx™ Spatial Molecular Imager (SMI) efficiently handles highly multiplex protein analysis on FFPE tissues at > 68-plex. The CosMx protein assays use oligonucleotide-conjugated antibodies that are detected using universal, multi-analyte CosMx readout reagents. Standard CosMx SMI assays make use of standard glass microscope slides with attached flow cells, with 300 mm^2 of the imaging area. The flow cell is mounted within an observation chamber fitted with control valves to precisely deliver cell preparation and analyte detection reagents, and a high-resolution fluorescent microscope linked to a high-capacity data collection computer for further computational analysis of captured images. However, the size, complexity, and heterogeneity of the human brain from one area to another further drive the need to sample much larger tissue sections. Here we demonstrate a novel Large Surface Area flow cell with > 1,600 mm^2 of the imaging area, representing a > 5-fold increase in the imaging area. To enable this large imaging surface area, we developed a custom flow cell larger in size than standard glass slides and optimized the flow-cell design and reagent-delivery conditions to ensure uniform reagent delivery throughout the large-sized samples being analyzed. Combining the Large Surface Area flow-cell imaging capabilities with our human-specific > 68-plex CosMx SMI Human Neural Cell Profiling and Alzheimer's Pathology Protein Panel, we demonstrate spatial imaging of key neural cell typing (GFAP, Iba1, NeuN), and disease-specific targets (APP, Amyloid Betas, Tau), and key post-translational modifications. Advanced neuronal segmentation algorithms allow for specific tracing and segmentation of single neurons, including axons, across hundreds of micrometers of space. The ability to image and evaluate 5-fold larger sections of human brain tissues with unparalleled spatial context and high-plex analyte content allows the grander scale of disease anatomy and processes to be cataloged with exquisite precision and accuracy.

Disclosures: **T. Rane:** A. Employment/Salary (full or part-time):; Nanostring Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nanostring Technologies. **T. Phan-Everson:** A. Employment/Salary (full or part-time):; Nanostring Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nanostring Technologies. **G. Ong:** A. Employment/Salary (full or part-time):; Nanostring Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nanostring Technologies. **Z. Lewis:** A. Employment/Salary (full or part-time):; Nanostring Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nanostring Technologies. **A. Rosenbloom:** A. Employment/Salary (full or part-time):; Nanostring Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nanostring Technologies. **S.A. Bonnett:** A. Employment/Salary (full or part-time):; Nanostring Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of

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Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR442.04/WW45

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Alzheimer's Association Research Grant

Title: A Flexible and Cost-Effective Method for Generating Custom 10x Beads to Capture Neuronal Splicing Events at Single Cell Resolution

Authors: K. IRWIN[1], I. SINHA[1], K. BOWDEN[2], P. C. WONG[3], ***J. LING**[1];
[1]Johns Hopkins Sch. of Med., Baltimore, MD; [2]Johns Hopkins Univ., Baltimore, MD; [3]Johns Hopkins Sch. of Med., Sch. of Med., Baltimore, MD

Abstract: Droplet-based RNA sequencing methods, such as the 10x Genomics platform, have revolutionized our ability to profile human tissues at single cell/nuclei resolution. However, 10x single nuclei RNA sequencing (snRNAseq) is currently limited to 5' or 3' capture and is unable to directly target the middle portions of mRNA transcripts. Internal sites of mRNAs contain many features that are relevant to disease such as alternative splicing variants. Our lab has developed a method that can generate gel beads that are coated with both poly(dT) oligos and targeted capture oligos (TaCOs), all of which contain cell barcodes and unique molecular identifiers. By designing TaCOs to anneal directly adjacent to internal sequences of interest, TaCO-Seq can profile any portion of an mRNA transcript. Meanwhile, the remaining polyT probes ensure that simultaneous capture of gene expression is unaffected. TaCO beads are compatible with 10x Genomics Chromium X controllers and can integrate seamlessly with downstream sequencing and analysis pipelines.
As a proof-of-concept, our lab has used TaCO-Seq to capture TDP-43-associated cryptic exons in human brain samples from ALS-FTD and identified differential cryptic exon burden in different cell types. Detection of splicing at single nuclei sequencing will help us clarify the genetic and environmental factors that underlie the onset and progression of TDP-43 cryptic exons in neurodegenerative diseases. Furthermore, TaCO-Seq is a highly flexible and cost-effective method that can be generalized for single cell targeting of any sequences in the genome or transcriptome. We believe TaCO-Seq will be a useful tool to study alternative splicing in the fields of neuroscience, oncology, and the broader research community.

Disclosures: K. Irwin: None. **I. Sinha:** None. **K. Bowden:** None. **P.C. Wong:** None. **J. Ling:** None.

Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR442.05/WW46

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Spatial validation of Evercode™ split-pool combinatorial barcoding with RNAscope™ multiplex assay in adult mouse brain cryosections

Authors: *A. SAPRE[1], S. DESHPANDE[2], S. MARRUJO[1], M. SAMBHI[2], L.-C. WANG[2], G. DEMIRKAN[1], M. SRINIVASAN[2], A. ROSENBERG[1];
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Abstract: Single nuclei RNA sequencing (snRNA-seq) using Evercode™ combinatorial barcoding is a powerful tool to capture transcriptomes from up to 1 million nuclei to identify both known and novel cell types. Capturing cellular heterogeneity is important for complex tissues such as the mammalian brain where there are defined cell-cell interactions within brain structures. However, traditional snRNA-seq sacrifices spatial information due to the dissociation of samples into single nuclei suspensions. Complementing snRNA-seq with RNAscope™ RNA in-situ hybridization (RNA-ISH) can provide an understanding of the spatial organization of transcriptomes and the ability to visually validate snRNA-seq findings.
To show the power of these complementary techniques we performed both Evercode™ snRNA-seq and RNA-ISH from alternating sagittal sections from a frozen, healthy, adult-mouse brain. First, we developed a protocol to extract nuclei from multiple cryosections to perform nuclei fixation. Fixed nuclei were processed with Evercode™ WT v2 and a subset of barcoded nuclei were sequenced resulting in transcriptomes from 13,800 nuclei with 2,300 median genes/cell. Using this snRNA-seq dataset, we selected a set of highly expressed and differentiating genes to perform RNAscope™ on adjacent sections for granule, Purkinje, oligodendrocyte precursor (OPC), endothelial, glutamatergic, and GABAergic cells. To validate and spatially confirm the presence of these cells, we used the RNAscope™ Multiplex method to detect cell-type markers determined by snRNA-seq and previously established canonical markers. From colocalization patterns, we observed a remarkable concordance of gene expression between snRNA-seq and RNA-ISH, exhibiting both cell specificity and brain region specificity.
In the snRNA-seq dataset, *Car8* was a highly specific and differentiating marker for Purkinje cells, representing only 0.5% of all cells processed. Using RNAscope™ LS Multiplex, we validated *Car8* to be uniquely specific and concordant with *Calb1*, another canonical marker for Purkinje cells. Similarly, we determined *Pde1a* as a marker for glutamatergic cells by snRNA-seq which was found to have region specific co-expression with *Slc17a7* in the cortical layers. Moreover, we determined *Vcan* as a specific marker of OPCs and OPC-like cells by snRNA-seq, which was found to co-localize with *Pdgfra* and both datasets revealed a subset of OPC-like cells that were *Vcan*+ and *Pdgfra*-. These examples highlight the power of complementing snRNA-seq and spatial RNA-ISH techniques to validate biologically relevant findings and understand region specific gene expression.

Disclosures: **A. Sapre:** A. Employment/Salary (full or part-time):; Parse Biosciences. **S. Deshpande:** A. Employment/Salary (full or part-time):; Bio-techne. **S. Marrujo:** A. Employment/Salary (full or part-time):; Parse Biosciences. **M. Sambhi:** A. Employment/Salary (full or part-time):; Bio-techne. **L. Wang:** A. Employment/Salary (full or part-time):; Bio-techne. **G. Demirkan:** A. Employment/Salary (full or part-time):; Parse Biosciences. **M. Srinivasan:** A. Employment/Salary (full or part-time):; Bio-techne. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bio-techne. **A. Rosenberg:** A. Employment/Salary (full or part-time):; Parse Biosciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Parse Biosciences.

Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR442.06/WW47

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Improving Yield and Cell Type Diversity in Single Nuclei RNAseq Studies

Authors: *D. J. ACRI[1], L. DABIN[2], R. MUSTAKLEM[3], J. KIM[4];
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Abstract: Single cell assays for DNA, RNA, and protein provide a rapidly expanding toolkit for understanding neurobiology. These new technologies have made it possible to test new hypotheses concerning rare cell types, cellular responses to disease, and cell-specific mechanisms. However, the accessibility of these types of studies is limited by the cost and quality of cells that can be isolated intact from the dense interconnected cellular matrix of the brain. To overcome this technical hurdle, one popular approach has been to isolate out nuclei from frozen tissue for transcriptomic profiling, termed single nuclei RNA sequencing (snRNAseq). While isolating out nuclei, the presence of ambient RNA and cellular debris can introduce technical artifacts and affect the quality of sequencing. For droplet-based microfluidic systems, this is especially important as debris can clog instruments and lead to the loss of multiple samples. Additionally, the presence of debris can lead to unintentional overloading and the presence of doublets. Therefore, the goal of our study is to test publicly available protocols for efficiency, cost, yield, and overall quality of the data using 10xGenomics Single Cell Gene Expression profiling method. In this study, we directly compare several centrifuge-based and machine-assisted protocols to isolate nuclei from the cortex of 6-month-old wild-type mice. Across all samples, we observed that the presence of debris significantly decreased yield in terms of total nuclei recovered and reads past quality control for ambient RNA via SoupX pipeline. Directly comparing between technologies, we found that centrifuge-based isolation protocols were cost-effective but were significantly variable in total nuclei recovered. In contrast, machine-assisted isolation protocols that did not require staining were the most time-efficient and were able to capture a wider diversity of cell types. Finally, ex-vivo activation of several glial cell types was found to be modulated by the protocol used to isolate nuclei. This study provides valuable benchmarking of cost, time, and quality control metrics associated with nuclei isolation for snRNAseq.

Disclosures: **D.J. Acri:** None. **L. Dabin:** None. **R. Mustaklem:** None. **J. Kim:** None.

Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR442.07/WW48

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Templeton Foundation Grant 62587
NINDS K08 NS128074

Title: Dissecting the impact of non-coding somatic mutations in the human brain via single cell Multiomics paired with duplex-sequencing (Duplex-Multiome)

Authors: *A. J. KRIZ[1], S. MAO[2,3,5,6], D. D. SHAO[1,4], D. A. SNELLINGS[1], R. E. ANDERSEN[1], E. A. LEE[2,3,5,6], C. A. WALSH[1];
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Abstract: While somatic variants have been heavily studied in tumors, their prevalence and significance to other diseases, and in healthy individuals, are less well-understood. Our lab and others revealed that somatic mutation is a widespread phenomenon throughout normal brains. Human neurons each contain 100 or more clonal somatic single nucleotide variants (sSNV) at birth, acquired during prenatal development, and gain 15-20 additional sSNVs arising per year per genome. Despite being the main source of genetic diversity between cells within an individual, the patterns and mechanisms by which somatic variants form in different brain cell types as well as their functional impact are not well understood. Typical methods for detecting somatic variants in single cells do not provide simultaneous gene expression, cell type, or chromatin state information. In order to bridge somatic variant calling, gene expression, and epigenetics we developed the Duplex-Multiome method. In Duplex-Multiome, a strand-tagging step is added to construction of 10X Single Cell Multiome ATAC + Gene Expression libraries. Consensus between both strands of tagmented DNA, or duplexes, can then be applied to single cell ATAC-seq libraries to filter out artifacts created by PCR and sequencing error while maintaining true somatic variant calls. Duplex-Multiome was performed on three post-mortem cortical brain samples ranging from gestational week 14 to 51 years of age, generating data in >10,000 single nuclei representing 13 different cell types. Requiring duplex consensus reduced variant calls likely to be artifacts by up to 10-fold, as well as eliminated mutational signatures resulting from sequencing error. Duplex consensus somatic variant calls further fit known contributors to somatic variation in the human brain such as the clock-like mutational signatures SBS1 and SBS5. Variant calls could be also assigned to cell type as determined by multimodal clustering of single cell ATAC + Gene Expression data. Duplex-Multiome thus enables association of somatic variants with cell type and gene expression in the human brain at a much larger scale than previously possible.

Disclosures: A.J. Kriz: None. **S. Mao:** None. **D.D. Shao:** None. **D.A. Snellings:** None. **R.E. Andersen:** None. **E.A. Lee:** None. **C.A. Walsh:** None.

Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR442.08/WW49

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH NINDS R01NS115017
NIH NINDS R01NS094596

Title: Determination of cell type specific variant burden in a somatic SLC35A2-associated drug resistant focal epilepsy case reveal variant enrichment in non-neuronal cell types

Authors: *D. LAI[1], M. GADE[1], B. E. PORTER[2], E. L. HEINZEN[1];
[1]Univ. of North Carolina, Chapel Hill, NC; [2]Stanford Univ., Stanford Univ., Stanford, CA

Abstract: Germline genetic variants in *SLC35A2*, a Golgi-localized, UDP-galactose transporter essential for cellular glycosylation, have been implicated in one type of congenital disorder of glycosylation associated with intractable seizures and X-linked developmental and epileptic encephalopathy. Recent evidence has recognized somatic, loss-of-function *SLC35A2* variants as an important contributor to drug resistant neocortical epilepsy and in mild malformation of cortical development with oligodendroglial hyperplasia in epilepsy (MOGHE). However, the specific cell types harboring *SLC35A2* variants remain largely under investigated. In this study, we demonstrate cell type specific variant burden in human somatic mosaic brain tissue can be determined using SoMoSeq (Somatic Mosaic tissue Sequencing); a modified G&T-seq method that enables parallel genotyping and full-length cDNA sequencing from single nuclei. Resected brain tissue was obtained from a male with drug resistant focal epilepsy harboring a pathogenic *SLC35A2* variant (c.C435A:p.Y145X) and an age-matched pediatric stroke case (control). Total nuclei were isolated, immunostained with an anti-NeuN antibody and underwent fluorescence-activated nuclei sorting. Forty-six NeuN+ and 46 NeuN- single nuclei were sorted into each well of a 96-well plate along with two mini-bulk (n=50 nuclei) and two blank wells. We collected twenty-four 96-well plates, for a total of 1104 NeuN+ nuclei and 1104 NeuN- nuclei, for SoMoSeq processing where DNA and RNA were physically separated for genotyping and full-length cDNA generation, respectively. We observed a genotyping dropout rate of 39% (857/2208) and a doublet rate of 0.5% (11/2208). Of those that genotyped (n=1340), 92% genotyped as wild-type and 8% genotyped as variant positive (variant allele fraction, VAF = 8%). This VAF is concordant with that in bulk tissue, which was determined as 10% using digital PCR. Interestingly, variant positive nuclei are more prominent in NeuN- (13.5%) compared to NeuN+ (3%) populations, suggesting that the variant is preferentially enriched in non-neuronal cell types. We have selected all *SLC35A2* variant harboring cells (n=91), matched variant negative cells (n=213), and stroke control (n=37) for cDNA library preparation and sequencing on the Illumina NovaSeq6000 to a targeted depth of 8-10 million reads per cell. Analysis of transcriptomic data to robustly classify the specific cell types harboring the *SLC35A2* variants is currently ongoing. This study demonstrates the successful application of SoMoSeq to human somatic brain tissue, providing a powerful tool to quantify cell type specific variant burden.

Disclosures: D. Lai: None. **M. Gade:** None. **B.E. Porter:** None. **E.L. Heinzen:** None.

Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR442.09/WW50

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Investigating Neuroinflammation in the human brain tumor microenvironment using a novel rna-protein co-detection assay

Authors: A. DIKSHIT[1], S. DESHPANDE[1], L.-C. WANG[1], G.-A. KIM[1], J. YU[1], **D. WAKHLOO**[1], *J. PULLIAM[2], M. SRINIVASAN[1];
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Abstract: Glioblastomas are one of the most aggressive forms of brain tumors characterized by distinct genetic and molecular signatures. The progression of these tumors as well as their response to therapy is impacted by the complex tumor-immune interactions in the microenvironment. Compared to other solid tumors, the role of immune cells in progression, invasion and prognosis is not well studied for CNS tumors. Assessing unique features of the brain tumor microenvironment requires a multi-omic strategy to identify unique immune cells infiltrating the tumor and their dynamic interactions with other cells within the tumor. Using the flagship single-cell spatial RNAscope technology, target gene and protein expression can be visualized to characterize cell types and tissue neighborhoods. Here, we demonstrate a novel method for the simultaneous detection of RNA and protein using a modified co-detection assay. With this novel TSA amplification-based co-detection assay we visualized a few combinations of 3 RNA and 3 protein marker panels on human FFPE normal brain and brain tumor tissues. Antibodies targeting key immune cell markers such as CD8, IBA1 and CD68 were used. In addition, neuronal and morphology marker antibodies for PanCK and NeuN were included in the panels. RNA probes targeting chemokines and cytokines such as CXCL10, IFNG, TNFA, CXCL2. IL-6 were also used in the panels. Immune cells infiltrating the brain tumor tissues were characterized by studying co-expression of key RNA and protein markers. The tumors demonstrated infiltration of immune cells such as T cells, microglia and macrophages represented by expression of CD8, IBA1 and CD68 protein markers. In addition, expression of cytokines was used to assess the activation status of these immune cells which is an important indicator for potential success of certain therapeutic interventions. Distinct differences in neuroinflammation signatures were also observed between the normal and tumor brain tissues. The assay offers a powerful technique for visualizing target RNA biomarkers in specific cell-types identified by cell-marker protein expression. This is a valuable tool for multiomic analysis and accurate interrogation of complex tissues such as the brain to obtain insights into novel prognostic and therapeutic biomarkers.

Disclosures: **A. Dikshit:** A. Employment/Salary (full or part-time):; Bio-Techne. **S. Deshpande:** A. Employment/Salary (full or part-time):; Bio-Techne. **L. Wang:** A. Employment/Salary (full or part-time):; Bio-Techne. **G. Kim:** A. Employment/Salary (full or part-time):; Bio-Techne. **J. Yu:** A. Employment/Salary (full or part-time):; Bio-Techne. **J. Pulliam:** None. **M. Srinivasan:** A. Employment/Salary (full or part-time):; Bio-Techne.

Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR442.10/WW51

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: High-plex analysis of expression profiles in human frontal cortex following ischemic stroke at single- cell resolution by spatial molecular imaging

Authors: *O. HO-SHING, M. PATRICK, C. WILLIAMS, A. WARDHANI, C. PHAN, S. ILCISIN, O. APPELBE, S. HE, H. ZHAI, J. LYSSAND, V. DEVGAN, M. RHODES, J. BEECHAM;
NanoString Technologies, Seattle, WA

Abstract: Stroke is ranked as the fifth leading cause of death and the principal cause of long-term disability for adults in the United States. Ischemic stroke occurs due to a blockage or rupture in blood vessels, leading to critical loss of oxygen and glucose to the brain. This triggers the release of free oxygen radicals, disruption of the blood-brain barrier, and ultimately the death of neuronal tissue (infarction). The progression of neural damage after ischemic stroke is mediated through the interaction of immune cells, glial cells, surviving neurons, and the surrounding extracellular matrix. Therefore, a deeper understanding of the impact of ischemic infarction on long-term neuronal function requires a high-resolution analysis of interactions between these spatially and functionally distinct cell types. Here we evaluate the differential expression in the frontal cortex of ischemic stroke patients compared to non-infarct areas, utilizing the CosMx™ Spatial Molecular Imager (SMI). SMI uses high-plex in situ imaging chemistry to provide high-throughput and high-sensitivity single-cell analysis of RNA and marker proteins. The CosMx™ SMI 6,000-plex assay targets critical genes involved in metabolic (>20), immunological (>70), and neurobiological (>80) pathways, providing a comprehensive investigation of single-cell expression in normal and neurodegenerative brain tissue. We characterize the loss of neuronal function in peri-infarct cortical tissue and elucidate post-infarction mechanisms within glial and immune cell types in the surrounding extracellular matrix. We find that peri-infarct cortical regions initiate regulatory expression profiles distinct from non-infarct regions, demonstrating higher integration of astrocytic processes, spatially identified throughout the tissue by immunohistochemical marker GFAP. We also distinguish the extent of the peri-infarct border and find a decrease in typical neuronal signaling pathways within surviving neuronal cells, but a persistent increase in immunological and inflammatory pathways. Altogether, these data demonstrate that spatially characterizing astrocytic and neuronal pathways in post-infarction tissue provides a granular examination of neurodegeneration. These findings may facilitate targeted cell type-specific therapies for patients following ischemic stroke injury. The CosMx™ 6,000-plex assay allowed us to simultaneously visualize RNA transcripts and relevant protein analytes at a single-cell level, providing a quantitative multiomic approach to surveying the heterogeneous environment of brain tissue. FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.

Disclosures: **O. Ho-Shing:** None. **M. Patrick:** None. **C. Williams:** None. **A. Wardhani:** None. **C. Phan:** None. **S. Ilcisin:** None. **O. Appelbe:** None. **S. He:** None. **H. Zhai:** None. **J. Lyssand:** None. **V. Devgan:** None. **M. Rhodes:** None. **J. Beecham:** None.

Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR442.11/WW52

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: 1R21MH126420-01A1

Title: Multimodal profiling of neurons in 3D human cortical organoids using patch-seq

Authors: ***E. BAT-ERDENE**[1], A. J. BORELAND[1], R. GABRIEL III[1], J. LIU[2], A. KREIMER[2], Z. P. PANG[1];
[1]Dept. of Neurosci. and cell biology, Child Hlth. Inst. of New Jersey, Rutgers Robert Wood Johnson Med. Sch., New Brunswick, NJ; [2]Dept. of Biochem. and Mol. Biol., Robert Wood Johnson Med. Sch., Piscataway, NJ

Abstract: The progress in understanding the pathophysiology of mental disorders is hampered by the lack of an integrative understanding of diverse cell types in human brain. Brain neurons exhibit diversity in gene expressions, morphology, and electrophysiology. However, it has been technically difficult to investigate all aspects of neuronal diversity in the same cells. We hypothesize that electrophysiological (and possibly morphological) features of human neurons can be predicted by single-cell transcriptomic profiles. Therefore, the aim of this study is to construct a comprehensive cell atlas of human neurons from 3D brain organoids using multimodal analyses. We used 3D cortical organoids derived from human induced pluripotent stem cells (hiPSC). Patch-seq combining patch-clamp recording, biotin staining, and single-cell RNA sequencing provided a morpho-electric annotation of most transcriptomically defined neural cell types. The electrophysiological features include cell membrane properties, resting membrane potential, action potential, after hyperpolarization potential, postsynaptic currents and, Na+ and K+ whole-cell currents. The morphological features include the soma, dendrites, axon, and axon terminals.We profiled more than 100 human neurons with electrophysiology and single-cell RNA sequencing using SMARTSeq with excellent quality control. The profiles include 3000 to 10000 unique genes from one cell. The neural clusters represented distinct subtypes, including upper and deep cortical neurons, progenitors, and radial-glial cells. Our results suggest that neuronal types in the neocortex represent a diverse pool of cells that consists of distinct non-overlapping subtypes. Ongoing experiments will significantly increase the number of cells with complete profiles to enrich the database.

Disclosures: **E. Bat-Erdene:** None. **A.J. Boreland:** None. **R. Gabriel III:** None. **J. Liu:** None. **A. Kreimer:** None. **Z.P. Pang:** None.

Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR442.12/WW53

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Uncover laminar organization of the developing human neocortex using 6000-plex RNA spatial imaging

Authors: *T. MUKHTAR[1], O. HO-SHING[2], Y. WANG[1], V. UPADHYAY[1], A. KLOCK[2], M. PATRICK[2], S. CHURCH[2], J. LI[1], J. BEECHEM[2], **S. HE**[2], A. R. KRIEGSTEIN[1];
[1]Univ. of California San Francisco, San Francisco, CA; [2]NanoString Technologies Inc., Seattle, WA

Abstract: The human cerebral cortex is composed of billions of morphologically and functionally distinct neurons. These neurons are produced in an organized fashion during development. The neocortex is a 6-layered laminar structure, with a precise anatomical organization ensuring proper function. Each layer is comprised of distinct populations of neurons distinguished by differences in size, shape, connectivity, and gene expression. The complex cognitive functions of the adult neocortex depend on the precise emergence of these properties during development. Elucidating the molecular mechanisms that regulate human neocortical development has been a challenge for many years. Development of the neocortex requires an orchestration of a series of processes including the appropriate generation, migration, positioning of the neurons, acquisition of layer-specific transcriptional hallmarks, and formation of precise axonal projections and networks. Over the past years, fate-mapping, genome-wide analysis, and transcriptome profiling has been used to characterize this neocortical cellular diversity. In our pilot study, we apply the 6000-plex NanoString's CosMx™ SMI, a spatial molecular imaging platform to detect RNA and protein markers in situ at single-cell and subcellular resolution. The pre-defined content from the 6000-plex panel has comprehensive coverage of genes and pathways. The assay utilizes standard IHC-grade antibodies or in situ RNA hybridization probes that are covalently linked to small (~20nm) high information content single-molecule imaging barcodes. The platform's ability to perform high-plex multi-omic imaging with sub-cellular resolution allows the visualization and quantification of targeted RNA and proteins directly from tissue samples. Using second trimester primary brain samples, focusing on the neurogenic and gliogenic periods, we visualize known and novel patterns of RNA transcripts across cell types. We identify transcripts involved in cell-cell interactions, synaptogenesis and cortical layering spanning cortical laminae. Using this platform reiterates fundamental differences between neurogenic and gliogenic radial glia, excitatory neurons, and other cell types, highlighting differences in local transcription between the two significant time periods, across the germinal zones and cortical plate

Disclosures: **T. Mukhtar:** None. **O. Ho-Shing:** None. **Y. Wang:** None. **V. Upadhyay:** None. **A. Klock:** None. **M. Patrick:** None. **S. Church:** None. **J. Li:** None. **J. Beechem:** None. **S. He:** None. **A.R. Kriegstein:** None.

Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR442.13/WW54

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: 1-U01-MH114812-01
1-UM1-MH130981-01

Title: Morphoelectric properties of Layer 4, 5 and 6 intratelencephalic transcriptomic types in the human neocortex

Authors: *R. DALLEY[1], K. HADLEY[2], X.-P. LIU[2], M. MALLORY[2], R. MANN[2], G. WILLIAMS[2], S. WALLING-BELL[2], H. D. MANSVELDER[3], C. P. DE KOCK[3], G. TAMAS[4], J. T. TING[2], T. JARSKY[2], E. LEIN[2], S. SORENSEN[2], B. E. KALMBACH[2], B. LEE[2];
[1]Allen Inst. For Brain Sci., Seattle, WA; [2]Allen Inst. for Brain Sci., Seattle, WA; [3]Vrije Univ., Amsterdam, Netherlands; [4]Univ. of Szeged, Szeged, Hungary

Abstract: Recent advances in transcriptomic studies of the human cortex have provided a universal framework to explore cell type diversity in the brain. Among these cell types, cortical excitatory neurons are known by their signature dendritic arbors, with a pronounced apical dendrite, which compartmentalize and integrate synaptic input to compute and ultimately translate signals to downstream neurons. Using Patch-seq sampling, which facilitates the collection of morphology, electrophysiology, and transcriptomic data from the same neuron, we investigated the morphoelectric and transcriptomic properties of human cortical, intratelencephalic (IT)-projecting excitatory neurons in Layer 4 through 6 (L4-6). IT neurons serve as the recipients of thalamocortical projections and are responsible for cortico-cortico feedback. They also have diverse transcriptomic properties and map to fourteen different IT transcriptomic types based on a reference taxonomy (Hodge et al 2019) and can be roughly aggregated based on their laminar position into L4 IT, L4/5 IT, L4/5 Near Projecting (NP), L5 IT, L6 IT and L6 IT Car3 types. Here we evaluated whether the transcriptomic diversity of these neurons, which account for over half (14 out of 24) of the excitatory transcriptomic types in the taxonomy corresponds to distinct morphoelectric properties. Our findings reveal differences in the tendency of apical dendrites to reach layer 1, apical dendrite orientation, and somato-dendritic morphology across transcriptomic types. We also see subtle shifts in dendrite and soma distribution patterns of different L4 IT transcriptomic types. These patterns suggest that cortical inputs to these neurons are highly topographic. The electrophysiological properties of these neurons also vary in a layer-specific and depth-dependent manner. In summary, the combined transcriptomic and morphoelectric properties of these neurons offer insights into how different

IT neuron types may contribute to the flow of information across cortical circuits within the human cortex.

Disclosures: **R. Dalley:** None. **K. Hadley:** None. **X. Liu:** None. **M. Mallory:** None. **R. Mann:** None. **G. Williams:** None. **S. Walling-Bell:** None. **H.D. Mansvelder:** None. **C.P. De Kock:** None. **G. Tamas:** None. **J.T. Ting:** None. **T. Jarsky:** None. **E. Lein:** None. **S. Sorensen:** None. **B.E. Kalmbach:** None. **B. Lee:** None.

Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR442.14/WW55

Topic: I.08. Methods to Modulate Neural Activity

Title: Spatial Transcriptomic Profiling of the human and mouse retina Prepared with the CryoJane Tape Transfer System using GeoMx DSP and CosMx SMI Spatial Analysis

Authors: *Y. LIANG;
NanoString Technologies, Seattle, WA

Abstract: Spatial Transcriptomic Profiling of the Human and Mouse Retina Prepared with the CryoJane Tape Transfer System using GeoMx DSP and CosMx SMI Spatial Analysis
Yan Liang[1], Charlie Glaser[1], Wei Yang[1], Su Ma[1], Bela Anand-Apte[2], Vera L. Bonilha[2], Sujata Rao[2], William Horrigan[2], Joseph Beechem[1]
[1]NanoString® Technologies, Seattle, WA, USA[2]Dept. of Ophthalmology, Cole Eye Institute, Cleveland, OH, USAThe goal of the study is to identify key transcriptomic markers in the retina by profiling retina layers using spatial transcriptomic analysis at cellular and subcellular levels. Additionally, transcriptomic results from each layer are compared to identify layer-specific characteristics. Human and mouse retina samples, prepared as fresh and fixed frozen, are analyzed using the GeoMx® Digital Spatial Profiler (DSP) with the Mouse Whole Transcriptome Atlas. Furthermore, FFPE mouse retina samples are analyzed to characterize transcriptomic profiles at single-cell and subcellular resolutions on CosMx™ Spatial Molecular Imager (SMI) using the 1,000-plex Mouse Neuroscience Panel.Retina is using Cryo-Jane Taper Transfer system and mounted on adhesive-coated slides using an adhesive tape. This method is used to secure fragile frozen tissue, such as the retina. Human and mouse samples were stained for immunofluorescence microscopy with antibodies that target NF-H, GFAP and NeuN for GeoMx DSP and 18s rRNA, amyloid-beta and GFAP for CosMx SMI. Staining allows for the identification of structural layers in the retina and regions of interest for spatial profiling. On DSP, each sample had three ROIs in each photoreceptor, inner nuclear, and ganglion cell layers, and then, photocleaved oligonucleotides from probes were collected and sequenced for readout. For SMI data analysis, six field of views were placed on each section to cover most regions of multiple layers.Using GeoMx DSP, ~6,000 genes were detected in human retina samples, and approximately 500 genes were detected between the photoreceptor and inner nuclear

layer.CosMx SMI results show we were able to identify cell types (e.g. amacrine, horizontal cell, bipolar cell, ganglion cell) and cell-specific markers for outer nuclear layer, inner nuclear layer and ganglion cell layer. Data between GeoMx DSP and CosMx SMI showed high concordance, identifying multiple biologically relevant genes in each layer. Overall, our data demonstrate that GeoMx DSP and CosMx SMI can identify distinctive, biologically relevant genes in the retina as specifically targeting different morphological structures. In addition, we show the validity of the CryoJane Tape Transfer System using GeoMx DSP and CosMx SMI RNA-based assays.

Disclosures: Y. Liang: None.

Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR442.15/WW56

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Chromatin organization of retinal development at the single-cell level

Authors: *V. TRINH[1], C. P. SANTIAGO[1], R. P. CARMEN[1], D. T. KIM[2], J. P. LING[1], S. BLACKSHAW[1];
[1]Johns Hopkins Univ., Baltimore, MD; [2]Aarhus Univ., Aarhus, Denmark

Abstract: The spatial organization of chromatin in the nucleus dictates gene expression, where interacting elements, such as enhancers and promoters, are situated in close proximity. These configurations can be measured through chromatin conformation capture (3C) and genome-wide Hi-C studies, which have revealed changes in chromatin architecture during lineage commitment, cell differentiation, and neural development. Large-scale changes in chromatin structure, therefore, play an important role in altering gene expression systems and cellular functions. The retina has long been used as a model for studying neurogenesis because it is an easily accessible extension of the CNS. However, since rod photoreceptors make up ~80% of cells in the murine retina, single-cell genomics is necessary to study low-abundance cell types. The Blackshaw Lab has developed an atlas of mouse retinogenesis at the single-cell level using scRNA-seq and scATAC-seq at multiple time points throughout retinal development. Hi-C and single-cell Hi-C has also been conducted in the retina, however, the previous studies only focused on rod photoreceptors and had sparse data on low-abundant cell types. Multiomic studies that correlate different genomic modalities allow for a more comprehensive understanding of gene regulation. Overall, there lacks a single-cell system that unifies measurements in chromatin organization with transcriptomic and other epigenomic modalities. To capture both 3D genome organizational and transcriptomic data in individual cells, we adapt the Hi-C protocol for use in a multiomic, droplet-microfluidic single-cell system. We demonstrate that our droplet-based single-cell Hi-C + RNA-seq protocol is able to capture and correlate DNA contacts and RNA profiles of cells. The method will enable the analysis of local and large-scale chromatin

conformational changes associated with cell type-specific gene expression changes in retinal cells and other heterogeneous tissues.

Disclosures: V. Trinh: None. **C.P. Santiago:** None. **R.P. Carmen:** None. **D.T. Kim:** None. **J.P. Ling:** None. **S. Blackshaw:** None.

Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR442.16/WW57

Topic: D.06. Vision

Support: WorldQuant Foundation
NASA

Title: Spatial and Single-Cell Multiomics of the Mouse Retina Reveals Cellular and Tissue-Level Changes Induced by Spaceflight

Authors: *J. PARK[1], E. OVERBEY[1], J. KIM[1], S. GAENSAN[1], A. KLEINMAN[1], J. PROSZYNSKI[1], G. INGHIRAMI[1], W. YANG[2], C. GLASER[2], Y. LIANG[2], X. W. MAO[3], J. BEECHEM[2], C. MASON[1];
[1]Cornell University: Weill Cornell Med. Col., New York, NY; [2]NanoString Technologies, Seattle, WA; [3]Loma Linda Univ., Loma Linda, CA

Abstract: Spaceflight causes a variety of physiological changes, including vision impairment known as spaceflight-associated neuro-ocular syndrome (SANS). Possible causes include radiation exposure, microgravity, and changes in intracranial venous flow. SANS can lead to optic disc edema, posterior globe flattening, chorioretinal folds, and hyperopic shifts. These changes pose a risk to astronaut health and will likely require mitigation for long-duration spaceflight missions.
To fully understand the pathogenesis of SANS, we profiled gene expression and chromatin profiles of mouse retina from the Rodent Research-6 (RR-6) mission, which flew on the International Space Station (ISS) for 29 days and was harvested after 4 days of return in 2017. Using the NanoString GeoMx® Digital Spatial Profiler, tissue architectures were characterized by Nf-H, GFAP, CD31, and Cyto13 staining to collect whole transcriptome profiles of distinct regions within the mouse retina, including lens, corneal stroma, ganglion, inner nuclear, outer nuclear, photoreceptor, and retinal pigment epithelium. Based on these region-specific expression profiles, the NanoString CosMx™ Spatial Molecular Imager with CosMx RNA Assays are currently being implemented to define associated receptor-ligand and cell-cell interactions.
To match the spatial findings to a complete cellular profile, we utilize 10X Genomics Multiome single-nuclei RNA- and ATAC-sequencing from the opposite (left) eyes that were preserved flash frozen. By integrating multimodal sequencing data with spatially resolved transcriptomics

data, we present a multimodal atlas of mouse retina and demonstrate the impact of spaceflight in a tissue microenvironment and cellular level. Our analysis is ongoing, and we expect to highlight perturbed pathways, including those involved in photoreceptor cell degeneration. We also expect to find molecular targets and insights related to countermeasures for SANS, which will be crucial in preparing and protecting astronaut vision for future spaceflight missions.

Disclosures: **J. Park:** None. **E. Overbey:** None. **J. Kim:** None. **S. Gaensan:** None. **A. Kleinman:** None. **J. Proszynski:** None. **G. Inghirami:** None. **W. Yang:** A. Employment/Salary (full or part-time):; NanoString Technologies. **C. Glaser:** A. Employment/Salary (full or part-time):; NanoString Technologies. **Y. Liang:** A. Employment/Salary (full or part-time):; NanoString Technologies. **X.W. Mao:** None. **J. Beechem:** A. Employment/Salary (full or part-time):; NanoString Technologies. **C. Mason:** F. Consulting Fees (e.g., advisory boards); NanoString Technologies.

Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR442.17/WW58

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Allen Distinguished Investigator Grant

Title: Antibody multiplexing and expansion microscopy enable synaptic-resolution molecularly-annotated connectomics

Authors: ***R. PARK**[1], S. TRUCKENBRODT[2], J. WINNUBST[2], F. RIEGER[3], B. AN[4], K. LEUNG[4], D. LEIBLE[4], S. PANDIT[4], K. LEEPER[2], N. BALASKAS[1], M. S. FEE[4], E. BOYDEN[4,5], J. KORNFELD[3], A. PAYNE[2], S. RODRIQUES[1];
[1]The Francis Crick Inst., London, United Kingdom; [2]E11 Bio, Convergent Res., Alameda, CA; [3]Max Planck Inst. for Biol. Intelligence, Planegg, Germany; [4]McGovern Inst. for Brain Res., [5]Howard Hughes Med. Inst., MIT, Cambridge, MA

Abstract: Recent years have seen extraordinary advances in synaptic-resolution connectomics using electron microscopy (EM). Despite enabling high fidelity reconstruction of synaptic connections, EM connectomics is not intrinsically robust to errors that may arise from tracing, sectioning, and imaging and lacks molecular details that define circuit function, such as ion channels and receptors. Here, we report progress on an effort to achieve scalable synaptic-resolution connectomics combining expansion microscopy, a recently-developed optical super-resolution imaging technique, with multiplexed antibody staining and cellular barcoding. With this approach, we are able to map nanoscale cell morphology and achieve rich *in situ* molecular annotation of cell type markers and synaptic proteins. This method should facilitate reconstruction of synaptic connections and make tracing error-robust via the input of cellular barcodes. Scaling properties for barcoded connectomics suggest the potential to eventually

overtake electron microscopy in volumetric reconstruction speed by 1 to 2 orders of magnitude while also delivering molecular annotations.

Disclosures: **R. Park:** F. Consulting Fees (e.g., advisory boards); E11 Bio, Convergent Research. **S. Truckenbrodt:** A. Employment/Salary (full or part-time):; E11 Bio, Convergent Research. **J. Winnubst:** A. Employment/Salary (full or part-time):; E11 Bio, Convergent Research. **F. Rieger:** None. **B. An:** None. **K. Leung:** None. **D. Leible:** None. **S. Pandit:** None. **K. Leeper:** A. Employment/Salary (full or part-time):; E11 Bio, Convergent Research. **N. Balaskas:** None. **M.S. Fee:** None. **E. Boyden:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Expansion Technologies. **J. Kornfeld:** F. Consulting Fees (e.g., advisory boards); E11 Bio. **A. Payne:** A. Employment/Salary (full or part-time):; E11 Bio, Convergent Research. **S. Rodriques:** F. Consulting Fees (e.g., advisory boards); E11 Bio, Convergent Research.

Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR442.18/WW59

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: U01MH114812
UM1MH130981

Title: Patch-seq technique: the method, parameters, and adaptations for optimal single neuron characterization

Authors: *K. NASIROVA, K. BLAKE, A. MCCUTCHEON, K. HADLEY, R. MANN, B. PAWAR, R. RAJANBABU, M. VANNESS, S. VARGAS, M. CLARK, J. GLOE, W. HO, N. DEE, K. SMITH, J. TING, R. DALLEY, E. LEIN, H. ZENG, T. JARSKY, S. SORENSEN, B. KALMBACH, B. R. LEE;
Allen Inst., Seattle, WA

Abstract: Patch-seq is a technique (Lee, Budzillo, et al. eLife 2021) that collects electrophysiological, transcriptomic, and morphological (MET) data from a single neuron. This multimodal characterization has been instrumental in defining the phenotypic properties of transcriptomic types across regions and species. As we have expanded to different species and brain regions, we have identified and optimized the key parameters to obtain high quality multimodal data from diverse cell types. These parameters include cell/slice health evaluations, an automated electrophysiology acquisition software package with custom modules, and adaptive nucleus extraction methods. Additionally, we have identified decision points in the Patch-seq process depending on the dataset to be acquired. For example, if the goal is to obtain electrophysiology and transcriptomics data (ET), the extraction/retraction period is more simplistic; however, if the goal is to obtain electrophysiology, transcriptomics and morphology

(MET), extra time and a more cautious pipette retraction is needed to preserve the somatic membrane for morphological evaluation. To improve the specificity of Patch-seq targeting, we utilized various forms of genetic tools to target specific cell types. For the mouse neurons, genetic tools such as Cre-lines, retro-orbital injections and intracranial injections of retrograde tracing were utilized to target cell types or projection neurons. For human and non-human primate, we optimized a culture paradigm to extend the use of each case and the opportunity to apply enhancer adeno-associated viruses (AAVs) to label and target cells in a cell type specific manner (Lee, Dalley, et al., BioRxiv 2022). The Patch-seq protocol, its key parameters, and much of the data collected by our team are publicly available. The aim of this project is to assist the larger scientific community in Patch-seq experiments with the hope to foster a collective effort to understand the multimodal properties of neurons across regions and species.

Disclosures: K. Nasirova: None. **K. Blake:** None. **A. McCutcheon:** None. **K. Hadley:** None. **R. Mann:** None. **B. Pawar:** None. **R. Rajanbabu:** None. **M. VanNess:** None. **S. Vargas:** None. **M. Clark:** None. **J. Gloe:** None. **W. Ho:** None. **N. Dee:** None. **K. Smith:** None. **J. Ting:** None. **R. Dalley:** None. **E. Lein:** None. **H. Zeng:** None. **T. Jarsky:** None. **S. Sorensen:** None. **B. Kalmbach:** None. **B.R. Lee:** None.

Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

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Program #/Poster #: PSTR442.19/WW60

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant U19MH114830

Title: The transcriptomic and spatial organization of telencephalic GABAergic neuron types

Authors: *C. T. J. VAN VELTHOVEN, M. KUNST, D. MCMILLEN, C. LEE, J. CAMPOS, J. CLOSE, S. DANIEL, B. LONG, J. MALONE, N. MARTIN, A. RUIZ, N. VALERA CUEVAS, C. PAGAN, L. KRUSE, J. WATERS, L. NG, K. A. SMITH, B. TASIC, Z. YAO, H. ZENG;
Allen Inst. for Brain Sci., Seattle, WA

Abstract: The telencephalon consists of an intricate set of structures that are required for some of the most complex and evolved functions of the mammalian brain. Gamma-aminobutyric-acidergic (GABAergic) cells form a very heterogeneous population of neurons throughout the telencephalon that play a crucial role in regulating the activity of neuronal networks. Recently, we generated a comprehensive, high-resolution transcriptomic and spatial cell type atlas for the whole mouse brain (Yao et al 2023). We leveraged this atlas to conduct an in-depth analysis of the transcriptomic diversity and spatial organization of GABAergic neuronal types in the mouse telencephalon. We analyzed ~615,000 GABAergic neurons from cerebral cortex, including isocortex, hippocampal formation, olfactory areas, and cortical subplate, and the cerebral nuclei

including striatum and pallidum. The neurons were clustered into 5 classes, 52 subclasses, 219 supertypes, and 1051 cell types. The classes and subclasses are mostly defined by their developmental origin and spatial location. Within this dataset, we identified a large set of highly distinct cell types as well as continuous molecular gradients within and across different regions. The discrete and continuous gene expression diversity collectively shape the cellular diversity that underlie the diverse function of the many regions and neural circuits in the telencephalon.

Disclosures: C.T.J. van Velthoven: None. **M. Kunst:** None. **D. McMillen:** None. **C. Lee:** None. **J. Campos:** None. **J. Close:** None. **S. Daniel:** None. **B. Long:** None. **J. Malone:** None. **N. Martin:** None. **A. Ruiz:** None. **N. Valera Cuevas:** None. **C. Pagan:** None. **L. Kruse:** None. **J. Waters:** None. **L. Ng:** None. **K.A. Smith:** None. **B. Tasic:** None. **Z. Yao:** None. **H. Zeng:** None.

Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR442.20/WW61

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: HHMI

Title: Analysis of neural activity in molecularly defined cell types in the mouse frontal cortex

Authors: *T. WANG[1], A. SINGH[1,2], K. DAIE[2], K. SVOBODA[2];
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Abstract: Neuronal cell type classification has been revolutionized by advances in genomics. It is now possible to profile the mRNA repertoire of individual cells and cluster populations of cells into ‘transcriptomic cell types’ (t-types). Individual brain regions can contain dozens of t-types. Large-scale functional imaging experiments have revealed diverse patterns of neural activity during behavior. The correspondence between t-types and behavior-related activity patterns is largely unknown. To establish this correspondence we developed FIRE (functional imaging registered to gene expression) to link transcriptomic identity with neuronal activity. Mice were trained to perform a sensory discrimination task that required short-term memory. Anterior lateral motor (ALM) cortex is necessary for this task. ALM neurons exhibit persistent activity that predicts upcoming movements (i.e. a neural correlate of motor planning), in addition to activity related to sensory stimuli, movements, rewards and other behavioral parameters. Population calcium imaging was performed using two-photon microscopy over the course of several weeks in excitatory (Slc17a7-cre, Emx1-cre, Rbp4-cre) and inhibitory (Vgat-cre) GCaMP-expressing transgenic lines. After in vivo imaging, thick (300 um) brain slices were cut and aligned to the imaged volume with single-cell precision. We performed RNA fluorescence in situ hybridization coupled with expansion microscopy (EASI-FISH). This approach relies on the hybridization chain reaction to generate bright fluorescent punctae corresponding to individual

mRNA molecules that can be resolved and counted. To rapidly image large sample volumes, we built a custom multispectral selective plane illumination (i.e. 'lightsheet') microscope that enables the simultaneous detection of 7 dyes species. We have also developed a multi-stage computational pipeline to efficiently process the resultant TB-scale raw image datasets. Finally, we analyzed the extent to which t-types statistically explain the diverse response patterns observed across thousands of neurons. Defining cell type-specific behaviorally-related signals is a necessary step towards understanding how computation is implemented in neural circuits.

Disclosures: **T. Wang:** None. **A. Singh:** None. **K. Daie:** None. **K. Svoboda:** None.

Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR442.21/WW62

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: National Institute on Aging Intramural Research Program

Title: Spatial transcriptomics of the aging mouse brain reveals origins of inflammation in the white matter

Authors: *L. WANG[1], I. BODOGAI[1], C. LEE[1], O. IRFANOGLU[2], N. YANG[1], C. SHI[1], D. BENJAMINI[3], E. MARAGKAKIS[1], C.-Y. CUI[1], P. SEN[1];
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Abstract: To systematically understand age-induced molecular changes, we performed spatial transcriptomics of young, middle-aged, and old mouse brains and identified seven transcriptionally distinct regions. All regions exhibited age-associated upregulation of inflammatory genes and downregulation of genes related to synaptic function. Notably, aging white matter fiber tracts showed the most prominent changes with pronounced effects in females. The inflammatory signatures indicated major ongoing events; microglia activation, astrogliosis, complement activation, and myeloid cell infiltration. Immunofluorescence and quantitative MRI analyses confirmed physical interaction of activated microglia with fiber tract and concomitant reduction of myelin in old mice. Machine learning algorithms identified transcription factor networks driving these changes. Our study provides a resourceful dataset of spatially resolved transcriptomic features in the naturally aging murine brain encompassing three age groups, and both sexes. The results link previous disjointed findings and provide a comprehensive overview of brain aging identifying fiber tracts as a focal point of inflammation.

Disclosures: **L. Wang:** None. **I. Bodogai:** None. **C. Lee:** None. **O. Irfanoglu:** None. **N. Yang:** None. **C. Shi:** None. **D. Benjamini:** None. **E. Maragkakis:** None. **C. Cui:** None. **P. Sen:** None.

Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR442.22/WW63

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Spatial multiomic evaluation reveals the expression profiles of subpopulations of microglia within differing microenvironments of the mouse brain

Authors: *C. PHAN, O. HO-SHING, A. ROSENBLOOM, C. WILLIAMS, J. REEVES, C. KANG, O. APPELBE, H. ZHAI, V. DEVGAN, M. RHODES, J. BEECHEM; NanoString Technologies, Inc., Seattle, WA

Abstract: Spatial multiomic profiling of the brain has the potential to augment our understanding of the nervous system with unprecedented detail, allowing researchers to profile thousands of targets at subcellular resolution. Investigations of this magnitude previously relied on a combination of immunohistochemistry and fluorescence-activated cell sorting or single-cell sequencing to understand the biology, but each of these approaches only captures individual aspects of the tissue structure and molecular biology happening within each cell. For high-plex spatial approaches to be successful, they require accurate localization of profiled targets, high enough target coverage to understand the underlying biology, and the ability to accurately capture the structure of cells in which these targets are found. The CosMx™ Spatial Molecular Imager (SMI) allows researchers the ability to examine the expression of 1,000 RNA targets and 64 proteins at a time at subcellular resolution.

In this study, we focus on microglia as these are a particularly challenging cell type to profile accurately but are critical as they are the brain's tightly regulated macrophages involved with brain development, neuronal maintenance, as well as pro- and anti-inflammatory activities. Microglia are both motile and are defined by complex cellular morphology with branching processes, and thus, these cells represent a key class to model, but one which is easily misinterpreted.

In the coronal and sagittal sections profiled here, we demonstrate that the CosMx SMI is able to successfully segment distinct microglial cells, which enables investigation of the substantial cellular diversity not just between brain regions but based on individual cell-to-cell interactions within the local microenvironment. Furthermore, distinct protein and gene expression profiles of these diverse activities can be mapped back to their location in tissues with single-cell precision, including the ability to trace morphological distributions of critical protein markers such as P2ry12, TMEM119, CD68, DAP12, and CD11b within individual glial processes.

As the RNA panel used covers 1,000 curated targets for neurobiology, we can explore novel insights based on complex spatial evaluation of expression data including cell typing, signaling pathway analysis, ligand-receptor analysis, and cell type co-localization. Identifying the differential expression of certain targets amongst different subtypes of cells will provide insights into the potential discovery of new biomarkers and targets for future therapeutics.

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Disclosures: **C. Phan:** A. Employment/Salary (full or part-time):; NanoString Technologies, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies, Inc. **O. Ho-Shing:** A. Employment/Salary (full or part-time):; NanoString Technologies, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies, Inc. **A. Rosenbloom:** A. Employment/Salary (full or part-time):; NanoString Technologies, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies, Inc. **C. Williams:** A. Employment/Salary (full or part-time):; NanoString Technologies, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies, Inc. **J. Reeves:** A. Employment/Salary (full or part-time):; NanoString Technologies, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies, Inc. **C. Kang:** A. Employment/Salary (full or part-time):; NanoString Technologies, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies, Inc. **O. Appelbe:** A. Employment/Salary (full or part-time):; NanoString Technologies, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies, Inc. **H. Zhai:** A. Employment/Salary (full or part-time):; NanoString Technologies, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies, Inc. **V. Devgan:** A. Employment/Salary (full or part-time):; NanoString Technologies, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies, Inc. **M. Rhodes:** A. Employment/Salary (full or part-time):; NanoString Technologies, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies, Inc. **J. Beechem:** A. Employment/Salary (full or part-time):; NanoString Technologies, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies, Inc..

Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR442.23/WW64

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Single-cell rna sequencing of cd45+ cells reveal an increased in lyve1+ macrophages in hind paws of diabetic mice

Authors: *V. PHAM, J. NICHOLS, R. MAHALINGAM, A. SHEPHERD;
Symptom Res. CAO, MD Anderson, Houston, TX

Abstract: Diabetic peripheral neuropathy (DPN) is one of the most common complication of diabetes. Symptoms of DPN can include tingling, numbing and burning sensation in the fingertips or toes. Based on previous studies, DPN involves a complex interaction of several factors such as hyperglycemia, dyslipidemia, microvasculature dysfunction, oxidative stress and inflammation. Immune cells, especially macrophages, have been shown to have pathological functions in DPN. Therefore, this study focused on performing an in-depth analysis of leukocytes, with specific attention to macrophages, in the microenvironment of the distal foot to determine potential pathological mechanisms that might affect the distal nerve endings of the hind limbs. We collected hind paws from male $Lepr^{db/db}$ and $Lepr^{wt/wt}$ (WT) mice aged up to 12 weeks (young mice) or 21 weeks (aged mice). After obtaining a single cell suspension from the hind paws of these mice, fluorescent staining and cell sorting was used to isolate $CD45^{+}$ cells for single-cell RNA sequencing. These sequences were integrated into clusters based on their similar biological state and annotated based on differential gene expression. Supervised subclustering of the "macrophage" cells was performed based on Lyve1 and MHCII expression to isolate cells with distinct functional roles. Histology was also performed using Lyve1 co-stained with CD68 and IBA1 antibodies in order to quantify $Lyve1^{+}$ macrophages. Using MHCII expression, we formed 4 groups that designate as $Lyve1^{+}MHCII^{HI}$, $Lyve1^{+}MHCII^{LO}$, $Lyve1^{-}MHCII^{HI}$, $Lyve1^{-}MHCII^{LO}$. We examined these 4 types and found an increase in $Lyve1^{+}MHCII^{LO}$ in aged $Lepr^{db/db}$ that did not occur in the other 3 samples and an overall decrease in the percentage of $Lyve1^{+}MHCII^{HI}$ and $Lyve1^{-}MHCII^{HI}$ in aged $Lepr^{db/db}$ sample. We also noticed that $Lyve1^{+}MHCII^{LO}$ cells upregulate pathways of vasculature development and mobility while the other 3 groups are more proinflammatory or activated immune response. This result would suggest a transition from a pro-inflammatory M1 phenotype to an anti-inflammatory M2/angiogenic phenotype in aged $Lepr^{db/db}$. Histology also showed an increase in mean intensity of Lyve1 in macrophages present in the foot pads of young and aged $Lepr^{db/db}$ mice as compared to WT mice from both age groups. Pearson's correlation showed that aged $Lepr^{db/db}$ have a greater overlap of Lyve1 with CD68/IBA1 compare to both young and aged WT. Based on these findings it is clear that there is a substantial difference between immune cells in the foot pads of WT and $Lepr^{db/db}$ and the immune response evolves as the mice age.

Disclosures: **V. Pham:** None. **J. Nichols:** None. **R. Mahalingam:** None. **A. Shepherd:** None.

Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR442.24/WW65

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant U19MH114831
NIH Grant U19MH114830
NIH SIG Grant #S10OD026929

Title: Comprehensive single-cell analysis of chromatin accessibility in the adult mouse brain

Authors: *S. ZU[1,2], Y. E. LI[2], K. WANG[2], M. MILLER[3], Y. WANG[2], M. L. AMARAL[2], X. HOU[3], B. LI[2], S. KUAN[2], H. LIU[4], J. ZHOU[4], A. PINTO-DUARTE[4], J. LUCERO[4], J. OSTEEN[4], M. NUNN[4], K. A. SMITH[5], B. TASIC[5], Z. YAO[5], H. ZENG[5], Z. WANG[2], J. SHANG[2], M. M. BEHRENS[4], J. R. ECKER[4], A. WANG[6], S. PREISSL[6,7], B. REN[6,2,8]; [1]Dept. of Cell. and Mol. Med., La Jolla, CA; [3]Ctr. for Epigenomics, [2]UCSD, La Jolla, CA; [4]The Salk Inst. for Biol. Studies, La Jolla, CA; [5]Allen Inst. for Brain Sci., Seattle, WA; [6]Ctr. for Epigenomics, La Jolla, CA; [7]Inst. of Exptl. and Clin. Pharmacol. and Toxicology, Univ. of Freiburg, Freiburg, Germany; [8]Ludwig Inst. for Cancer Res., La Jolla, CA

Abstract: The mouse brain comprises tens of millions of neurons and nonneuronal cells organized into numerous circuits and structures that are responsible for complex behaviors and neurological functions. Advances in single-cell technologies have led to the discovery of thousands of brain cell types. However, our understanding of the gene regulatory programs in these cell types is still limited. Here we report a comprehensive atlas of candidate cis-regulatory DNA elements (cCREs) in the adult mouse brain, generated through examination of the chromatin accessibility in 2.3 million individual brain cells from 117 anatomical dissections. The atlas includes 1.3 million cCREs and their chromatin accessibility across 602 distinct brain cell populations, adding over 640,000 new cCREs to the most recent such annotation in the mouse genome. The mouse brain cCREs are moderately conserved in the human brain. The mouse specific cCREs, in particular those identified from a subset of cortical excitatory neurons, are strongly enriched for transposable elements, suggesting a potential role for transposable elements in the emergence of new regulatory programs and neuronal diversity. Finally, we infer the gene regulatory networks in over 200 subclasses of mouse brain cells, and develop deep learning models to predict, from DNA sequence alone, the activities of gene regulatory elements in different brain cell types. Our results provide a resource for analysis of cell-type-specific gene regulation programs in both mouse and human brains.

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Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR442.25/WW66

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant U19MH114830

Title: A high-resolution transcriptomic and spatial atlas 1 of cell types in the whole mouse brain

Authors: *Z. YAO[1], C. V. VELTHOVEN[1], M. KUNST[2], M. ZHANG[3], D. MCMILLEN[1], C. LEE[4], W. JUNG[7], J. GOLDY[1], C. PAGAN[1], L. KRUSE[1], N. DEE[1], S. M. SUNKIN[1], L. ESPOSITO[1], M. J. HAWRYLYCZ[8], J. WATERS[5], L. NG[1], K. SMITH[1], B. TASIC[6], X. ZHUANG[9], H. ZENG[5];
[1]Allen Inst. for Brain Sci., Seattle, WA; [2]Imaging, Alle Inst. for Brain Sci., Seattle, WA; [3]Harvard University/Howard Hughes Med. Inst., Cambridge, MA; [4]Modeling Analysis and Theory, [6]Cell and Circuit Genet., [5]Allen Inst. For Brain Sci., Seattle, WA; [7]Harvard Univ., Boston, MA; [8]Modeling, Analysis, and Theory, Allen Inst. Brain Sci., Seattle, WA; [9]HHMI / Harvard Univ., Cambridge, MA

Abstract: The mammalian brain is composed of millions to billions of cells that are organized into numerous cell types with specific spatial distribution patterns and structural and functional properties. An essential step towards understanding brain function is to obtain a parts list, i.e., a catalog of cell types, of the brain. Here, we report a comprehensive and high-resolution transcriptomic and spatial cell type atlas for the whole adult mouse brain. The cell type atlas was created based on the combination of two single-cell-level, whole-brain-scale datasets: a single-cell RNA-sequencing (scRNA-seq) dataset of ~7 million cells profiled, and a spatially resolved transcriptomic dataset of ~4.3 million cells using MERFISH. The atlas is hierarchically organized into four nested levels of classification: 32 classes, 306 subclasses, 1,045 supertypes and 5,200 clusters. We systematically analyzed the neuronal, non-neuronal, and immature neuronal cell types across the brain and identified a high degree of correspondence between transcriptomic identity and spatial specificity for each cell type. The results reveal unique features of cell type organization in different brain regions, in particular, a dichotomy between the dorsal and ventral parts of the brain: the dorsal part contains relatively fewer yet highly divergent neuronal types, whereas the ventral part contains more numerous neuronal types that are more closely related to each other. We also systematically characterized cell-type specific expression of neurotransmitters, neuropeptides, and transcription factors. The study uncovered extraordinary diversity and heterogeneity in neurotransmitter and neuropeptide expression and co-expression patterns in different cell types across the brain, suggesting they mediate a myriad of modes of intercellular communications. Finally, we found that transcription factors are major determinants of cell type classification in the adult mouse brain and identified a combinatorial transcription factor code that defines cell types across all parts of the brain. The whole-mouse-brain transcriptomic and spatial cell type atlas establishes a benchmark reference atlas and a foundational resource for deep and integrative investigations of cell type and circuit function, development, and evolution of the mammalian brain.

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Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR442.26/WW67

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: 5U19MH114830-05
1U01MH130962-01

Title: Generation of a mouse brain spatial atlas for cell types

Authors: *M. KUNST[1], D. MCMILLEN[2], J. CAMPOS[2], C. LEE[5], M. CHEN[7], B. R. LONG[6], S. DANIEL[3], J. GEE[8], N. VALERA[2], C. PAGAN[2], S. M. SUNKIN[4], M. J. HAWRYLYCZ[9], L. NG[5], C. VAN VELTHOVEN[4], Z. YAO[4], H. ZENG[4];
[1]Imaging, Alle Inst. for Brain Sci., Seattle, WA; [2]Imaging, [4]Allen Inst. for Brain Sci., [3]Allen Inst. for Brain Sci., Seattle, WA; [5]Allen Inst. For Brain Sci., [6]Imaging, Allen Inst. For Brain Sci., Seattle, WA; [7]Univ. of Pennsylvania, [8]Univ. of Pennsylvania, Philadelphia, PA; [9]Allen Inst. Brain Sci., Allen Inst. Brain Sci., Seattle, WA

Abstract: In recent years the advent of single-cell RNA sequencing (RNAseq) technologies gave scientists an unprecedented ability to define cell types by their gene expression profiles. However, the spatial information retained does not go beyond broader brain regions, and finer details (i.e. subnuclear or layer-specific localization and gradients) are lost in the dissociation process. Spatial transcriptomics has the potential to fill in this gap by mapping of cell types defined by a transcriptomic atlas to the location of individual cells within a histological section. The Allen Institute recently generated a transcriptomic atlas of cell types for the entire mouse brain. To further our understanding of cell type distribution within the brain we generated a spatial transcriptomics dataset covering the entire mouse brain using the commercial MERFISH platform MERSCOPE. Here we describe our workflow of processing these data from experimental outputs to cells mapped to the reference taxonomy and aligned to the Allen Institute Common Coordinate Framework (CCF). Furthermore, we will present preliminary analysis on how these data can be used to quantify the distribution of cell types across the brain and describe the complexity of brain regions with respect to cell type distribution.

Disclosures: M. Kunst: None. **D. McMillen:** None. **J. Campos:** None. **C. Lee:** None. **M. Chen:** None. **B.R. Long:** None. **S. Daniel:** None. **J. Gee:** None. **N. Valera:** None. **C. Pagan:** None. **S.M. Sunkin:** None. **M.J. Hawrylycz:** None. **L. Ng:** None. **C. van Velthoven:** None. **Z. Yao:** None. **H. Zeng:** None.

Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR442.27/WW68

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Toward a whole-brain cell type atlas of the cuttlefish Sepia officinalis

Authors: *S. RENCKEN[1], D. HAIN[1], G. TUSHEV[1], E. CIIRDAEVA[1], T. KAPURUGE[2], G. LAURENT[1];
[1]Max Planck Inst. for Brain Res., Frankfurt, Germany; [2]Ludwig-Maximilians-Universität, Munich, Germany

Abstract: Many cephalopods camouflage, evading predators and surprising prey by matching their appearance to their surroundings. This behavior requires animals to observe their environment, process texture information, and reproduce a matching texture on their skin. Not only is this behavior a remarkable feat of visual perception, but the lineages giving rise to cephalopods and vertebrates diverged very early during animal evolution, providing a fascinating opportunity to study the convergent evolution of sensory computations. In addition, the camouflage output is realized by a unique motor system operating at single-cell resolution, providing insight into ultra-fine motor control. While the brain areas involved in camouflage have been coarsely mapped, the cell types underlying the behavior remain largely unknown. To understand the genetic basis of camouflage in the European cuttlefish *Sepia officinalis*, we are assembling the genome of an adult cuttlefish using various sequencing techniques. Using the genome as a reference, we profile the cell type diversity in the cuttlefish brain using single-nucleus RNA sequencing, enabling evolutionary comparisons to different cephalopod and model species. Further, we visualize the cell types by mapping the spatial expression of marker genes using *in situ* hybridization and spatial sequencing methods to generate a comprehensive molecular map of the cuttlefish brain. Ultimately, we aim to inform physiological studies of the identified cell types in order to link their molecular profiles to their role in camouflage behavior.

Disclosures: S. Rencken: None. **D. Hain:** None. **G. Tushev:** None. **E. Ciirdaeva:** None. **T. Kapuruge:** None. **G. Laurent:** None.

Poster

PSTR442. Single-Cell Approaches for Understanding Brain Structure and Function

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR442.28/WW69

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Nellie Ball Trust
OVPR DEI Supplement

Title: Single-cell transcriptomic analysis on the cerebellum of mice with the 16p11.2 microduplication mutation

Authors: *K. D. TISON[1], H. HALVERSON[2], M. GAINE[3], K. PARKER[4], A. WILLIAMS[2];
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Exptl. Therapeutics, Col. of Pharm., [4]Dept. of Psychiatry, Iowa Neurosci. Inst., Univ. of Iowa, Iowa City,, IA

Abstract: Schizophrenia (SCZ) is a complex genetic disorder and mutations such as the copy number variant 16p11.2 microduplication (16p11.2dup) can cause SCZ. 16p11.2dup is associated with microcephaly, suggesting that the duplicated genes are involved in neurodevelopment. Computational analysis of publicly available bulk RNA-seq data from mice with 16p11.2dup shows significant transcriptional dysregulation in the cerebellum, but the specific cell types affected are unknown, and there are no published analyses of cerebellum-dependent behaviors in this mouse line. Therefore, we performed delay eyeblink conditioning, which is a cerebellum-dependent form of learning, as well as single nucleus RNA-seq, to address these knowledge gaps. We observed that 16p11.2dup mice show impaired learning ($p<0.01$) and conditioned response timing ($p<0.05$) compared with WT littermates. We also performed single nucleus RNA-seq using the 10X Genomics 3' expression kit on the whole cerebellum of male and female 16p11.2dup mice and WT littermates. Using the R package toolkit Seurat, we will cluster nuclei with similar gene expression profiles which is crucial for unraveling the cellular heterogeneity present in the cerebellum. Dimensionality reduction techniques, such as PCA and t-SNE, will be used to look at the dataset in a lower-dimensional space. By condensing the complex gene expression data, these techniques will allow for the identification of patterns and relationships among the different cell populations. Overall, our data show that 16p11.2 dup is associated with a deficit in cerebellum dependent behavior. The single nucleus RNA-seq data will help specify which cerebellar cell types are most affected by 16p11.2dup and may accelerate the discovery and development of new diagnostic and therapeutic strategies for schizophrenia.

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Poster

PSTR443. Light and Electron Microscopy

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR443.01/WW70

Topic: I.03. Anatomical Methods

Support: Max Planck Society

Title: Development of a miniaturized microscope for two-photon lifetime imaging in freely behaving mice

Authors: *T. SALEMI[1], L. YAN[1], Y. NAKAHATA[1], L. CHEN[2], R. YASUDA[1];
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Abstract: Intracellular signaling plays a critical role in synaptic plasticity, learning, and memory. However, a tool to monitor neuronal signal transduction with subcellular spatial

resolution in freely behaving animals is lacking. Fluorescent resonance energy transfer (FRET) based biosensors, in combination with 2-photon fluorescence lifetime imaging (2pFLIM), provide means to monitor conformational changes or translocation of donor and acceptor fluorescent protein as a proxy for intracellular protein dynamics in live brain. Here, we developed a miniaturized 2-photon head-mounted microscope to perform in vivo FRET-FLIM (mini-2pFLIM). The 4g body of the mini-2pFLIM did not impair the ability of the animal to locomote. Using a genetically encoded calcium indicator (GCaMP6s) we were able to image a field of view of 350um x 350um with a sufficient resolution to image the soma and dendrites in freely moving animals while performing calcium imaging and reliably measuring the lifetime of the excited state. An electrotunable lens enabled us to image the supragranular layers within a range of 200 um of depth. Next, we used in-utero electroporation to sparsely transfect layer 2/3 pyramidal neurons in the mouse somatosensory cortex with an improved mEGFP-based FRET biosensor (2dvCamuiα) able to report Ca2+/calmodulin-dependent protein kinase II (CaMKII) activation. The fluorescence of mEGFP in the 2dvCamuiα-expressing neurons had a significantly shorter lifetime of the excited state and a higher degree of cell-to-cell variability compared to cells expressing mEGFP alone. These results demonstrate that our system can efficiently measure FRET at subcellular resolution in freely moving animals. Future work will aim to apply this mini-2pFLIM in different behavioral paradigms to clarify the spatiotemporal dynamic of CaMKII activation in freely behaving animals during plasticity, memory formation and learning. Our system will allow to image a wide variety of other biosensors during naturalistic behaviors in mice and has thepotentiall to reveal the complex intracellular protein machinery that regulates neuronal steady state and plasticity.

Disclosures: **T. Salemi:** None. **L. Yan:** None. **Y. Nakahata:** None. **L. Chen:** None. **R. Yasuda:** None.

Poster

PSTR443. Light and Electron Microscopy

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR443.02/WW71

Topic: I.03. Anatomical Methods

Support: NIH BRAIN Initiative F32MH120872
Max Planck Florida Institute for Neuroscience

Title: Ten color identification within miniscope acquired functional imaging reveals mPFC coding of social identity

Authors: *M. L. PHILLIPS[1,2], N. T. URBAN[2], T. SALEMI[2], R. YASUDA[2];
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Abstract: Current capabilities of state-of-the-art in vivo imaging during freely moving behavior can record two, spectrally distinct, fluorophores. This severely limits the number of cell types identifiable in a functional imaging experiment. Here we present a pipeline that enables the distinction of nine neuronal subtypes from regions defined by behaviorally relevant cells during *in vivo* GCaMP imaging. These subtypes are identified utilizing unique fluorophores that are co-expressed with GCaMP, unmixed by multispectral lambda imaging on a confocal, and spectral fingerprints co-registered with functional data obtained on miniaturized microscopes. Using this method, we recorded pyramidal neurons in the medial prefrontal cortex categorized by projection region during a freely moving social memory task. As neural activity in each category was concurrently imaged, we can determine which projection neurons most preferentially encode identity versus novelty/familiarity and what types of social behaviors are most important for this distinction. This method will not only increase efficiency for calcium imaging experiments by enabling nine neuronal populations to be investigated simultaneously but also enhances the statistical power of the results by utilizing within subject comparisons.

Disclosures: **M.L. Phillips:** A. Employment/Salary (full or part-time):; ZEISS Research Microscopy Solutions. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); ZEISS Research Microscopy Solutions. **N.T. Urban:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); ZEISS Research Microscopy Solutions. **T. Salemi:** None. **R. Yasuda:** None.

Poster

PSTR443. Light and Electron Microscopy

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Program #/Poster #: PSTR443.03/WW72

Topic:

Support: NIH BRAIN Initiative F32MH120872
Max Planck Florida Institute for Neuroscience

Title: Co-registering ten genetically expressed fluorophores during in-vivo GRIN lens imaging

Authors: ***N. T. URBAN**[1], M. L. PHILLIPS[3,2], T. SALEMI[2], R. YASUDA[2];
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Abstract: By imaging genetically expressed calcium sensors in the brain, we can simultaneously record and correlate the activity of hundreds of neurons with observable behavior. Using head-mounted miniscopes enables this without restricting the animal's free movement, at the expense of image quality and versatility. Multicolor imaging, in particular, becomes a challenge, even more so when using GRIN lenses to image deeper within the brain. But without the ability to use additional fluorescent labels to identify specific cell types, genetic traits, or connectivity within the brain, we are left with frightfully little knowledge about the neurons whose activity we are

trying to interpret.
Recent dual-color miniscopes are capable of visualizing one additional color alongside GCaMP, but adding a third or more colors seems elusive. It is possible, however, to circumvent these limitations and restore full multicolor capabilities by supplementing functional miniscope data with a single, multispectral volumetric image taken with a confocal microscope. By creating a high-resolution, multicolor map of the visible neurons, we can identify which were active during behavior, along with any secondary labels they might be expressing. Furthermore, by combining multispectral lambda-detection with excitation multiplexing, we can differentiate fluorophores based on both their emission and their excitation spectra. This opens up the use of spectrally adjacent fluorescent proteins, allowing more fluorescent labels to be used simultaneously.
Even though GRIN lenses notoriously complicate multicolor imaging by inducing a multitude of spatial and chromatic distortions, these aberrations are highly reproducible and can be precisely measured, allowing them to be overcome. We characterized the aberrations of the most common GRIN lenses used for miniscope imaging, and used these corrections to image with wavelengths across the visible spectrum, and co-register each color channels in x,y, and z with high accuracy. Finally, we used an upright Zeiss LSM 980 to image the mPFC of head-fixed mice, expressing nine spectrally distinct fluorescent proteins alongside GCaMP. After co-registering the miniscope and confocal images, we measured the multispectral fingerprint of each active neuron in order to detect and identify secondary fluorophores. This technique allows us to not only overcome the optical limitations on spatial resolution and volumetric imaging, but to further allow the simultaneous recording of multiple neuronal subtypes at once, enabling the direct comparison of activity patterns during behavior.

Disclosures: **N.T. Urban:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); ZEISS Research Microscopy Solutions. **M.L. Phillips:** A. Employment/Salary (full or part-time):; ZEISS Research Microscopy Solutions. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); ZEISS Research Microscopy Solutions. **T. Salemi:** None. **R. Yasuda:** None.

Poster

PSTR443. Light and Electron Microscopy

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR443.04/WW73

Topic: I.03. Anatomical Methods

Title: Evaluation and characterization of Styrylbenzene analogue-K114 and its use for high contrast and resolution fluorescent localization of plaques in rodent and plaques and tangles in human brain tissue

Authors: *S. SARKAR[1,2], S. PADALA[2], J. B. RAYMICK[3];
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Abstract: Plethora of studies showed utilities of several chemical dyes due to their affinity to bind amyloid abeta to show plaques under light or fluorescence microscope, and some of them show affinity to bind and neurofibrillary tangles (NFT) at the end of 19th century. However, only few of them has the propensity to bind both senile plaques (SP) and neurofibrillary tangles (NFT). In our current study, we have modified the method of K114 staining procedure and our modified method yielded substantial improvement in the labeling of plaques and tangles in the human brain. Additionally, our modified method could detect SP in rodent brains in 15 min and NFT in human brains within 20 min. We have also compared whether different drying methods or mounting medium can affect the resolution and contrast of the plaque and tangle staining in the brain. To further evaluate the target of the modified K114, we performed double labeling of K114 and abeta antibodies raised against three different epitopes. Our results demonstrated that modified K114 can be used as a very sensitive dye to detect various forms of senile plaques in the brain. Regarding detecting tauopathy in the AD brain, we have used five different phosphorylated Tau to understand the potential binding targets. We have found more than 80% hyperphosphorylated Tau against AT8, PTau and TNT1 colocalized with K114 labeled tangles, whereas more than 70% of the hyperphosphorylated Tau colocalized with modified K114 tangles. On the other hand, more than 90% of the plaques that are stained with abeta MOAB-2 were colocalized with modified K114. Our results indicate that modified K114 could be used as a valuable tool which can be used to detect AD pathologies in human and rodent brains with high contrast and resolution relative to other conventional fluorescence markers.

Disclosures: S. Sarkar: None. **S. Padala:** None. **J.B. Raymick:** None.

Poster

PSTR443. Light and Electron Microscopy

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR443.05/WW74

Topic: I.03. Anatomical Methods

Title: Three-dimensional visualization and quantitative analysis of retinal degeneration for hereditary retinal dystrophies with retina-PACT

Authors: *Z. WANG, J. B. TREWEEK;
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Abstract: Retinal neurodegenerative diseases which are often distinguished by dysfunction or loss of cell subsets, are the leading cause of vision deficits. However, the genetic and pathological heterogeneity of retinal dystrophies complicates the development of effective treatments. Royal College of Surgeons (RCS) rat model serves as a powerful tool for investigating the pathogenesis of retinitis pigmentosa, characterized by loss of photoreceptor (PR) cells, and for testing candidate therapies for their ability to slow or reverse degeneration. To accelerate and improve these preclinical studies, we developed a novel tissue clearing technique (retina-PACT or rPACT) to enable high-resolution visualization of intact retinas by light sheet

fluorescence microscopy (LSFM), avoiding tissue damage and loss of 3D-constructional information. In addition to rendering retinas optically transparent, rPACT protects the 3D architecture and laminar structure of retinas, permitting researchers to evaluate topographical differences in retinal degeneration. Because rPACT supports immunolabeling and high-resolution imaging at depth, it enables the quantification of major pathological signs of retinal degeneration across all retinal layers at multiple positions. A semi-automated computational pipeline was created to facilitate quantitative analysis for whole-retina datasets. Greater PR cell loss was found at positions close to the optic nerve, suggesting that the progression of degeneration originated from the central retina. Compared to PR cells, slower degeneration of bipolar cells indicated that their degeneration progressed from outer to inner layers. Evidence of dendrite withdrawal of bipolar cells prior to their significant cell loss hints at the potential importance of synaptic connectivity for cell survival. The outward migration of glial cells from ganglion cell layer, which has been observed in previous studies, was confirmed in rPACT-cleared RCS rat retinas. But more interestingly, our results revealed an earlier onset of gliosis in female RCS rats relative to male counterparts. In addition to these sex differences, although the spatial uniformity of retinas was demonstrated at postnatal day 21 (P21), position-related disparities of degeneration were found at P49 and P60, uncovering the spatial heterogeneity in the progression of retinal degeneration. This work demonstrates the utility of rPACT in granting researchers access to high-quality, whole-retina image datasets for quantitative evaluation, either to forward vision research more broadly or to characterize dynamic developmental and disease processes of retina.

Disclosures: Z. Wang: None. **J.B. Treweek:** None.

Poster

PSTR443. Light and Electron Microscopy

Location: WCC Halls A-C

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Program #/Poster #: PSTR443.06/WW75

Topic: I.03. Anatomical Methods

Support: FWF Grant P35614
FWF Grant P31263
WWTF Grant CS18-019

Title: Ultramicroscopy of human tissue

Authors: *H.-U. DODT[1,2], J. A. OAKES[1,2], C. FUCHSSTEINER[2], M. FOROUGHIPOUR[1], M. FOROUGHIPOUR[1], K. BECKER[1], S. SAGHAFI[1];
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Abstract: Ultramicroscopy has been used very successfully to investigate chemically cleared mouse brains. However its application to human tissue has been hampered by difficulties to

obtain specific cellular staining. The use of endogenous fluorescent marker is not possible in humans and staining with antibodies is difficult due to diffusion problems. Furthermore standard clearing protocols like 3DISCO take weeks to months to clear human tissue. Notoriously difficult is clearing of tumors due to their high cell density. We therefore developed in the last years a new clearing protocol, pathoDISCO, which allows clearing of tumor tissue in the centimeter range within days[1]. This method uses boosted autofluorescence for cellular visualization but does not yet provide specific subcellular staining. We found specific fluorescent stainings for cytoplasm and nuclei which also survive our harsh tumor clearing. Interestingly modern stainings were very sensitive to our active clearing with a chemical reaction. So we had to resort to simple chemical stainings developed long time ago which were more stable. Applying suitable image processing we could generate Hematoxilin/Eosin (HE) stained like images of various human tissues. With our ultramicroscope volume recordings of pathology cassette sized tumor pieces of 4 mm thickness could be obtained with subcellular resolution. Our single optical sections provide a resolution and appearance equivalent to histological slides but now for whole tissue volumes. We were able to specifically stain cytoplasm, cell nuclei, collagen and blood vessels. The ability to stain blood vessels in tumors like glioblastoma offers a completely new parameter for malignancy staging in pathology. Standard histological sections can not provide this information as blood vessels appear in these sections only as rings or stripes. Thus with our 3D recordings the important aspect of neovascularisation can be quantified and may be used in future as predictive marker. We applied our technology to various neuronal and non neuronal tumors. We are confident that this approach will open up completely new ways for diagnostics in pathology. [1]Sabdyusheva-Litschauer I et al. (2020) 3D histopathology of human tumours by fast clearing and ultramicroscopy, Sci Rep.10:17619

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Poster

PSTR443. Light and Electron Microscopy

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Topic: I.03. Anatomical Methods

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Leducq 22CVD0

Title: Cell type resolved proteomic imaging of whole human brains using transcriptomics-guided combinatorial coding and multiplexing

Authors: *K. SWAYZE[1], M. J. CASPER[1], P. SIMKO[2], P. MANOJ[1], W. WANG[3], I. VASYLIEVA[4], A. WATSON[4], C. PEREZ CAMPOS[2], W. LI[2], Z. WU[3], E. M. C. HILLMAN[2]; [2]Mortimer B. Zuckerman Mind Brain Behavior Inst., [1]Columbia Univ., New York, NY; [3]Appel Alzheimer's Dis. Res. Institute, Feil Family Brain and Mind Res. Inst., Weill Cornell Med., New York, NY; [4]Ctr. for Biologic Imaging, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: An interplay of genetic factors and experiences shape a network of nearly 200 billion cells in the human brain making up who we are. Recent advances in tissue clearing, immunostaining and high-speed microscopy have opened up the possibility of imaging all of these cells throughout the entire human brain. However, transcriptomic studies have revealed that cells in the brain belong to numerous subtypes. To label each cell-type individually would require hundreds of antibodies. Limitations on the number of secondary antibodies and fluorophores that can be imaged in parallel would require laborious repeated imaging rounds and computationally expensive registration of images between each staining round, making the task of imaging an entire human brain, let alone many brains, nearly impossible. A smaller number of specific antibodies could be used, but data would then only cover a small number of cell types, overlooking the complexity of distributions and differences between individual cell types throughout a sample. To overcome these problems, we devised a combinatorial coding framework to resolve many cell types with a limited subset of antibodies. This approach recognizes that relative amounts of select proteins in each cell may be sufficient to encode a much larger number of cell types. Human and mouse brain transcriptomic datasets provide the opportunity to predict how many cell-types could potentially be resolved using subsets of expressed genes. Data-driven analysis can predict optimal marker combinations, while modeling can predict the number and range of cell types that could be resolved using a given subset of genes (e.g. constrained antibody availability). We also recognized that wider coverage may be achieved by pooling multiple antibodies into a reduced number of spectral channels. Pooling can be guided by consideration of the hierarchical nature of cell type taxonomies. For example, if markers in one spectral channel define whether a detected cell is a neuron (e.g., NeuN), an interneuron marker (e.g., GAD1) can be pooled with a microglia marker (e.g., Iba-1) in a second spectral channel. An independent nuclear marker can be used to locate all cells within the sample in order to extract each cell's 'proteomic code'. This approach has been tested by imaging mouse brain samples with 7 antibodies pooled into 4 spectral channels (plus a 5th nuclear label channel). This 'compressed sensing' approach to large-scale immunohistochemistry holds the potential to resolve hundreds of cell types within the whole human brain with highly feasible single-round staining and high-speed 9-color spectrally multiplexed acquisition.

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Poster

PSTR443. Light and Electron Microscopy

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR443.08/WW77

Topic: I.03. Anatomical Methods

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NIH MH123994
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Title: Ultraplex microscopy: Serial multiplexing of molecular labeling techniques and imaging modalities using ultrathin sections

Authors: *J. PEREZ-GARZA[1], Z. DEANE[2], G. RAIMONDI[3], J. OREA[3], R. TRIPP[4], E. GASTEYER[5], I. CHARLES[3], M. P. KENNY[3], L. OSTROFF[3];
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Abstract: The molecular organization of cells and tissue is challenging to study due to the inefficiency of multiplexed molecular labeling methods and the limited options for combining staining and imaging modalities in a single specimen. Here we present ultraplex microscopy, a versatile strategy that combines ultrathin sectioning with reversible embedding to allow multiplexing of molecular probes, staining protocols, and imaging methods with subcellular resolution. We show that 50 nm-thick sections of brain tissue are compatible with immunofluorescence (IF), fluorescence in situ hybridization (FISH), histological staining, and direct imaging of fluorescent reporter proteins, as well as imaging by brightfield, epifluorescence, super-resolution, and electron microscopy. We also demonstrate the use of click chemistry on ultrathin sections to detect metabolic labeling of RNA as well as immunoreagents. Arbitrary combinations of staining and imaging methods can be applied to serial sections from a single tissue sample to allow multiplexed labeling and imaging at the subcellular level, bypassing incompatibilities between reagents and protocols. High-resolution molecular detection is possible, as ultrathin sectioning enhances spatial resolution and signal-to-noise relative to standard preparations for light microscopy, while reversible embedding permits molecular labeling, most notably of mRNA, that is impossible on conventional resin sections. Labeling is performed using routine protocols for standard fixed tissue sections, and no custom or specialized regents are needed. Samples as large as an intact rat brain can be embedded and sections are shelf-stable, so a single sample can be used for many experiments across time to allow collection of rich multimodal datasets.

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Poster

PSTR443. Light and Electron Microscopy

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Program #/Poster #: PSTR443.09/WW78

Topic: I.03. Anatomical Methods

Support: NIH KL2TR001432
NIH 1R01NS133441

Title: Microwave-assisted acceleration of protein retention expansion microscopy in neural tissue of Xenopus laevis tadpoles

Authors: M. R. BULLARD[1], N. B. QUAICOE[1], D. A. ADAMS[1], A. JIN[1], J. M. LIN[1], *H. HE[2];
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Abstract: Protein retention expansion microscopy (pro-ExM) retains genetically-encoded fluorescent proteins or antibody-conjugated fluorescent probes in the fixed tissue and isotropically expands the tissue through a swellable polymer network to allow nanoscale (<70 nm) resolution on diffraction-limited confocal microscopes. Despite numerous advantages pro-ExM brings to biological studies, the complete protocol is time-consuming and can take multiple days to complete. Here, we adapted the pro-ExM protocol to the brain tissue of *Xenopus laevis* tadpoles and significantly accelerated the workflow using a commercially available specialized microwave processor. Microwave radiation has long been successfully used to accelerate and, in some case, enhance various steps of the conventional immunohistochemistry (IHC) and electron microscopy (EM) protocols, from fixation to antibody incubation. With automated pulse duration and wattage control, as well as precise temperature-regulation, microwave-assisted processing can effectively facilitate the diffusion of reagent molecules in the tissue in aqueous solutions, while preventing overheating hot spots inside the specimen. We tested the feasibility of microwave-assisted expansion microscopy using vibratome sections (40-80um) of tadpole brain tissues, and successfully reduced the hours-long Acx incubation (>6hr) and protease digestion (8-13 hr) steps to <30 minutes, making it possible to finish the entire pro-ExM protocol within a day. In addition to the significantly accelerated processing time, our microwave-assisted pro-ExM protocol maintains the superior resolution and signal-to-noise ratio of the original pro-ExM protocol, which we verified with cytoskeletal and synaptic markers. Furthermore, microwave-assisted pro-ExM protocol consistently yielded higher expansion factor, suggesting that microwave radiation may also facilitate the expansion process, aside from the increased diffusion rate. With appropriate adjustment of the microwaving parameters (wattage, pulse duration and interval, and number of cycles), this protocol can be adapted to other model organisms and tissue types and would greatly increase the efficiency of pro-ExM experiments.

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Poster

PSTR443. Light and Electron Microscopy

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Topic: I.03. Anatomical Methods

Support: NIH grant U01 MH117023
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Union's Horizon 2020 grant Laserlab-Europe 654148
Fondazione CR Firenze grant

Title: A multimodal pipeline for creating a cellular resolution atlas of human Broca's area

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Abstract: Cells are not uniformly distributed in the human cerebral cortex. Rather, they are arranged in a regional and laminar fashion that spans a range of scales. We present an innovative imaging and analysis pipeline to construct a reliable cell census of human Broca's area. Magnetic resonance imaging (MRI) is used to establish a macroscopic reference coordinate system of laminar and estimated cytoarchitectural boundaries. Cell counting is obtained with a digital stereological approach performed on the 3D reconstruction of the sample at cellular resolution using a custom-made inverted confocal light-sheet fluorescence microscope (LSFM). Mesoscale optical coherence tomography (OCT) is used to register the distorted histological cell typing obtained with LSFM to the MRI-based atlas coordinate system. The result is an integrated high-resolution cellular census of Broca's area within a whole-brain reference space atlas. In particular, a whole hemisphere is first imaged at 150 µm isotropic resolution with a 7 T MRI, and then Broca's area is dissected out. The block is cleared with TDE for 500-µm in-depth imaging with OCT. The system is equipped with a custom-made vibratome that enables serial sectioning of the block after each acquisition. The resulting slabs are treated with the SHORT method for immunofluorescence staining and optical clearing. LSFM imaging allows obtaining 3D reconstruction of the slabs with an isotropic resolution of 3,3 µm. Finally, digital stereology is performed to obtain the count of various neuronal markers.

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Poster

PSTR443. Light and Electron Microscopy

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Topic: I.03. Anatomical Methods

Support: NIDA U01DA043098
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The Hope for Depression Research Foundation (HDRF)
The Pritzker Neuropsychiatric Research Consortium

Title: Assessment of multiplexed quantitative ability of in situ hybridization chain reaction based upon fluorescent intensity and mRNA grain count correlation

Authors: *V. KUMAR[1], D. M. KROLEWSKI[2], M. FOLTZ[2], H. AKIL[2], S. J. WATSON[3]; [2]Univ. of Michigan, [1]Univ. of Michigan, Ann Arbor, MI; [3]Univ. of Michigan, Michigan Neurosci. Inst., Ann Arbor, MI

Abstract: The cost effectiveness of in situ hybridization chain reaction (HCR) compared to other multiplexing in situ hybridization approaches (e.g., RNAscope) makes it a desirable method for RNA expression analysis. However, variations in the individual probe binding and the extent of linear chain amplification, can influence both the fluorescence intensity and the mRNA grain (copy number) count. Such variations could become more prominent in mammalian brain tissues, particularly in the postmortem human brain tissues where disease conditions can alter the RNA integrity and thus the overall binding sensitivity of probes. Here, we assess the multiplexed quantitative ability of HCR for mRNA analysis in multiple cell types in fresh-frozen brain tissue, and explore key factors including the choice of fluorophores, tissue preparation, experimental conditions, and imaging parameters which critically determine the overall sensitivity and effectiveness of the HCR quantitation. Using multi-round HCR, we analyze *Vglut1*, *Gad2*, *Fgf12* transcripts based upon fluorescence intensity and grain count estimation. These genes under basal conditions in rat brain, represent a dynamic range of expression pattern in terms of cell number and mRNA copies. Our preliminary tests for *Fgf12* and *Gad2* suggest a linear association between voxel intensity and grain counts for hairpin concentration range 2-30 nM and incubation times 1-2 hrs. Thereafter, increasing the number of probe pairs, longer hairpin incubation times and/or higher concentrations appears to yield brighter and larger spots which fail to resolve properly, thus, inversely effects the segmentation of individual grains particularly in high grain density (subcellular) locations. We further explore and highlight the key factors which determine consistency, sensitivity, and optimum signal to noise ratio, essential for both qualitative and quantitative analysis. Based upon our observations, choice of fluorophore (AlexaFluor-647 or -546 vs. AF-488) hairpin conjugates, tissue fixation (fresh frozen vs.

perfused) and the abundance of transcript level (grain density and cell number) critically determines the effectiveness of the HCR for accurate grain counting with respect to the mRNA expression. For example, *Gad2* in fresh-frozen tissues and *Npy* when detected with AF-647 or -546 consistently showed biologically accurate cell counts in comparison to perfused tissues using AF-488 or -405, respectively. Similarly, genes with high density of grains such as *Sst* present challenge to accurately resolve individual grains and count despite using low concentration of hairpins (0.6nM to 1.25nM) or fewer probe pairs (1-2 pairs).

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Poster

PSTR443. Light and Electron Microscopy

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Topic: I.03. Anatomical Methods

Title: Pku tag, a genetically-encoded shape tag for light and electron microscopy

Authors: *R. SUN, L. WANG, Y. HU, Y. LI;
Peking Univ. Sch. of Life Sci., Beijing City, China

Abstract: The neural network in the brain is composed of hundreds or thousands of neuronal types with different morphology, electrophysiological activity and gene expression profiles. In order to dissect the large number of neuronal types in the brain, understand their gene expression and connectomic characteristics, and explore the role of these neurons in complex animal behavior, a sufficient number of tags are needed for both optically cellular labeling under light microscopy (LM) and ultrastructural labeling under electron microscopy (EM). However, tags that currently exist are still limited, constraining our understanding of the immense diversity of neuronal types. Here, we have developed a series of new genetically-encoded tags, named PKU (Polymer King-size Unit) tags, which make use of the polymerization of self-assembling proteins to generate easily visible and distinguishable shapes to encode diverse cell type identities. By combining the multi-shape PKU tags with fluorescent colors and subcellular localizations, we have further expanded the number of labeling tags. We also demonstrated that the combinations of tags could, in principle, expand the labeling patterns to up to thousands of types, far exceeding the number of known neuronal types. Additionally, shape tags are suitable for neuronal circuit tracing between multiple brain regions. Finally, to test whether PKU tag could be used as a versatile tag for brain connectomic studies in EM, we fused APEX2, a genetically-encoded EM reporter, with distinct PKU tags. The results showed that PKU tags could preserve their distinct shape features under extreme fixation condition of EM and could be used to tag multiple neurons as a “brainbow” for EM.

Disclosures: R. Sun: None. **L. Wang:** None. **Y. Hu:** None. **Y. Li:** None.

Poster

PSTR443. Light and Electron Microscopy

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR443.13/WW82

Topic: I.03. Anatomical Methods

Support: NIH/NIMH Grant 1DP2MH119423-01
NIH Grant 5UH3TR002151-05

Title: Scalable projected light sheet microscopy for high-resolution imaging of large samples

Authors: *Y. CHEN[1,2,3], C. GONG[2,3,1], S. CHAUHAN[3,1], E. D. DE LA CRUZ[1,3], M. S. DATTA[4,1], R. TOMER[1,2,3,4];
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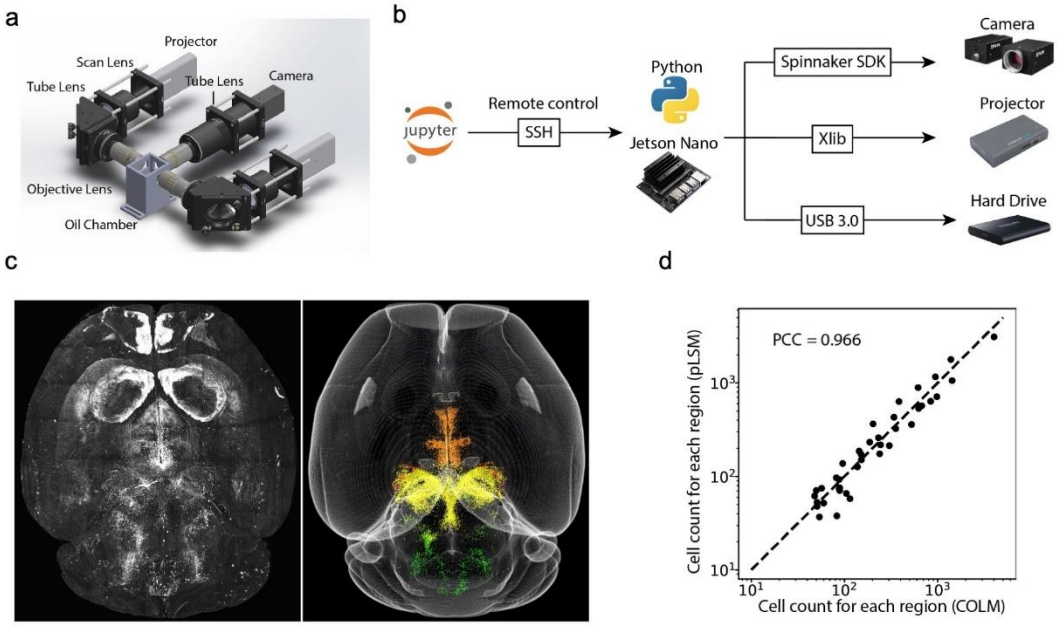
Abstract: Light sheet fluorescence microscopy (LSFM) is a versatile imaging technique used for live and fixed samples, particularly for high-resolution mapping of large cleared intact organs. However, existing high-performance LSFM implementations are often prohibitively expensive and lack scalability needed for high-throughput applications. Here, we introduce a low-cost and highly scalable framework, called projected Light Sheet Microscopy (pLSM), while maintaining high imaging quality. By leveraging consumer-grade off-the-shelf components, including pocket laser projectors, inexpensive CMOS cameras, Nvidia nano board, and optimized optical paths, pLSM offers comparable imaging performance to high-end systems at just one twentieth of the cost. One of pLSM's key strengths lies in its over-the-network control architecture and compatibility with various sample types, including brains cleared with multiple tissue clearing methods as well as live imaging application. The plug-and-play architecture and remote operability from anywhere, make pLSM highly scalable for high-throughput imaging applications. We demonstrate pLSM’s capabilities by performing high-resolution, multi-color imaging of intact whole mouse brains and post-mortem human brain samples cleared using various methods. Moreover, we showcase its potential for comprehensive phenotyping by quantitative mapping of the brain-wide distribution of entire dopamine system in mouse brain. Overall, the open access pLSM framework has the potential to further democratize LSFM and facilitate greater scalable access to high-resolution light sheet microscopy.Figure. (a) 3D model of the pLSM system. (b) pLSM control architecture. (c) High resolution pLSM imaging of TH+ dopamine neurons in intact mouse brain followed with quantitative mapping by brain-wide neuron segmentation. (d) Region-specific dopamine neuron counts comparison between pLSM and COLM (a high-end system). A high degree of agreement achieved.



Disclosures: **Y. Chen:** None. **C. Gong:** None. **S. Chauhan:** None. **E.D. De La Cruz:** None. **M.S. Datta:** None. **R. Tomer:** None.

Poster

PSTR443. Light and Electron Microscopy

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR443.14/WW83

Topic: I.03. Anatomical Methods

Support: R00MH116100

Title: Validation of light-sheet microscopy for identifying the laminar distribution of parvalbumin-, calbindin-, and calretinin-positive neurons in the macaque cortex

Authors: ***P. D. MENG**[1], M. LICHTENFELD[1], A. G. MULVEY[1], B. M. CARLSON[1], B. MITCHELL[1], A. V. MAIER[2], A. BASTOS[3];
[1]Psychology, [2]Dept. of Psychology, [3]Psychology and Vanderbilt Brain Inst., Vanderbilt Univ., Nashville, TN

Abstract: Inhibitory interneurons are hypothesized to play critical roles in cortical circuit operations such as spatial summation, prediction, and attention. Specific classes of interneurons may also be involved in controlling oscillatory dynamics. These neurons can now be precisely studied in transgenic mice and other genetically accessible species using light sheet microscopy. However, in classical primate histology, anatomical analysis is done by slicing. This

permanently deforms the sample orthogonal to the slicing plane, making analysis possible in only one plane per sample. Light sheet microscopy works by loading in specific antibodies into delipidated tissue, and imaging is performed non-destructively in three dimensions. This technique has been mostly applied to small brains (e.g., mouse brains, ~0.4 cm3). The size, scarcity of primate tissue, and the lack of methodological validation have mostly prevented primate researchers from using light sheet imaging. We seek to validate the methodology of light sheet microscopy for high-throughput anatomical imaging for the three main classes of inhibitory neurons (Parvalbumin-PV, Calbindin-CB, and Calretinin-CB positive interneurons) in the macaque brain. In two rounds, we performed staining and imaging of neurons expressing fluorescent markers for NeuN (a non-specific indicator for neurons) and PV, followed by CR and CB in the same block (7mm x 11mm x 8mm) of macaque parietal cortex. Antibody penetration and expression in the tissue was high and approximately uniform in the tissue for Neun, CB, and CR, but weak for PV. We developed an automated cell counting pipeline which avoids the drawbacks of manual counting. For this analysis we used LIP as our region of interest. We used MATLAB functions to run edge detection on the 3D matrix, followed by pixel-connectivity on the binary output to identify potential neurons. We used volume and solidity (measure of roundness) statistics to differentiate neurons from noise. We validated the automated statistics by overlaying the computer generated centroids on the raw data, and adjusted thresholds to obtain the best match. Finally we compared the laminar profile of CB+, CR+, and PV+ interneurons from light sheet to classical histological cell counting. In both datasets we found expression of CB/CR was high in superficial layers (with a peak in layer 2), and PV expression peaked in middle layers. This demonstrates the feasibility of light sheet microscopy and automated cell counting to study the composition of inhibitory neurons in the non-human primate brain. This will lead to both increased throughput of data collection, as well as enabling discovery of new neural patterns.

Disclosures: P.D. Meng: None. **M. Lichtenfeld:** None. **A.G. Mulvey:** None. **B.M. Carlson:** None. **B. Mitchell:** None. **A.V. Maier:** None. **A. Bastos:** None.

Poster

PSTR443. Light and Electron Microscopy

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR443.15/XX1

Topic: I.03. Anatomical Methods

Support: DFG : SPP1926
DFG : MBExC
DFG: Leibniz program

Title: Quantification of cochlear neurons based on light sheet microscopy

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[2]InnerEarLab, Inst. for Auditory Neuroscience, Univ. Med. Ctr., Goettingen, Germany; [3]Auditory Circuit Lab, Inst. for Auditory Neuroscience, Univ. Med. Ctr., Göttingen, Germany; [4]Göttingen Grad. Sch. for Neurosciences, Biophysics and Mol. Biosci. (GGNB), Univ. of Göttingen, Göttingen, Germany; [5]Auditory Neurosci. and Optogenetics Laboratory, German Primate Ctr., Göttingen, Germany; [6]Cluster of Excellence "Multiscale Bioimaging: From Mol. Machines to Networks of Excitable Cells,"; Univ. of Göttingen, Göttingen, Germany; [7]Auditory Neurosci. and Nanophysiology Group, Max Planck Inst. of Multidisciplinary Sci., Göttingen, Germany

Abstract: The world health organisation (WHO) indicates that about half a billion of people suffer from disabling hearing loss. The most common form is sensorineural hearing impairment, which arises from degeneration or dysfunction of cochlear cells (World report on hearing, WHO, 2021). Novel therapeutic strategies involving gene or cell therapies, aimed at restoring hearing (Wolf et al., 2022; Ma et al., 2019) necessitate unbiased quantification of targeted cells in the intact cochlea. This becomes challenging, especially when there are more than hundreds of targeted cells present.

Our group is developing optical cochlear implants, where the cochlear neurons (spiral ganglion neurons, SGNS) are optogenetically modified and tagged by a fluorescent protein to further investigate the transduction rate by immunohistochemistry (Hernandez et al., 2014; Wrobel et al., 2018; Huet et al., 2021). Since each cochlea contains thousands of SGNs, we have developed a pipeline to fully automate cell quantification based on volumetric imaging of intact cochleae by light sheet fluorescence microscopy.

Previously, we developed a semi-automatic approach using Arivis Vision4D based on automatic seed finding and watershed-based algorithm to segment SGNs of intact mouse cochleae and estimated the performance evaluation based on the F1 score (~0.97). However, this approach was time consuming, dependent on sample quality and required manual inputs (Keppeler et al., 2021).

In this study, we are developing a fully automatic segmentation approach based on the machine learning algorithm-Stardist (Schmidt et al.,2018; Weigert et al.,2020). This technique uses a convolutional neural network to predict cells shaped as star convex polyhedrons, considering the object probability and star-convex distances. From the data obtained from the previous semi-automatic approach, we cropped out 21 smaller sections of images and their corresponding segmented results as annotations, to generate the first model. Despite the very small number of training data used to generate this model, we achieved a high F1 score of 0.91. However, several rounds of training cycles are required to increase the quality of predicting SGNs. We will employ the NAPARI framework to combine the first model's predictions and incorporate manual corrections, optimizing the precision of the SGN quantification. In this poster, we would discuss the methodology, recent findings, and prospects of fully automated quantification of SGNs. The use of such tools based on machine learning algorithms contributes to the advancement of hearing research and the development of innovative therapeutic approaches to deafness.

Disclosures: A. Thirumalai: None. **T. Quilitz:** None. **A. Huet:** None. **T. Moser:** Other; founder of OptoGenTech GmbH.

Poster

PSTR443. Light and Electron Microscopy

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR443.16/XX2

Topic: I.03. Anatomical Methods

Support: NIMH R00MH116100
R01-EY029666
R01EY027402
T32EY007135

Title: The Laminar Distribution of Inhibitory Cell Types in Macaque Cortex and its Implications for Hierarchical Information Processing

Authors: *M. J. LICHTENFELD[1], A. G. MULVEY[1], H. NEJAT[1], Y. XIONG[1], B. M. CARLSON[1], B. MITCHELL[1], D. MENDOZA-HALLIDAY[2], P. D. MENG[1], R. DESIMONE[2], A. MAIER[1], J. H. KAAS[1], A. BASTOS[1];
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Abstract: The Canonical Microcircuit (CMC) has been hypothesized to be the fundamental unit of information processing in laminar cortex. Each CMC contains (with some variation) each of the six cortical layers along with their specific cellular characteristics and organization. Importantly, the characteristic oscillatory activity spanning across the CMC is produced by interplay between GABAergic inhibitory interneurons and excitatory neurons. However, the anatomical and physiological basis of the CMC has primarily been established in excitatory neuron anatomy and neurophysiology. We sought to describe the laminar distribution of inhibitory cell types across the cortex, and in this work, we investigate the role of inhibitory cells in producing laminar electrophysiology. Brain tissue was collected from six macaques. Electrophysiology was performed in six macaques using laminar probes placed perpendicular to the cortical sheet. Anatomy and electrophysiology were collected in 11 distinct cortical areas spanning the cortical hierarchy: Primary visual cortex (V1), V2, V3, V4, lateral interparietal cortex (LIP), medial superior temporal cortex (MST), medial temporal area (MT), 8A/FEF, TEO, premotor cortex (PMD), and lateral prefrontal cortex (LPFC). Additional anatomical data was collected in two areas: 7A and dorsal prelunate (DP). We stained representative slices for Parvalbumin (PV), Calbindin (CB), and Calretinin (CR) positive neurons, which comprise the three main inhibitory cell types found in macaque cortex. Nissl and NeuN staining were also performed to provide contextual information about laminar position. We found a significant laminar structure of PV+, CB+, and CR+ neurons across the cortical hierarchy and across animals. PV+ neurons consistently peaked in layer 4, and CB+ and CR+ neurons peaked in layer 2 and upper parts of layer 3. We further found a consistent relationship between the laminar distribution of these inhibitory neurons with power in the local field potential. PV+ distributions correlated positively with theta (4-8 Hz) and gamma (40-150 Hz) oscillations. CR+ and CB+ neuron distributions correlated negatively with theta, alpha (8-12 Hz), and beta (15-30 Hz) oscillations. Additionally, CR+ neurons were found to significantly increase in proportion moving up the hierarchy, while CB+ neurons significantly decreased. These findings, of patterns

of inhibition across cortex and their alignment with laminar neurophysiology at specific frequencies, provide a richer functional and anatomical basis for the CMC as the fundamental unit of information processing.

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Poster

PSTR443. Light and Electron Microscopy

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR443.17/XX3

Topic: I.03. Anatomical Methods

Support: NIH R00CA240681
NIH RF1MH128841
NIH R01NS123959
NIH U42OD011123
NIH P51OD010425
Paul G. Allen Foundation

Title: Expansion-assisted selective plane illumination microscopy for nanoscale imaging of centimeter-scale tissues

Authors: *A. GLASER[1], J. CHANDRASHEKAR[1], J. VASQUEZ[1], C. ARSHADI[1], N. OUELLETTE[1], X. JIANG[1], J. BAKA[1], G. KOVACS[1], M. WOODARD[1], S. SESHAMANI[1], K. CAO[1], N. CLACK[2], A. GRIM[1], P. BALARAM[3], E. TURSCHAK[3], A. LIDDELL[2], J. ROHDE[1], A. HELLEVIK[3], K. TAKASAKI[3], L. ERION BARNER[1], M. LOGSDON[1], C. CHRONOPOULOS[1], S. DE VRIES[1], J. TING[3], S. I. PERLMUTTER[4], B. KALMBACH[3], N. DEMBROW[3], C. REID[3], D. FENG[1], K. SVOBODA[1];
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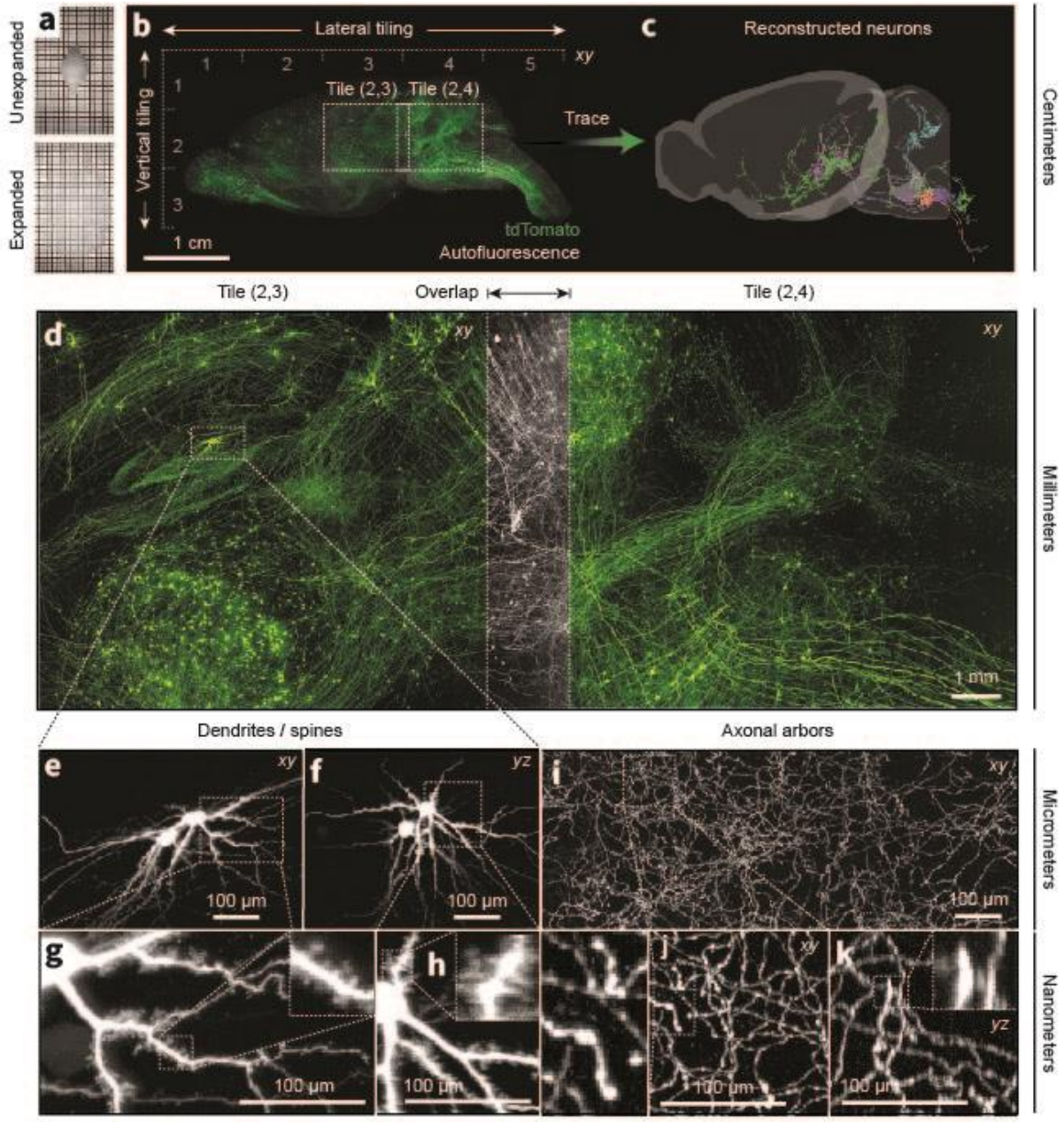
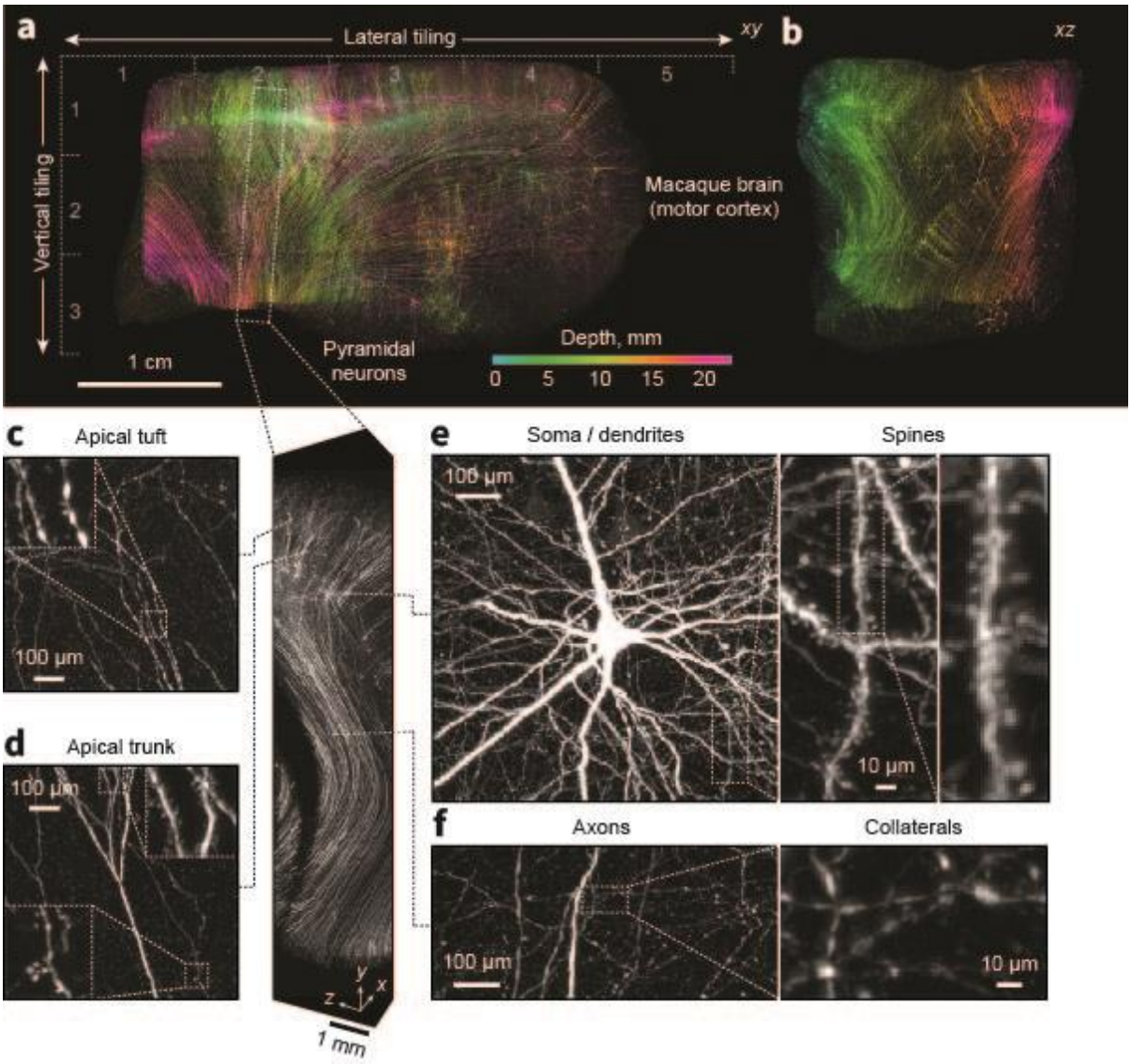
Abstract: Recent advances in tissue processing, labeling, and fluorescence microscopy are providing unprecedented views of the structure of cells and tissues at sub-diffraction resolutions and near single molecule sensitivity, driving discoveries in diverse fields of biology, including neuroscience. Biological tissue is organized over a wide range of spatial scales. Understanding cellular function and multi-cellular organization often requires simultaneous probing of tissue architecture over scales of nanometers to centimeters. Harnessing molecular imaging across three-dimensional samples on this scale requires new types of microscopes with larger fields of view and working distance, as well as higher imaging throughput. We present a new expansion-assisted selective plane illumination microscope (ExA-SPIM) with diffraction-limited and

aberration-free performance over a large field of view (85 mm^2) and working distance (35 mm). Combined with new tissue clearing and expansion methods, the microscope allows nanoscale imaging of centimeter-scale samples, including entire mouse brains, with high isotropy, no sectioning, and at speeds approaching 1 gigavoxel/sec. We illustrate ExA-SPIM by reconstructing individual neurons, including complete axonal arbors across the entire mouse brain, imaging xfp-expressing cortico-spinal neurons in the macaque motor cortex, and tracking axon pathways in the human white matter fluorescently labeled for heavy chain neurofilaments.





Disclosures: **A. Glaser:** None. **J. Chandrashekar:** None. **J. Vasquez:** None. **C. Arshadi:** None. **N. Ouellette:** None. **X. Jiang:** None. **J. Baka:** None. **G. Kovacs:** None. **M. Woodard:** None. **S. Seshamani:** None. **K. Cao:** None. **N. Clack:** None. **A. Grim:** None. **P. Balaram:** None. **E. Turschak:** None. **A. Liddell:** None. **J. Rohde:** None. **A. Hellevik:** None. **K. Takasaki:** None. **L. Erion Barner:** None. **M. Logsdon:** None. **C. Chronopoulos:** None. **S. de Vries:** None. **J. Ting:** None. **S.I. Perlmutter:** None. **B. Kalmbach:** None. **N. Dembrow:** None. **C. Reid:** None. **D. Feng:** None. **K. Svoboda:** None.

Poster

PSTR443. Light and Electron Microscopy

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR443.18/XX4

Topic: I.03. Anatomical Methods

Title: Bone tissue clearing reveals new neuronal structures in teeth and jawbones

Authors: *Y. YUN[1], S. KANG[2], J.-Y. PARK[3], H. CHOI[1];
[1]Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of; [2]Dent. reserch institute, Seoul, Korea, Republic of; [3]Oral Maxillofacial Surgery, Seoul Natl. Univ. Col. of Dent., Seoul, Korea, Republic of

Abstract: As the demand for dental work such as tooth extraction and implant surgery increase, trigeminal nerve injuries are becoming more common. Despite efforts to generate microscopic images of the nerves in dental pulp using conventional neurohistological analysis, the whole maxilla and mandible visualization in a whole, undamaged tooth has remained a technically challenging task. The neural architecture of the branch of the peripheral nerve that travels through the alveolar bone where the teeth are rooted is unknown in particular. In this study, we developed a quick and easy technique that combines decalcification, tissue clearing, immunohistochemistry, confocal microscopy, light-sheet fluorescence microscopy, and quantitative analysis of full-thickness bowel for high-resolution 3D imaging of the mouse maxilla and mandible. In order to explain the neural network and offer novel insights into neuronal anatomy, statistical techniques and three-dimensional image reconstruction were applied. The findings of this investigation supported the idea that at least 40% EDTA for two weeks was necessary for the complete decalcification process. After 16 hours of electrophoretic tissue clearing mode and another 14 hours of immersion in SDS buffer at 60°C, the overall structural details and staining properties were at their best. We were able to see the mouse mandible and maxilla in three dimensions at the macroscale-level, as well as the dental pulp's whole peripheral nerve branch that runs through the alveolar bone. When examined using a 10x objective lens, full-thickness mandible pictures might reach sizes of 8 x 3 x 1 mm^3. Quantitative data for the maxilla and mandible revealed comparatively distinct features. In conclusion, the developed techniques could be able to offer a thorough 3D view of the nerve fibers found in soft tissues surrounded by hard tissues. By giving a 3D image of numerous immune cells in an intact mouse maxilla and mandible, this technique could be used to facilitate a variety of research methodologies on neural-immune interaction.

Disclosures: **Y. Yun:** None. **S. Kang:** None. **J. Park:** None. **H. Choi:** None.

Poster

PSTR443. Light and Electron Microscopy

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR443.19/XX5

Topic: I.03. Anatomical Methods

Title: Deep learning-assisted automated image analysis of toxicant-induced changes in brain regions and nerve fibers

Authors: *M. STAUP[1], O. MENDES[1], A. ŻURAW[2], J. LENSEN[3], V. PICCICUTO[3], E. RAMAKER-ERLANDSON[6], A. HOLMEN[4], T. MASINDE[1], E. TREMBLAY-ROUSSEAU[5]; [1]Charles River, Durham, NC; [2]Charles River, Frederick, MD; [3]Charles River, S-Hertogenbosch, Netherlands; [4]Charles River, Reno, NV; [5]Charles River, Senneville, QC, Canada; [6]North Carolina State Univ., Raleigh, NC

Abstract: Neuropathological assessment of the effect of toxic compounds on the central and peripheral nervous systems requires classification and quantification of a variety of anatomical and histological endpoints. Many of these methods require time-consuming, manual measurements subject to considerable variability within and between raters. Automated deep learning-assisted digital image analysis provides an opportunity to decrease turnaround time for these studies and increase the precision of the data. At Charles River, we have validated a method of generating qualified algorithms, which leverages the depth of expertise from our wide field of pathologists across the globe and our own team of developers. This approach is very efficient and flexible enough to create and adapt algorithms on a study-by-study basis. Here, we show qualification data demonstrating the predictive strength and statistical power of automated methods for brain region and nerve fiber segmentation relative to the traditional, manual measurements used for assessing trophic effects of toxicants in several brain regions and peripheral, myelinated fibers.

Disclosures: **M. Staup:** A. Employment/Salary (full or part-time):; Charles River Laboratories. **O. Mendes:** A. Employment/Salary (full or part-time):; Charles River Laboraties. **A. Żuraw:** A. Employment/Salary (full or part-time):; Charles River Labs. **J. Lensen:** A. Employment/Salary (full or part-time):; Charles River Labs. **V. Piccicuto:** A. Employment/Salary (full or part-time):; Charles River Labs. **E. Ramaker-Erlandson:** None. **A. Holmen:** A. Employment/Salary (full or part-time):; Charles River Labs. **T. Masinde:** A. Employment/Salary (full or part-time):; Charles River Labs. **E. Tremblay-Rousseau:** A. Employment/Salary (full or part-time):; Charles River Labs.

Poster

PSTR443. Light and Electron Microscopy

Location: WCC Halls A-C

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Program #/Poster #: PSTR443.20/XX6

Topic: I.03. Anatomical Methods

Support: NIH: UF1 NS108213 U01NS094296 U19NS104649 RF1 MH114276 R01 NS063226 U01 CA236554
Simons Collaboration on Global Brain: 542991
Leducq: 22CVD0

Title: High-speed two-photon light sheet microscopy for volumetric functional imaging in the mouse brain.

Authors: *O. DERMANCI[1], H. YU[2], W. LI[2], W. LIANG[4], M. CASPER[3], R. YAN[3], E. ÖZEN[3], C. CAMPOS[2], E. M. HILLMAN[3];
[2]Mortimer B. Zuckerman Mind Brain Behavior Inst., [3]Biomed. Engin., [1]Columbia Univ., New York, NY; [4]Univ. of Sci. and Technol. of China, Hefei, China

Abstract: High-speed two-photon light sheet microscopy for volumetric functional imaging of somatic and dendritic activity in the awake mouse brain
Authors
Ö. DERMANCI, H. YU, W. LI, W. LIANG, M. J CASPER, R. W. YAN, E. ÖZEN, C. PEREZ CAMPOS, E.M.C. HILLMAN
Laboratory for Functional Optical Imaging, Department of Biomedical Engineering, Columbia University, New York, NY, USA;Mortimer B. Zuckerman Mind Brain Behavior Institute, Columbia University, New York, NY, USA
Disclosures
Ö. DERMANCI: None. H. YU: Leica W. LI: Leica. W. LIANG: Leica M. J CASPER : None. R. W. YAN: None E. ÖZEN: None C.P CAMPOS: Leica, E.M.C. HILLMAN: Leica
Two photon calcium imaging is widely used for recording neural activity in the living mammalian brain. However, extending high-speed two-photon imaging to 3-dimensions has proven difficult. In conventional 2-photon microscopy studies which are confined to 2D, imaging can only capture in-plane structures and usually focus on imaging somatic activity. While dendritic segments may be visible in 2D images, it is almost impossible to relate their activity to specific cells, or to simultaneously record the activity of multiple soma and their dendrites in parallel. Meanwhile, attempts to image activity throughout complex 3D dendritic projections have needed significant and precise a-priori knowledge, and are sensitive to natural movements. Here, we demonstrate that two-photon light-sheet microscopy offers unique advantages for high-speed, volumetric functional brain imaging, even in the scattering in-vivo mouse brain. We describe and characterize a two-photon version of swept confocally aligned planar excitation microscopy (2P-SCAPE), a single-objective light sheet approach capable of high-speed volumetric imaging to depths exceeding 400 microns below the cortical surface. 2P-SCAPE can achieve near-isotropic sampling of large volumes spanning depth ranges of over 300 microns at up to 20 volumes per second. Recordings of in-vivo GCaMP activity at cellular resolution in awake mice capture neural activity in apical dendrites and soma in awake behaving mice. Our results demonstrate that a single-objective two-photon light-sheet is a powerful alternative to point-scanning two-photon for high-speed volumetric imaging of mouse brains.

Disclosures: O. Dermanci: None. **H. Yu:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Leica. **W. Li:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Leica. **W. Liang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Leica. **M. Casper:** None. **R. Yan:** None. **E. Özen:** None. **C. Campos:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Leica. **E.M. Hillman:** E. Ownership

Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Leica.

Poster

PSTR443. Light and Electron Microscopy

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR443.21/XX7

Topic: I.03. Anatomical Methods

Title: Vascular Network Analysis across the Whole Mouse Brain via Light-sheet Microscopy and AI-Based Image Analysis

Authors: *K. MINATOHARA[1], K. SUGAWARA[2], M. OITATE[1];
[1]Daiichi Sankyo, Tokyo, Japan; [2]LPIXEL Inc., Tokyo, Japan

Abstract: In this study, we performed fluorescent staining and light-sheet microscopy imaging of cleared mouse brain to acquire fluorescently labeled vascular images throughout the whole brain. Furthermore, we established a method for vascular image analysis using AI, successfully achieving vascular image analysis across the whole brain. First, fluorescent labeling of blood vessels and tissue clearing of mouse brains were visualized by light-sheet microscopy. This technique allowed us to capture the vascular network of the whole brain with high spatial resolution. The acquired images enabled the observation of both the overall brain structure and the detailed morphology of individual vessels. Specifically, we extracted 3D features from the vascular data, enabling the characterization of complex vascular structures throughout the brain. Furthermore, we developed a machine learning model that required minimal annotation for training. This model effectively registered and aligned the vascular structures in the 3D brain volume, enabling accurate visualization and analysis of vascular network across the whole brain. Our results demonstrate the effectiveness of combining fluorescent labeling and imaging of the whole-brain vasculature in cleared mouse brains, highlighting the potential of this novel approach. Additionally, the AI-based vascular image analysis method represents a groundbreaking advancement for 3D analysis of the whole-brain vasculature. Through this study, we aim to disseminate knowledge of this innovative approach for vascular analysis in cleared mouse brains, providing researchers with new insights and prospects in brain vascular research.

Disclosures: K. Minatohara: A. Employment/Salary (full or part-time):; Daiichi Sankyo Co., Ltd. **K. Sugawara:** A. Employment/Salary (full or part-time):; LPIXEL Inc., RIKEN. **M. Oitate:** A. Employment/Salary (full or part-time):; Daiichi Sankyo Co., Ltd..

Poster

PSTR443. Light and Electron Microscopy

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR443.22/XX8

Topic: I.03. Anatomical Methods

Support: Ministry of University and Research (MUR), National Recovery and Resilience Plan (NRRP), project MNESYS (PE0000006)
Ministry of Foreign Affairs and International Cooperation (MAECI) - Strategic Projects of cooperation with US

Title: Smart light-sheet fluorescence expansion microscopy for imaging the full-intact mouse nervous system

Authors: N. BRADY[1], E. IMBIMBO[1], J. E. RODRIGUEZ GATICA[2], A. FRANCESCHINI[4], G. MAZZAMUTO[5], F. S. PAVONE[6], U. KUBITSCHECK[3], ***L. SILVESTRI**[4];
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Abstract: At the very basis of all physiological research is the ability to image the anatomy of the tissue in question, facilitated by the tools available. Each organ system has its own defined structure corresponding to its functionality which gives rise to the need to image these systems in an unbiased and complete manner. Analysing large volumes of organ tissue, however, remains a palpable challenge within science. Despite the variety of sample preparation and imaging techniques, many require complex protocols and expensive equipment and reagents as well as being limited by the size of the sample able to be acquired as a whole. Organ systems and, specifically, neuronal circuits often extend beyond this limited data field. Here we present a pipeline that utilizes the resolution capabilities of fluorescent expansion microscopy techniques, in which the sample is physical and isotopically enlarged, and targets of interest are fluorescently labelled using assorted techniques, creating the data field. Adding to the pipeline is customized dual-sided light sheet microscope capable of imaging large samples, made from standard optical equipment, along with targeted data acquisition software which specifically targets the data field. The large sample chamber and the choice of different refractive index matching solutions provide the grounds for imaging at several resolutions, from the sub-micron to the mesoscale. Since the samples are able to undergo various rounds of testing under different expansion factors, a hierarchal approach can be employed: first imaging at low resolution in order to acquire the entire data field and proceeding to increase the resolution and reducing the data field to image finer structures at higher-resolution.The physical capabilities of the system facilitate the imaging of neuronal morphology and extended to the central nervous system. Moreover, this system has the potential to extend to other large tissue volumes, providing the basis for further anatomical research. The fundamental goal of this pipeline is to stay within the parameters accessible to most laboratories in terms of cost and the ability to be replicated, fulfilling the ultimate goal of expanding knowledge and enhancing opportunities for the research community.

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Poster

PSTR443. Light and Electron Microscopy

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Topic: I.03. Anatomical Methods

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Title: Two-photon GRIN microendoscope for stereotactic neurosurgery

Authors: T. WELTON[1], ***S. A. SUAREZ**[1], N. M. GEORGE[2], B. OZBAY[5], A. GENTILE POLESE[3], G. OSBORNE[4], G. L. FUTIA[1], J. KUSHNER[2,4], B. KLEINSCHMIDT-DEMASTERS[6,7,8], A. L. ALEXANDER[8,9], A. ABOSCH[10], S. OJEMANN[8], D. RESTREPO[3], E. GIBSON[1];
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Abstract: During Deep Brain Stimulation (DBS) surgery for Parkinson’s Disease (PD), it is critically important to accurately place the electrodes within specific deep brain targets, such as the subthalamic nucleus (STN). Current methods of evaluating electrode position in the operating room include: 1) microelectrode recordings, which increase the risk of intracranial hemorrhage and often require the patient to be awake during invasive brain surgery, and 2) intraoperative imaging, which has a limited resolution. Our group proposes to use two-photon microendoscopy to perform high resolution, real-time imaging along the surgical trajectory through a narrow stereotactic cannula to assist with neuronavigation and detect blood vessels using Second Harmonic Generation (SHG). Here we show that conventional, two-photon microscopy and several rudimentary image classifiers can be used to distinguish the human STN from the surrounding tissue due to the spatial distribution of autofluorescence. We also develop a 186 mm long and 1.2 mm diameter microendosocpe composed of Gradient Refractive Index (GRIN) lenses. This device has a magnification of ~2.8X, a field of view of ~180 microns, and a

resolution of 0.86 microns and 9.6 microns in the lateral and axial directions, respectively. The prototype was used to image endogenous autofluorescence and blood vessels in *ex-vivo* human brain tissue. We also report on our progress towards a second-generation prototype.

Disclosures: T. Welton: None. **S.A. Suarez:** None. **N.M. George:** None. **B. Ozbay:** None. **A. Gentile Polese:** None. **G. Osborne:** None. **G.L. Futia:** None. **J. Kushner:** None. **B. Kleinschmidt-DeMasters:** None. **A.L. Alexander:** None. **A. Abosch:** None. **S. Ojemann:** None. **D. Restrepo:** None. **E. Gibson:** None.

Poster

PSTR443. Light and Electron Microscopy

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Program #/Poster #: PSTR443.24/Web Only

Topic: I.03. Anatomical Methods

Support: NIDDK/NIH grant U24 DK116195

Title: The Expression Profile Of Selected Class A, B And C Orphan GPCRs And CNS Localizations

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Abstract: Containing more than 800 members, G protein coupled receptors (GPCRs) represent the largest class of membrane proteins in the human genome. It is estimated that 20-30% of FDA approved drugs target GPCRs making them the largest druggable family in the genome. However, the endogenous ligand/function remains unknown for more than 100 orphan GPCRs (oGPCRs). GPCRs are expressed in every major organ system in the body. Located in the cell membrane, GPCRs transduce extracellular signals into important physiological effects. Upon ligand dependent activation of a GPCR, signal transduction involves binding to and stimulating heterotrimeric G protein dissociation which in turn couples to various downstream effectors that modulate secondary messenger signaling molecules including cAMP, IP3, Ca^{+2} and others. Human GPCRs are classified into five major families: Rhodopsin (Class A), Secretin and Adhesion (Class B), Glutamate (Class C), and Frizzled/taste receptors 2 (TAS2). This study focuses on characterizing some selected understudied members of class A, Class B and class C GPCRs namely Gpr173, *MTNR1B, Adgrb2, Adgra3 and GprC5b* . For this study we generated CRISPR GPCR-*Cre* mouse lines (i) *Gpr173* (*Gpr173-EGFP-CT-IRES-ERT2-Cre*), (ii) *Gprc5b* (*Gprc5b-Ires-Cre*), (iii) Ar2 (*Adgrb2-EGFP-CT-IRES-ERT2-Cre*), (iv) Gpr125 (*Adgra3-mGL-CT-IRES-Cre*) as well as *Flpo* mouse line (v) *Mtnr1b* (*Mtnr1b-mCherry-Ires-Flpo*). The

Cre^{ERT2}mouse line is then crossed with reporter *Ai9-TdTomato* (Td-Tomato) mouse line. The animals were treated with tamoxifen (150mg/kg) for 4 days between postnatal (P) days 39 and 42 after which 22 different organs were harvested on P56. The *Gprc5b* mouse line was crossed with *Td-Tomato* mouse line and the *Mtnr1b* mouse line was crossed with *FRT-EGFP* mouse line. For *Gprc5b*, tissues were harvested on p56 and on p120. Histological analyses were performed to identify tissue-specific distribution of the Td-Tomato and EGFP reporter. Here we show the whole-body organ screening of *Gpr173*, *Mtnr1b* and *Gprc5b*. All results will be available on AMIS (http://amis2.docking.org/). AMIS is an open resource for mouse imaging data generated by NIH Commonfund Program for Illuminating Druggable Genome (IDG) that provides access to whole body distribution of understudied genes via engineered mouse lines. Following the tissue-specific distribution profile, multiplexing IHC was employed for further analysis to identify downstream signaling pathways. This would add to the knowledge and establish oGPCRs as suitable therapeutic targets.

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Poster

PSTR443. Light and Electron Microscopy

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Topic: I.03. Anatomical Methods

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UF1 NS108213
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Kavli Institute for Brain Sciences Pilot Grant
Leducq: 22CVD0

Title: Holis: a high-throughput multispectral imaging pipeline for cell-type atlasing of whole human brains

Authors: *M. J. CASPER[1], W. LI[2], C. PÉREZ CAMPOS[2], P. SIMKO[2], K. SWAYZE[1], R. YAN[1], W. WANG[4], I. VASYLIEVA[5], A. M. WATSON[5], J. F. CRARY[6], Z. WU[4], E. M. HILLMAN[3];
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Abstract: In a human brain around 200 billion cells are working together shaping who we are. Grasping their complex organization, even in a single brain remains a major challenge. Advances in tissue clearing provide a window onto cells inside of intact brains, and have recently been optimized for clearing and immunostaining human brain tissue. However, imaging cleared tissues on the scale of the whole human brain presents many challenges, with conventional methods likely to require many months or years of acquisition time per brain. Here, we present a complete imaging and analysis pipeline developed specifically to achieve high-throughput, multispectral imaging of entire, cleared and immunolabelled human brains at cellular resolution for cell-type atlasing with acquisition times of less than 2 weeks. Our human brain optimized light-sheet (HOLiS) microscopy system is a form of oblique-plane single objective light-sheet, capable of imaging 5mm thick, optically cleared, complete coronal sections of human brain. By imaging thick sections, we can reduce tissue deformations and cut-edge effects, although this necessitated novel optical solutions to enable implementation of a long working distance, multi-immersion primary objective lens. Another feature of HOLiS is its ability to image multiple spectral channels in parallel to enable multiplexed antibody labeling of the different cell-types. Combining multiple laser lines for simultaneous excitation, emitted fluorescence is spectrally divided by our novel 4-way image splitter for simultaneous detection of currently 5 spectral channels with the capacity for 9 channels imaged in parallel. The 5^{th} channel images a nuclear dye, providing fiducials for every cell. Although spectrally multiplexing adds complexity, it greatly accelerates HOLiS acquisition time and reduces data processing burden. Using ultra-fast cameras, we acquire multi-spectral images up to at 0.75 mm^3/s with micron sampling translating to image an entire human brain in < 2 weeks. At modest sampling density and 9 spectral channels, the data of a single human brain scan is expected to exceed 3 PB, imposing constraints on transfer, storage, data pre-processing, and accessibility. We developed a parallelizable analysis pipeline which locates every nucleus, and extracts cell-type information from the spectral channels to provide compressed point-cloud representations of the data that can be quantitatively analyzed, shared and compared between brains. These rich datasets can be clustered to explore cell type distributions and used to guide more complex feature-based analysis, neuroanatomical segmentation and efficient visualization of raw data.

Disclosures: **M.J. Casper:** None. **W. Li:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Leica Microsystems. **C. Pérez Campos:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Leica Microsystems. **P. Simko:** None. **K. Swayze:** None. **R. Yan:** None. **W. Wang:** None. **I. Vasylieva:** None. **A.M. Watson:** None. **J.F. Crary:** None. **Z. Wu:** None. **E.M. Hillman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Leica Microsystems.

Poster

PSTR443. Light and Electron Microscopy

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Topic: I.03. Anatomical Methods

Support: R01 CA208623
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1 R21 CA270902
WashU T32 Imaging Sciences Pathway

Title: In vivo microscopy of peripheral nerve regeneration

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Abstract: Visualization of axons, Schwann cells, and other intraneural cells in peripheral nerve injury models will facilitate investigation of the mechanisms of nerve regeneration. Here, we introduce a technique for chronic high-resolution microscopy of a murine peripheral nerve trunk. We surgically displace the biceps femoris to expose up to 5 mm of the sciatic nerve around the trifurcation and implant a transparent, flexible, sutureless skin-embedded optical window. This enables chronic observation of the sciatic nerve for more than 8 weeks with negligible impact on behavior and nerve function. We also developed an optically friendly nerve repair conduit to enable intravital monitoring of regeneration in a peripheral nerve transection repair model with up to 6 mm gap. Using our system in transgenic mice with fluorescent protein-expressing axons, Schwann cells, or vascular endothelial cells, as well as exogenously introduced fluorescently labeled fibrin and dyes to label myelin and vasculature, we observed a variety of cellular-scale events in uninjured nerve and in nerves post-injury via two-photon confocal microscopy. We successfully captured fascicle-specific Wallerian degeneration, Schwann cell differentiation, angiogenesis, and growth cone-led axonal elongation. We also visualized the in-vivo formation of a tissue cable across the nerve gap and intraneural fibrin degradation and collagen formation. In summary, we demonstrate a longitudinally intravitally imageable small-mammal model of major peripheral nerve injury. This model can be applied to elucidate molecular healing mechanisms by facilitating measurement of migrational and morphological dynamics in injured nerve within the same animal over time.

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Poster

PSTR444. Electrophysiology-Cellular

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Topic: I.04. Physiological Methods

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Title: Spatiotemporal backpropagation patterns of human single unit waveforms revealed by intraoperative high-density Neuropixels recordings

Authors: *D. MESZENA[1], A. C. PAULK[1], W. MUÑOZ[2], I. CAPRARA[2], M. JAMALI[2], B. F. COUGHLIN[1], C. WINDOLF[3], E. VAROL[4], Z. M. WILLIAMS[2], S. S. CASH[1];
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Abstract: High-density laminar silicon probes have been widely used in rodent electrophysiology but were virtually absent from human research until recently. Clinical research devices have so far been able to detect single-unit activity (SUA) with a poor spatial resolution (due to large inter-contact distances) or they have been limited to recording only the low-frequency component of the extracellular neural activity (local field potentials). We pioneered a rare opportunity to test state-of-the-art silicon probes (namely the custom-designed, backside-thickened Neuropixels 1.0-S probes) in the operation room setting during resective surgeries in the cases of tumors or epilepsy or the implantation of a deep brain stimulator (DBS) in patients with Parkinson's disease. These probes feature hundreds of closely packed active contact sites enabling for multi-channel oversampling of extracellular SUAs. Spatiotemporally resolved morpho-electric properties of single units allow us for sophisticated clustering of neural cell types. We focus on a particular spatial feature of cortical neurons called the backpropagating action potential (bAP). Intracellular spikes can propagate backward along the somatodendritic axis. This phenomenon can also be detected extracellularly on spike-triggered averages (STAs) of the corresponding SUAs after spike sorting. These bAPs have been observed in rodents (using standard Neuropixels 1.0 probes) in vivo with distinct spatiotemporal patterns across brain regions, putative cell types, and morphological orientations. By using algorithms and criteria from published rodent literature, we report the first in vivo evidence for human neural action potential backpropagation from awake and anesthetized human patients. Interestingly, the appearance of human bAP did not depend on the spike amplitude, firing frequency, or spike count of the clustered SUA, but was correlated with the cell type. Putative cell types were predicted by their peak-to-trough ratio on the channel with the largest amplitude. Our preliminary results on the detection of multi-channel bAP patterns have shown consistency with the rodent findings, as we were able to identify canonical regular spiking (RS) putative principal cells expressing reliable bAPs (over 5 vertical channels, or over 100 μm), as well as fast-spiking (FS) putative interneurons which completely lack this spatial propagation. These results highlight the importance of high-density sampling in translational human in vivo recordings for disentangling cell types and morphological contributions to the local neocortical microcircuit.

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Poster

PSTR444. Electrophysiology-Cellular

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Program #/Poster #: PSTR444.02/XX13

Topic: I.04. Physiological Methods

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Conte - NIH/NIMH P50 MH119467
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Title: Extracting cellular spike activity from synchronous measurements of electrical and chemical neural signals

Authors: *U. AMJAD[1], J. CHOI[1], D. J. GIBSON[2], A. M. GRAYBIEL[2], H. N. SCHWERDT[1]; [1]Dept. of Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA; [2]McGovern Inst. for Brain Res. and Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: Dopamine plays an important role in synaptic plasticity and modulating neural activity (i.e., spike activity). Such synaptic plasticity is thought to underly many forms of learning. However, the role of these neuromodulators in regulating plasticity has yet to be fully explored in behaving animals. Measurements of synchronous dopamine and neural spike activity are needed to precisely examine how dopamine shapes neuroplasticity during learning. We previously demonstrated concurrent recording and analysis for local field potentials and dopamine in behaving non-human primates (Schwerdt et al., 2020); however, these methods did not naturally extend to extracting concurrent neural spike activity and dopamine. Artifacts introduced into the electrophysiological (EPHYS) recording by our neurochemical recording technique, fast scan cyclic voltammetry (FSCV), hinder concurrent analysis. FSCV applies a small periodic voltage (every 100 ms) to the implanted carbon fiber electrode to induce redox reactions of surrounding electroactive molecules, such as dopamine, which then produce a measurable current that is proportional to the dopamine concentration. However, the applied FSCV voltage couples directly to neighboring implanted EPHYS electrodes in the form of an artifact due to the brain's conductive properties. Artifact noise can be erroneously labeled as spikes using standard thresholding and clustering-based spike sorting methods. Consequent analytical measures, like spike firing rates, are then susceptible to error and misrepresentation of the actual neural environment. We developed an automated algorithm capable of interpolating away FSCV artifacts from raw EPHYS waveforms in the time-domain to reliably extract spike firing data. We compared spike sorting with and without interpolation by calculating a percent

spike recovery (%PSR) on spike timestamps. %PSR was 87.35% of the spikes (with a std:7.59%, n=6, 3 sessions, 2 channels/session). FSCV artifacts were simulated on isolated EPHYS signals (without concurrent FSCV) to make these calculations. The algorithm was tested on multi-modal FSCV-EPHYS recordings where we were able to successfully isolate and identify task-modulated neural units in the primate striatum in combination with dopamine signals, allowing us to directly compare neuronal spike and dopamine activity during online behavior. This work paves a way forward for extracting meaningful interactions between striatal dopamine and neural spike activity overtime.

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Poster

PSTR444. Electrophysiology-Cellular

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Program #/Poster #: PSTR444.03/XX14

Topic: I.04. Physiological Methods

Support: CIHR PJT-173423

Title: Spatiotemporal profile of extracellular action potentials in posterior parietal cortex area LIP of the macaque monkey

Authors: P. THIRUNAVUKKARASU[1], *M. PARE[2];
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Abstract: Extracellular spike waveforms recorded from neurons are known to reflect the intracellular process of action potential generation through the activation and inactivation of ion channels. The variability between the shape of spike waveforms is thought to be a direct result of the differential ion channel expression between these subtypes. In cerebral cortex, spike waveforms recorded from pyramidal neurons are known to be ‘broader’ than the ‘narrow’ spike waveforms exhibited by fast-spiking interneurons. To capture this temporal variability in primarily biphasic waveforms, where a negative deflection occurs before a positive deflection, researchers have used the spike width—the time between the trough and peak of the waveform. However, this uni-dimensional approach often fails to discriminate putative interneurons from pyramidal neurons and to account for further nuanced classes of neurons. Here we report on our efforts to refine classification approaches by examining the spatiotemporal profile of action potentials recorded from neurons within the lateral intraparietal (LIP) area in one rhesus monkey. We refined the standard approach to calculating the template mean waveform. We aligned each individual waveform, not on the time of the trough, but on the maximum of the first derivative between the trough and peak, which corresponds to the instance of maximum change in voltage of the waveform. Using unsupervised classification with both timing and relative amplitude

measures, we derived a three-class provisional model comprised of one narrow-spike class robustly separated from two broad-spike classes. Consolidating this classification, we identified a phenomenon observed exclusively in all broad-spike neurons, a knee-like change in the voltage close to the waveform peak. In addition, narrow-spike neurons were characterized by high firing rates, more irregular firing, and generally lacking bursting. While displaying converse firing statistics from narrow-spike neurons, there was no identifiable difference in the discharge properties between the two identified broad-spike classes. Nevertheless, several waveform features involving the knee-like change were highly discriminating between these two classes. Moreover, the class with the broader spikes exhibited more highly unusual, W-shaped troughs (observed in 15% of the sample), which has been associated with multiple Na+ influxes thanks to larger dendritic tree arborization. This novel analysis provides a fresh perspective and new tools to help identify distinct spatiotemporal and functional signatures of three different types of cortical neurons.

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Poster

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Topic: I.04. Physiological Methods

Support: NEI Grant R00EY029326
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Title: Longitudinal electrophysiological assessment of visual ability in mice

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Abstract: Over the past few decades, mice have emerged as a premier model for studying visual cortical function. To evaluate visual ability, experimental designs often employ behavioral assessments which either require extensive training and may drive unintended cortical plasticity, or rely on subcortical reflexes rather than cortical processing. To circumvent these limitations, one method for evaluating cortex-dependent visual ability is to measure the response in the primary visual cortex (V1) to precisely-timed visual stimuli. Here we present a low-cost, extensible pipeline for longitudinal monitoring of V1 responses. Our system leverages Bonsai Rx, an open-source programming language, to develop a suite of tools for the presentation of visual stimuli and synchronization with electrophysiological data recorded via the Open Ephys acquisition system. Using these tools, we mapped receptive fields and quantified spatial acuity, contrast sensitivity, and orientation selectivity in V1 of mice across a range of developmental ages (P23-P120). We demonstrate that this platform can reliably track visual cortical responses

using the local field potential recorded via chronically-implanted electrodes over months. Finally, we highlight the extensibility of this platform to include other forms of data acquisition (e.g. video, motor tracking, pupil monitoring, calcium imaging, high channel count electrophysiology) and real-time stimulation (e.g. optical, electrical, auditory). This work may be of interest to researchers seeking to build simple and affordable systems for measuring visual ability in mice over time, with the potential to scale to more complex experimental paradigms.

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Poster

PSTR444. Electrophysiology-Cellular

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR444.05/XX16

Topic: I.04. Physiological Methods

Support: Grant-in-Aid for JSPS Fellows (22J22779)
KAKENHI (20H03545)
JST CREST (JPMJCR21P1)
JST moonshot (JPMJMS2292)
Institute for AI and Beyond of the University of Tokyo

Title: A method to analyze gene expression profiles of a hippocampal neuron recorded from head-fixed mice

Authors: *H. YAGISHITA[1], K. OKAMOTO[2,3], Y. GO[4,5], Y. IKEGAYA[6,7,8], T. SASAKI[1]; [1]Grad. Sch. of Pharmaceut. Sci., Tohoku Univ., Sendai-city, Japan; [2]Dept. of Neuroanatomy, Grad. Sch. of Med., [3]Dept. of Cell Biol. and Neuroscience, Grad. Sch. of Med., Juntendo Univ., Tokyo, Japan; [4]Grad. Sch. of Information Sci., Univ. of Hyogo, Hyogo, Japan; [5]Natl. Inst. of Natural Sci., Natl. Inst. for Physiological Sci., Aichi, Japan; [6]Lab. of Chem. Pharmacology, Grad. Sch. of Pharmaceut. Sci., [7]Inst. of AI and Beyond, The Univ. of Tokyo, Tokyo, Japan; [8]Ctr. for Information and Neural Networks, Inst. Natl. of Information and Communications Technol., Osaka, Japan

Abstract: Neurons in the central nervous system exhibit diverse electrophysiological, morphological, and molecular characteristics. These diverse neurons form neural circuits that underlie a variety of brain functions and behaviors. The physiological properties of neurons are influenced by their molecular properties. However, methods for comprehensively analyzing these properties at multiple levels from a single neuron have not been established. To address this issue, we developed an experimental technique to analyze gene expression profiles of a hippocampal neuron recorded from *in vivo* conditions by integrating existing techniques including *in vivo* juxtacellular recording, cell labeling, brain slicing, patch clamping, and single-cell RNA sequencing. In detail, a juxtacellular recording of spike patterns from a neuron is performed from the hippocampus in a living mouse. Subsequently, a soluble fluorophore, Alexa

488/594 hydrazide, is electroporated into the recorded neuron for cell labeling. Acute hippocampal slices with a thickness of 200 μm are prepared from the mouse. In the slice, the labeled neuron is visualized and collected using a glass pipette under a microscope. Finally, a single-cell RNA sequencing is performed from the collected cell to identify its gene expression profiles. The accuracy of our methods was confirmed by comparing spike patterns and cellular localization with expression levels of marker genes in individual neurons. So far, we performed electrophysiological recordings and gene expression analysis from forty hippocampal CA1 neurons recorded under urethane anesthesia. By focusing on spike patterns related to hippocampus sharp wave ripples, a transient local field potential signal representing synchronous spikes of a large number of neurons, we are now analyzing a relationship between the degree of participation of individual neurons into sharp wave ripples and their gene expression profiles. Our method is useful to elucidate molecular mechanisms linked to physiological signatures of neurons.

Disclosures: H. Yagishita: None. **K. Okamoto:** None. **Y. Go:** None. **Y. Ikegaya:** None. **T. Sasaki:** None.

Poster

PSTR444. Electrophysiology-Cellular

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR444.06/XX17

Topic: I.04. Physiological Methods

Support: Research Grant from Sophion Bioscience

Title: Automated high throughput patch clamp studies of Piezo1 channel

Authors: S. PAZ[1], ***D. NAGY**[2], S. KARATSIOMPANI[3], J. KEFAUVER[4], R. PAK[5], D. SAUTER[2];
[1]Univ. of East Anglia, Norwich, United Kingdom; [2]Sophion Biosci., Bedford, MA; [3]Sophion Biosci., Copenhagen, Denmark; [4]Univ. of Geneva, Geneva, Switzerland; [5]Scripps Res., La Jolla, CA

Abstract: Piezo ion channels convert mechanical stimuli into various biological activities through a process called mechanotransduction. Piezo1 and Piezo2 were identified in 2010 as molecular mediators of the mechanically activated current found across multiple cell types. While the main function of Piezo2 is the mediation of gentle touch sensation, Piezo1 has functions in numerous physiological processes, including sensing shear stress of blood flow for proper blood vessel development, regulating urinary osmotic pressure, controlling blood pressure and exercise performance. Previous studies have shown that over 25 mutations in Piezo1 cause human disease. However, most mutations have yet to be extensively studied. Piezo1 is activated through cell membrane deformations caused by mechanical forces, such as osmotic pressure, fluid shear stress, substrate stiffness, and confinement. Known stimuli activating Piezo1 *in vitro*

are shear stress, as well as the indentation of a cell with a glass probe and membrane stretching. In this study, we aimed to develop a feature to perform Piezo1 experiments on a high throughput automated patch clamp platform. The stimulus applied here was shear stress produced by liquid flow injection containing Yoda1, the Piezo1 agonist, at 26 and 30 °C. Fluid flow shear stress is achieved by applying a defined solution flow through the microfluidic recording chamber, creating a uniform shear force for the study's cells. In addition, Yoda 1 slows the inactivation phase of transient currents, enabling better detection of Piezo1-mediated mechanically induced responses. HEK293T cells stably expressing mPiezo1-GFP fusion protein and HEK cell line lacking Piezo1were used to develop the assay and ensure the specificity of Piezo1 responses. We demonstrated the feasibility of mechanically stimulating Piezo1 using the high-speed liquid handling feature (or standard speed) in the Qube384 platform using either Yoda1 at 5 µM or 10 µM. Success rate of over 80% was achieved using Yoda1 10 µM and shear stress of 5 dyne/cm^2 regardless of the temperature (26/30°C) with an amplitude current around -2 nA. Importantly, no current response was observed in the HEK cell line lacking Piezo1. In addition, we evaluated whether responses could be observed by repeated stimulation. Indeed, a 20-minute wait time between stimulations allowed us to obtain current responses of similar amplitude. Our results demonstrate the Qube384 is capable of characterizing Piezo1 in vitro. These assays open the door to a new set of features that allow the identification of antagonists at Piezo1 in the presence of Yoda1 using automated high throughput electrophysiology platforms.

Disclosures: **S. Paz:** 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.; Sophion Bioscience. **D. Nagy:** A. Employment/Salary (full or part-time):; Sophion Bioscience. **S. Karatsiompani:** A. Employment/Salary (full or part-time):; Sophion Bioscience. **J. Kefauver:** None. **R. Pak:** None. **D. Sauter:** A. Employment/Salary (full or part-time):; Sophion Bioscience.

Poster

PSTR444. Electrophysiology-Cellular

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR444.07/XX18

Topic: I.04. Physiological Methods

Support: R21NS119671 (R.M., X.Z.)
R21NS124936 (R.M.)
ABCD Charitable Trust (R.M.)
Karen Toffler Charitable Trust (X.Z.)

Title: Operationalization of long-duration patch clamp recording of striatal medium spiny neurons in juvenile and old mice

Authors: *X. ZHAN[1,2], S. M. JOHNSON[1], R. MARGOLIS[2];
[1]Physiol. and Biophysics, Howard Univ., Washington, DC; [2]Psychiatry and Behavioral Sci., Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Long-duration recording of ion currents in brain slices has been challenging. Several efforts to extend the duration of recordings have been made in the past few decades, including the introduction of NMDG (*N*-Methyl-D-glucamine), HEPES, sucrose, and choline-based cutting solutions. Despite these improvements, long-duration recording has proven elusive, especially for brain slices from older animals. In this study, we attempted to develop a protocol to increase the duration of slice recording by focusing on depolarization dependent after hyperpolarization (AHP), and low-voltage threshold activated Ca currents in striatal medium spiny neurons. We induced I_{AHP}, and low-voltage threshold activated Ca dependent currents, using a hyperpolarization prepulse of -105 mV. In comparison to previous protocols, 1) lactate was used in the cutting solution (in mM: NMDG, 102; HEPES, 20; $NaHCO_3$, 26; NaH_2PO_4 1.25; KCl, 2.5; $MgSO_4.7H_2O$, 10; $CaCl_2$, 0.5; Na-L-ascorbate; 5; Na pyruvate, 3; Thiourea, 2; glucose, 20, at 4^oC, pH 7.4) and recording perfusate (in mM: NaCl, 124; KCl, 2.1; $MgSO_4.7H2O$, 1; $CaCl_2$, 2; KH_2PO_4, 1.2; $NaHCO_3$, 20; glucose, 10; Na-HEPES, 4; Sodium lactate, 1; Sodium pyruvate, 2; Sodium ascorbate, 0.4; pH 7.4, at room temperature); 2) R-CPP, a NMDA blocker, was used only in the recovery solution: 3) the intracellular Ca level was 5 mM. The currents were significantly greater than the currents induced without a prepulse or in a lower intracellular Ca level (0.25 mM). In young mice, the recorded currents were stable for up to nine hours after the slice was prepared. Stable recordings of three hours were achieved in mice as old as twelve months in both C57BL/6 and heterozygous JPH3 knockout mice (JPH3 KO). No significant run-down was observed even in JPH3 KO mice with partially compromised function of junctophilins, a critical protein for homeostasis modulation. These modifications, by preserving the physiological health of brain slices for as long as nine hours after slice preparation, will facilitate investigation of neural plasticity, pharmacological studies, and aging.

Disclosures: X. Zhan: None. **S.M. Johnson:** None. **R. Margolis:** None.

Poster

PSTR444. Electrophysiology-Cellular

Location: WCC Halls A-C

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Program #/Poster #: PSTR444.08/XX19

Topic: I.04. Physiological Methods

Support: NIH R01DA029639
NIH R01NS102727
NIH RF1AG079269

Title: Patch clamping video game: An interactive simulation to teach and train electrophysiology students

Authors: *N. J. MALTA[1], C. VAN ZYL[1], A. DUNNUM[1], W. D. HUNT[1], M. ROWAN[2], M. STIMBERG[3], R. BRETTE[3], C. R. FOREST[1];
[1]Georgia Inst. of Technol., Atlanta, GA; [2]Emory Univ., Atlanta, GA; [3]Inst. de la Vision, Paris, France

Abstract: Patch clamping is a well-established technique in electrophysiology for investigating the electrical properties of neurons and other cells. Unfortunately, the equipment required for patch clamping is often large, expensive, and limited in availability, making it challenging to teach and train students on the technique. Further, the hardware and software components involved in this technique have a steep learning curve for new researchers. There is a need to expose and engage many hundreds of learners each year to this gold-standard technique.
To address this barrier, we introduce a user-friendly software simulation, a video game, that emulates the process of working with a patching rig, providing an accessible learning tool for students interested in exploring this technique.
The software offers a virtual environment that replicates key aspects of patch clamping procedures. Users can establish gigaseals, break into cells, and take simulated electrical recordings. Further, the software replicates various failure modes encountered during real experiments, like pipette tip clogging, breakage, and variations in resistance between different tips.
Through the utilization of this software, in experiments with dozens of users in lab and course environments we record telemetry data to observe common challenges faced by novice patchers. By tracking the performance improvements of users over time, we demonstrate the effectiveness of our simulation-based approach in facilitating skill development and improving understanding of patch clamping techniques.
By providing an accessible and free solution, our simulation software aims to empower students to explore and gain familiarity with patch clamping. It serves as a valuable educational tool that bridges the gap between theoretical knowledge and practical experience, enabling students to enhance their understanding of the technique and its applications.

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Poster

PSTR444. Electrophysiology-Cellular

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR444.09/XX20

Topic: I.04. Physiological Methods

Support: NIH R01DA029639
NIH R01NS102727
NIH RF1AG079269

Title: Low noise, high-throughput patch clamping with microfabricated quartz electrodes

Authors: *C. P. VAN ZYL[1], M. C. YIP[1], R. E. PERSZYK[2], B. YANG[1], M. M. GONZALEZ[1], S. F. TRAYNELIS[2], C. R. FOREST[1];
[1]Georgia Inst. of Technol., Atlanta, GA; [2]Dept Pharmacol, Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Single-ion channel recordings are critical to understanding fundamental activities and behaviors of individual cells, such as electrophysiological response to drug exposure or functional information about neuronal synaptic transmission. Patch-clamping is the gold-standard, Nobel-prize-winning technique and highest precision method to facilitate single-cell or ion channel electrical recordings through contact between a glass micropipette electrode and cell membrane. However, it remains a low-throughput technique, as the process of sealing onto the cell membrane (and even touching any organic membranes) leaves organic debris within the electrode, preventing its reuse. In addition, high-resolution, single channel recordings require meticulous attention when patching to eliminate sources of noise and tedious repetition to obtain a single ion channel in a patch recording. Patch electrodes pulled from quartz glass have reduced electrical noise and isolate electrical signals more effectively than their more widely-used borosilicate glass counterparts. Electrode cleaning for reuse and electrode polishing are novel techniques applied to borosilicate electrodes to increase throughput and reduce noise. However, the application of these techniques to lower-noise quartz electrodes has yet to be attempted and would enable higher resolution and throughput of patch clamp experiments. Thus, we have demonstrated more efficient and accurate recordings of cell activity by applying electrode microfabrication and detergent-based cleaning techniques to quartz electrodes. First, we demonstrate the efficacy of cleaning quartz electrodes by successfully collecting greater than ten consecutive patch clamp recordings per single electrode. Then, we show increased gigaseal resistance and reduced noise recordings near the system limitations (i.e. baseline noise of patch clamp electronics) through the use of quartz electrodes with flat, sub-nanometer annular tip openings microfabricated using a focused ion beam. This combination of quartz electrodes, detergent-based cleaning, and electrode microfabrication can be readily applied to high-precision single-ion channel recordings and will advance the field of patch clamp electrophysiology by simultaneously increasing the quality and throughput of experiments at a lower cost per attempt.

Disclosures: C.P. van Zyl: None. **M.C. Yip:** None. **R.E. Perszyk:** None. **B. Yang:** None. **M.M. Gonzalez:** None. **S.F. Traynelis:** None. **C.R. Forest:** None.

Poster

PSTR444. Electrophysiology-Cellular

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Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR444.10/XX21

Topic: I.04. Physiological Methods

Support: NIH R01DA029639
NIH R01NS102727
NIH RF1AG079269

Title: Fluorescent-guided automated patch clamp electrophysiology to systematically study Parvalbumin interneurons in Alzheimer's Disease

Authors: *M. M. GONZALEZ[1], B. MAGONDU[2], M. J. ROWAN[3], C. R. FOREST[1]; [1]Woodruff Sch. of Mechanical Engin., [2]Coulter Dept. of Biomed. Engin., Georgia Inst. of Technol., Atlanta, GA; [3]Dept. of Cell Biol., Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive cognitive decline, affecting millions worldwide. Understanding the underlying mechanisms of AD is crucial for the development of effective therapeutic interventions. Tau and amyloid beta are key hallmarks of AD, however the underlying cause of cognitive decline and memory impairment remains elusive. As a result, alternative hypotheses regarding the cause of cognitive decline in AD are gaining attention. Namely, cognitive decline in early Alzheimer's disease (AD) may be attributed to disrupted neuronal circuits rather than the accumulation of amyloid plaques or tau tangles. Initial findings indicate that circuit hyperexcitability precedes plaque formation in AD mouse models. The dysregulation of GABAergic interneurons, specifically parvalbumin-expressing interneurons, is believed to contribute to circuit dysfunction. The spatial and temporal progression of interneuron dysregulation in AD is still largely unstudied. To effectively investigate the intrinsic properties of PV interneurons with precise spatial and temporal resolution, patch clamp electrophysiology is the recommended approach. However, manual patch clamp experiments are known for their low throughput and labor-intensive nature. To overcome these limitations, we have developed an automated patch clamp rig, known as the patcherBot, which enables automated fluorescent-guided patch clamp recordings. To demonstrate the utility of this technology, we focused on investigating the role of PV interneurons in a human amyloid precursor protein model of AD (hAbeta^{SAA}, Jax Strain #034711) and corresponding control strain (B6J hAbeta, Jax Strain #033013). To measure the intrinsic properties of PV interneurons, we utilized an adeno-associated virus (Addgene Plasmid #135631) to tag these neurons with green fluorescent protein (GFP). Utilizing the advanced capabilities of the enhanced patcherBot, we conducted 50 automated patch clamp recordings over several days on GFP-tagged interneurons derived from the lateral entorhinal cortex and somatosensory cortex of 2-month-old subjects. In this study, we present the intrinsic properties of these PV interneurons and highlight the invaluable contribution of automation in systematically investigating Alzheimer's Disease. The automation employed in this study will greatly accelerate our comprehension of the specific role played by PV interneurons in AD, offering the potential to unveil innovative therapeutic targets and strategies for alleviating cognitive decline.

Disclosures: **M.M. Gonzalez:** None. **B. Magondu:** None. **M.J. Rowan:** None. **C.R. Forest:** None.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

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Program #/Poster #: PSTR445.01/XX22

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

Support: NSF Grant 1942963
NSF Grant 2014862
NSF Grant 2020312
NSF Grant 2031985
NIH Grant 091018

Title: Why do networks need Excitation-Inhibition balance? A representation capacity perspective

Authors: *Q. WANG[1], M. A. POWELL[4], E. W. BRIDGEFORD[2], J. VOGELSTEIN[3]; [1]Neurosci., [2]Biostatistics, [3]Biomed. Engin., Johns Hopkins Univ., Baltimore, MD; [4]Mathematical Sci., United States Military Acad., West Point, NY

Abstract: Why do brains have inhibitory connections? Why do brains need Excitation-Inhibition (E-I) balance to avoid disorders like Schizophrenia and Autism Spectrum Disorders? Do feedforward deep neural networks also need E-I balance? We propose an answer from the perspective of representation capacity. We believe representing functions is the primary role of both (i) the brain in natural intelligence and (ii) deep networks in artificial intelligence. Our answer to why there are inhibitory/negative weights is: to represent more functions. We mathematically prove that, in the absence of negative weights, neural networks with non-decreasing activation functions are *not* universal approximators. We also provide insights on the geometric properties of the representation space that non-negative networks cannot represent. Our answer to how networks benefit from E-I balance is: to represent functions more efficiently. We mathematically prove that, when a fixed ratio of inhibition is allowed, neural networks of infinite size *are* actually universal approximators; However, the farther this ratio deviates from balance, the more resources neural networks waste on silent neurons and monotone neurons: when excitation ratio is low, networks tend to form silent neurons that are always quiescent regardless of how strong the input may be; when excitation ratio is high, networks tend to form monotone sub-networks that can only represent simple functions. Given an E-I ratio of the entire network, we mathematically deduce individual neuron's synaptic input E-I ratio distribution and quantify the probability of silent and monotone unit formation. In short, when networks are not E-I balanced, they cannot make efficient usage of the available neurons to represent complex functions, as many of the neurons are quiescent (low-E) or can only represent simple functions (high-E). Apart from theoretical derivations, we also experimentally demonstrate that 1) even feedforward deep neural networks need balanced E-I ratio to achieve better performance, and 2) the majority of state-of-the-art pre-trained deep neural networks have balanced E-I ratio. We expect these insights will yield a deeper understanding of inhibition and E-I balance that leads to more efficient machine learning and better solutions for disorders associated with E-I imbalance.

Disclosures: Q. Wang: None. **M.A. Powell:** None. **E.W. Bridgeford:** None. **J. Vogelstein:** None.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR445.02/XX23

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

Support: Gatsby Charitable Foundation
Simons Foundation (SCGB 543039)

Title: Spatial planning in the presence of positional uncertainty

Authors: *M. SALMASI, M. SAHANI;
Gatsby Computat. Neurosci. Unit, Univ. Col. London, London, United Kingdom

Abstract: Spatial navigation is conducted through combining path integration signals with incoming sensory modalities. The noise and intrinsic ambiguity of the sensory signals together with the noisy path integration system lead to positional uncertainty. To behave optimally in a navigation task, the animals/agents should take into account the uncertainty of the input signals, and infer a distributional belief about their location. Different frameworks have been suggested for the representation of distributional beliefs in the brain, such as sampling, probabilistic population codes, and distributed distributional codes (DDC). However, it remains unclear how the brain represents positional beliefs and how the hippocampus employs these representations to perform spatial planning. DDC suggests that a probability distribution is represented by the expected values of some encoding functions. We assume that given the history of sensory observations and efference copies of the motor commands, the posterior distribution over the location is represented by the DDC values. We have previously shown that the animal can update the DDC values recursively and conduct probabilistic localization and structural learning. Here we show how spatial planning can be performed using the codes of uncertainty. After learning the transition structure of the environment, the value iteration algorithm can be used to derive the optimal value function for each location in the environment. In the absence of positional uncertainty, the animal could follow the action that maximizes the value at each location and reach the desired goal. However, the true location is a latent variable, and the animal has only access to the DDC values of the posterior distribution over the location. We show that for each action, a weighted sum of the DDC values provides the expected value of that action, and by choosing the action corresponding to the highest expected value function, the animal can find an effective policy for planning. The simulations confirm that by following this DDC-based policy, the animal can take the positional uncertainty into account and reach the goal efficiently. Our results provide a general framework for planning under uncertainty and can be employed beyond spatial planning to any action selection task in which there is uncertainty about the task's (latent) variables.

Disclosures: **M. Salmasi:** None. **M. Sahani:** None.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

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Program #/Poster #: PSTR445.03/XX24

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

Support: NIH Grant R01NS085200
NIH Grant R01NS101353
NIH Grant RF1MH114224

Title: Deriving causal relationship in resting state functional connectivity using SSFO-based optogenetic fMRI

Authors: *X. HAN[1], S. R. CRAMER[2], N. ZHANG[1];
[2]Biomed. Engin., [1]The Pennsylvania State Univ., State College, PA

Abstract: The brain network has been extensively studied as a collection of brain regions that are functionally interconnected. However, the study of the causal relationship in brain-wide functional connectivity, which is central to brain function, remains challenging. Here we combined stabilized step-function opsin (SSFO)-based optogenetics with fMRI in a resting-state rodent model to study how a local increase of excitability affects brain-wide neural activity and resting-state functional connectivity (RSFC). We further examined the feasibility of using this method to infer the causal relationship in the brain network based on the modulated RSFC. When the dentate gyrus (DG) was sensitized by SSFO activation, we found significantly changed brain activity and connectivity in several brain regions associated with the DG, particularly in the mPFC. To derive the directional information in these connections, we incorporated Pearson's correlation and partial correlation analyses in a graphic model. Our causal inference result shows an 85-100% accuracy rate compared to the directional information based on anatomical tracing data. This study established a system to investigate the relationship between local region activity and RSFC modulation and provide a way to analyze the underlying causal relationship between brain regions.

Disclosures: X. Han: None. **S.R. Cramer:** None. **N. Zhang:** None.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

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Program #/Poster #: PSTR445.04/XX25

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

Support: Private Donations to the Western Institute for Advanced Study

Title: A mean-field approach to modeling energetic efficiency and non-deterministic signaling outcomes in cortical neural networks

Authors: *E. A. STOLL;
Western Inst. for Advanced Study, Denver, CO

Abstract: Mean field theory has been usefully employed to model probabilistic coding in cortical neural networks [Gabrie, 2020]. This methodology allows an exploration of the solution space, leading to the selection of an optimal system state from a probability distribution [Mei et al., 2019]. At the mean field limit, the network achieves a fixed state, where excitatory and inhibitory contributions are balanced, so that fluctuations dominate network level dynamics [Brunel, 2000]. As a result, applications of mean field theory have promoted a better understanding of how spontaneous membrane potential fluctuations influence signaling outcomes [Geisler et al., 2005] and how stochastic events shape network-level dynamics [Bandyopadhyay et al., 2022]. Yet a mechanistic connection between noisy coding and energy efficiency in cortical neurons has remained elusive.
This report presents a thermodynamic basis for mean-field theory, with the Hamiltonian being modeled not only as a computational quantity but also as an energetic quantity. In this approach, cortical neurons integrate upstream signals with random electrical noise to generate a physical quantity of information. This complex-valued probability distribution is then reduced as the trial Hamiltonian is resolved. As information is compressed, free energy is released, driving a shift in the neuronal membrane potential. Here, the optimal neuronal ensemble to encode the state of the surrounding environment is also the most energy-efficient solution, since the extraction of predictive value from a thermodynamic quantity of information both maximizes energetic efficiency and drives a system-wide non-deterministic computation.

Disclosures: E.A. Stoll: None.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR445.05/XX26

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

Support: PD Soros Fellowship

Title: Dendrites increase computational efficiency in physically constrained neural networks

Authors: *E. H. S. TOLOZA[1,3], M. T. HARNETT[2];
[1]MIT, Brookline, MA; [2]McGovern Inst. for Brain Res., MIT, Cambridge, MA; [3]Harvard Med. Sch., Boston, MA

Abstract: Unlike traditional artificial neural networks, biological networks must overcome energetic and spatial costs associated with sampling inputs in physical space. Specifically, the number of possible synaptic connections per unit volume should strongly influence how different types of networks are functionally organized. We constructed recurrent neural networks consisting of either rate-based or biophysically-realistic spiking units trained via gradient descent

and subjected them to synapse sampling limits. We discovered that the addition of dendrites enhances performance across a range of tasks that represent different classes of computation, but only under realistic spatial constraints. Using intuitive models of network energy and physical volume, we identified specific parameter spaces where dendrites represent an energetically and/or spatially efficient means to increase the computational power of a network. Our results provide a new general principle for explaining why some biological networks favor fewer principal neurons with extensive dendritic trees (e.g. cortex) while others favor an increased number of neurons with little to no dendritic arborization (e.g. cerebellar granule cells).

Disclosures: E.H.S. Toloza: None. **M.T. Harnett:** None.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR445.06/XX27

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

Title: Graph Theory Based Parcellation of Brain Networks

Authors: *K. XHINDI[1], J. C. NINO[2], M. FEBO[3];
[1]Biomed. Engin., [2]Material Sci. and Engin., [3]Psychiatry Dept., Univ. of Florida, Gainesville, FL

Abstract: The identification and characterization of modules in functional brain networks can be approached through two methodologies: community-finding algorithms and parcellation from functional Magnetic Resonance Image (fMRI) analysis. Both methodologies seek to uncover the organization of the brain, yet they differ in their utilization of methods and models. Community-finding algorithms are founded on graph theory principles and aim to identify communities of highly interconnected brain regions within a graph. Parcellation from fMRI analysis, on the other hand, employs statistical methods to identify brain regions exhibiting similar patterns of activation in response to a specific task. 128 human derived functional connectivity graphs were initially parcellated from the original methodology of the data by Schaefer et al, 2017.The current study evaluates the quality of the partitions derived from Louvain's Algorithm, k-means, and Principal Component Analysis by using mathematical measures such as modularity, Normalized Mutual Information (NMI), Surprise and Conductance Values. Furthermore, In Alzheimer's Disease (AD) and Cognitive Normal (CN) Groups, nodes are assigned different roles based on their within-module degree (Z) and participation coefficient (P), which measure connectivity within modules and link distribution among different modules.Louvain and PCA algorithms show higher NMI and surprise values compared to the Schaefer algorithm, indicating stronger agreement and statistical significance in their community structures, while Spectral and k-means algorithms exhibit the highest maximum values of conductance, reflecting more cohesive and well-defined communities. Based on the P and Z score combination the CN Group has 29 nodes with different functions as compared to the AD counterparts, with more than 50% of them being assigned to connecter nodes in the CN Group.The graph theory-based parcellation

approach offers several advantages for analyzing brain connectivity compared to other algorithms as shown by improved metric score. By applying graph partitioning algorithms, such as Louvain, k-means, and PCA it may enable the identification of distinct regions or communities within the brain network as well as difference in organization between AD and CN Groups.

Disclosures: **K. Xhindi:** None. **J.C. Nino:** None. **M. Febo:** None.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

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Program #/Poster #: PSTR445.07/XX28

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

Support: NSFC-32071008
ECS-26303921
STI2030-Major Projects 2022ZD0211900

Title: The scale-invariant covariance spectrum of brain-wide activity

Authors: *Z. WANG[1], W. MAI[2], Y. CHAI[1], K. QI[1], H. REN[1], C. SHEN[1], S. ZHANG[1], G. TAN[1], Y. HU[2], Q. WEN[1];
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Abstract: The structure of high-dimensional neural activity plays a pivotal role in various sensory and behavioral processes. Here, we analyze whole-brain calcium activity in larval zebrafish (huc:h2b -GCaMP6f), captured by fast light-field volumetric imaging during hunting and spontaneous behavior. We find that brain-wide activity is distributed across many principal component dimensions described by the covariance spectrum. Intriguingly, this spectrum shows an invariance to spatial subsampling: That is, the distribution of the eigenvalues of a smaller and randomly sampled cell assembly is statistically similar to that of the entire brain. We propose that this property can be understood in the spirit of multidimensional scaling (MDS), whereby pairwise correlation between neurons can be mapped onto a distance function between two points in a low-dimensional functional space. We numerically and analytically calculate the eigenspectrum in our model and identify three key factors that lead to the experimentally observed scale invariance: (i) the slow decay of the distance-correlation function, (ii) the higher dimension of the functional space, and (iii) the heterogeneity of neural activity. Our theory can quantitatively recapitulate the scale-invariant spectrum in zebrafish data, as well as two-photon and multi-area electrode recordings in mice. Furthermore, fitting the model to the experimental data uncovers a reorganization of neurons in the functional space when the zebrafish is engaged in hunting behavior. Our results therefore provide new insights and interpretations of brain-wide

neural activity and offer clues about circuit mechanisms for coordinating global neural activity patterns.

Disclosures: **Z. Wang:** None. **W. Mai:** None. **Y. Chai:** None. **K. Qi:** None. **H. Ren:** None. **C. Shen:** None. **S. Zhang:** None. **G. Tan:** None. **Y. Hu:** None. **Q. Wen:** None.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR445.08/XX29

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

Support: NRF-2022R1A2C3008991
NRF-2021M3E5D2A01019544
NRF-2019M3E5D2A01058328 (to S.P.)

Title: Balancing stable and unstable synapses for continual learning in deep neural networks

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Abstract: Modern deep neural networks (DNNs) can perform various tasks at the human level (Kiela, 2021). However, most DNNs encounter a critical issue when learning multiple tasks, known as catastrophic forgetting; at a certain point, the network drastically loses the ability to process old information while learning new tasks (McCloskey, 1989), which is not observed in human brains. On the other hand, when multiple items are memorized in the brain, a characteristic phenomenon known as the serial position effect is observed in which items learned first (primacy effect) and last (recency effect) are memorized better than those trained in between (Atkinson, 1968). Previous studies proposed that the serial position effect can arise in a network composed of two distinct types of synapses, stable and unstable types, where stable synapses consolidate their weights only when they change significantly, while unstable synapses change their weights freely (Lee, 2020). Importantly, it was reported that control of the ratio of stable and unstable synapses enabled the network to memorize both previously learned and newly learned information. Here, by advancing this notion, we show that the implementation of both stable and unstable synapses in DNNs can induce the serial position effect, with minimizing catastrophic forgetting to enable continual learning. Specifically, we trained AlexNet to classify images of different objects while the composition of stable and unstable synapses in the network was varied. As a result, we found that the model with only unstable synapses showed recency effect while the model with only stable synapses showed primacy effect. The model with both types of synapses showed evidence of the serial position effect in which both recency and primacy effects appeared. Moreover, we found that the model with both types of synapses could learn significantly more items than conventional DNNs, which show only recency effect. As the

number of sequentially learned items was increased, the model with only stable synapses failed to learn new items at a certain threshold, suggesting that its memory resources were overloaded. Similarly, the model with only unstable synapses failed to retain old items at a certain point, revealing catastrophic forgetting. In contrast, the model with both stable and unstable synapses maintained the serial position effect by learning new items while retaining old ones simultaneously, adaptively redistributing its memory resources. Overall, our results demonstrate that the coexistence of stable and unstable synapses in DNNs enables human-like flexible memory resource allocation, suggesting a possible solution for catastrophic forgetting.

Disclosures: **S. Cho:** None. **S. Baek:** None. **S. Paik:** None.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR445.09/XX30

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

Support: Marie Skłodowska-Curie grant agreement Nº 945371

Title: Topological structure of population activity in mouse visual cortex encodes densely sampled visual scene rotations

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Abstract: The primary visual cortex is one of the most well understood regions supporting the processing involved in sensory computation. Historically, our understanding of this part of the brain has been driven by describing the features to which individual neurons respond. An alternative approach, made possible by the availability of high-density neural recordings, has suggested that neural activity is often constrained to low dimensional representations, known as neural manifolds. However, responses in visual cortex seem to have very complex and nonlinear structure and the neural manifold they generate remains elusive.
In this work, we apply a precise quantification of the structure of such neural manifolds and address some of the problems the community has to face when conducting topological data analysis on neural data. We do this by analyzing publicly available two-photon optical recordings of primary mouse visual cortex in response to visual stimuli with a densely sampled rotation angle. Since the set of rotations of two-dimensional objects lives on a circle, one would hypothesize that they induce a circle-like manifold in neural activity. We discovered a circle-like neural manifold in the population activity of primary visual cortex, supporting the manifold hypothesis. To achieve this, we applied a shortest-path (geodesic) approximation algorithm for computing the persistent homology groups of neural activity in response to visual stimuli. It is

important to note that the manifold is highly curved and standard Euclidean approaches failed to recover the correct topology.
Furthermore, we identified subpopulations of neurons which generate both circular and non-circular representations of the rotated stimuli, with the circular representations being better for angle decoding. We found that some of these subpopulations, made up of orientationally selective neurons, wrap the original set of rotations on itself which implies that the visual cortex also represents rotations up to 180 degrees.
Given these results we propose that population activity can represent the angle of rotation of a visual scene, in analogy with how individual direction-selective neurons represent the angle of direction in local patches of the visual field. Finally, we discuss some of the obstacles to reliably retrieving the truthful topology generated by a neural population.

Disclosures: C. Bechkov: None. **M. Fyhn:** None. **T. Hafting-Fyhn:** None. **G.T. Einevoll:** None.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR445.10/XX31

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

Support: SFB 1089, German government funding

Title: Simulating cortical dynamics in anatomically detailed network models

Authors: *U.-U. NARANTSATSRALT[1], P. EKELMANS[1], A. BAST[2], M. R. CANO[2], T. TCHUMATCHENKO[1], M. OBERLAENDER[2];
[1]Inst. of Exptl. Epileptology and Cognition Res., Univ. Clin. of Bonn, Bonn, Germany; [2]Max Planck Inst. for Neurobio. of Behavior, Max Planck Inst. for Neurobio. of Behavior, Bonn, Germany

Abstract: The rodent barrel cortex processes information from whisker stimulus. The cortical structure and cell-type specificity strongly impact the network dynamics of the barrel cortex. However, the mechanisms of how structural details and cell-type specificity impact the network dynamics are not fully understood. We asked whether a connectomically constrained rate model can interpret the role of the cell-type specificity of the barrel cortex dynamics. To answer this question, we simulated the stabilized supralinear network (SSN) rate model and the spiking network activity of approximately 4000 leaky-integrate-and-fire (LIF) neurons. The parameters of SSN and LIF neurons were based on biologically plausible data for each specific cell type. We found that the SSN rate model and LIF network activity were consistent with each other, which showed that our approximation of population rate is biologically constrained. The connectivity from the excitatory neural population changed the amplitude of the dynamics, while connectivity from the inhibitory neural population changed the dynamic's amplitude and width. We conclude

that a connectomically constrained SSN rate model can explain the cell-type-specific network dynamics. Furthermore, the connectivity from excitatory and inhibitory neural populations has a different impact on the network dynamics. We can now use the model to derive predictions for future experiments and identify cell-type-specific activity patterns that contribute to sensory perception.

Disclosures: **U. Narantsatsralt:** None. **P. Ekelmans:** None. **A. Bast:** None. **M.R. Cano:** None. **T. Tchumatchenko:** None. **M. Oberlaender:** None.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

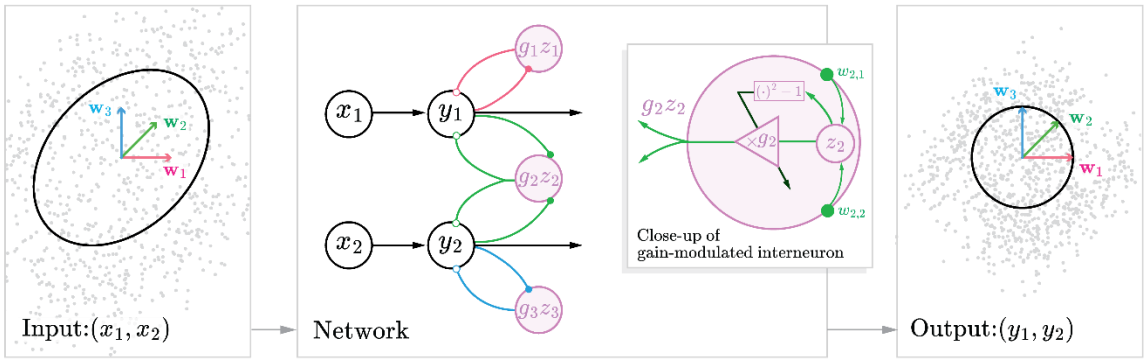
Program #/Poster #: PSTR445.11/XX32

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

Title: Adaptive coding efficiency in neural populations with gain modulation

Authors: L. R. DUONG[1,2], ***D. LIPSHUTZ**[2], D. J. HEEGER[1], D. B. CHKLOVSKII[2,3], E. P. SIMONCELLI[1,2];
[1]Ctr. for Neural Sci., NYU, New York, NY; [2]Flatiron Inst., New York, NY; [3]Neurosci. Inst., NYU Med. Ctr., New York, NY

Abstract: Efficient transmission of sensory information from dynamic environments necessitates systems that rapidly and reversibly adapt to changes in sensory statistics. Individual neurons in a variety of early sensory areas have been shown to rapidly adapt their input-output gains to normalize the variances of their responses. At the neural population level, neurons rapidly adapt to decorrelate, or whiten, their responses; however, the mechanism for decorrelation is not known. Existing models rely on synaptic plasticity mechanisms, which are unlikely to operate as transiently or reversibly as gain modulation. Here, we propose a novel circuit model which uses single neuron adaptive gain modulation to decorrelate population response.



Our model is derived from a novel objective, which reformulates whitening of neural responses in terms of the variances of a fixed *overcomplete* projection of the neural responses. Optimization of this objective yields an online algorithm that maps onto a recurrent neural network comprised of primary neurons, gain-modulating local interneurons and fixed synaptic weights. Each interneuron receives weighted inputs from the primary neurons, adjusts its input-output gain based on the variance of its input and feeds back onto the primary neurons, whitening their outputs. Our framework can be generalized to handle biophysical constraints that improve robustness of the network to ill-conditioned inputs, and we demonstrate its use in whitening local image patches using convolutional weights. We also consider a multi-timescale extension of our circuit model in which the synapses learn on a slow timescale. In this case, gains rapidly adapt to changing sensory statistics whereas synapses slowly adjust to learn invariant features of the sensory statistics.

Disclosures: L.R. Duong: None. **D. Lipshutz:** None. **D.J. Heeger:** None. **D.B. Chklovskii:** None. **E.P. Simoncelli:** None.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR445.12/XX33

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

Support: NIH Grant NS115821

Title: Emergence of cross-tuned inhibition promotes task solution in trained spiking neural network models

Authors: Y. ZHU[1,2], C. M. B. SMITH[1], M. TANG[1], ***J. N. MACLEAN**[3,4,2,5];
[1]Univ. of Chicago, Chicago, IL; [2]Committee on Computat. Neurosci., Chicago, IL; [3]The Univ. of Chicago, Chicago, IL; [4]Neurosci. Inst., Chicago, IL; [5]Inst. for Biophysical Dynamics, Chicago, IL

Abstract: Neocortex is composed of spiking neuronal units interconnected in a sparse, recurrent network. Spiking activity in such a network of neurons supports computations which transform sensory input to appropriate behavioral output. In this study, we use biofidelic, task-optimized spiking neural network (SNN) models to evaluate how recurrent networks of spiking units change to achieve task computations. Networks are composed of excitatory and inhibitory units randomly interconnected with likelihoods and strengths matched to mouse neocortex. We employ a task that requires a binary report of moving visual stimuli, analogous to tasks that mice perform. We find that, through training, SNNs selectively adjust firing rates in response to the stimulus input, and that excitatory and inhibitory connectivity between input and recurrent layers change in accordance with rate modulation. Input channels that exhibit bias to one specific input

developed stronger connections to recurrent excitatory units during training, while channels that exhibit bias to the other input developed stronger connections to inhibitory units. Furthermore, recurrent inhibitory units which were tuned to one input strengthened their connections to recurrent units of the opposite tuning. The convergence of trained network models on the specific pattern of cross-tuned inhibition highlights the significance of interneurons and their connectivity pattern in local circuit computations within neocortex.

Disclosures: **Y. Zhu:** None. **C.M.B. Smith:** None. **M. Tang:** None. **J.N. MacLean:** None.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR445.13/XX34

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

Support: 5 T32 EY 18080-15

Title: Investigating Multi-Feature Structure Learning in Recurrent Neural Networks for Visual Predictive Inference

Authors: *A. P. RASHED AHMED[1], T. SERRE[2], M. R. NASSAR[3];
[1]Neurosci., [3]Metcalf Hall, [2]Brown Univ., Providence, RI

Abstract: Flexible behavior depends critically on recognizing environmental changes and rapidly updating relevant internal representations. However, inferring which internal representations should be updated in high-dimensional real-world environments can be a difficult problem. Here we explore the computational basis of human flexibility in adapting to environmental cues through a novel visual predictive inference task called the Bouncing Ball task. Our task involves bouncing ball stimuli with separate color and velocity dimensions that undergo occasional discrete changepoints. The goal of the task is to predict the future position and color of the ball based on current observations, where the position is always observable, but the color is occluded in a specific region. By manipulating the degree of coupling between velocity and color changes, we investigate two conditions requiring different gating strategies to achieve optimal performance. In the independent condition, where the features change independently, disentangled feature representations are necessary to avoid interference. However, in the contingent condition, color changes co-occur with velocity changes, requiring coupled resetting of the features while maintaining independent representations of the features themselves. To explore the computational learning of these strategies, we trained three recurrent neural network (RNN) models: a vanilla RNN, a Long Short-Term Memory (LSTM) network, and a Hierarchical Multiscale-LSTM (HM-LSTM). Both LSTM variants models achieved optimal performance in the independent condition, demonstrating successful learning of disentangled representations. However, none of the models performed optimally in the contingent condition, exhibiting significant positional jitter following color changes. Separately,

the vanilla LSTM slightly outperformed the HM-LSTM on both conditions, indicating that the additional gates of the HM-LSTM are not well-suited for this task. Our findings suggest that the models successfully learn disentangled representations when explicitly required but struggle with contingently changing features, which lead to significant shifts in internal state representations upon color changes and impacting position predictions. We propose potential solutions, including adding representational constraints to promote disentangled representations and the refinement of boundary gates to provide more precise reset signals. Going forward, we plan to employ a wider battery of computational models and adapt the task for human participants.

Disclosures: **A.P. Rashed Ahmed:** None. **T. Serre:** None. **M.R. Nassar:** None.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR445.14/XX35

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

Title: Ai alignment and Goodhart's rule.

Authors: *Y. TAMORI[1,2], K. MOGI[3];
[1]Grad. Sch. of Arts and Sci., The Univ. of Tokyo, Meguro-ku, Japan; [2]Neurocreative Laboratory, NPO, Tokyo, Japan; [3]Sony Computer Sci. Labs., Tokyo, Japan

Abstract: With the advancement of artificial intelligence systems, alignment between AI and humans (Gabriel 2020) is becoming an important research theme, from Large Language Models (Open AI 2023), generative AI (Walters and Murcko 2020), to self-driving cars (Badue et al. 2021). Reinforcement learning from human feedback (RLHF) (Griffith et al. 2013) is an attempt to facilitate alignment with humans through the learning process. One of the limits of AI is the inability to be flexible in the application of evaluation function, or to think "outside the box". When considering collective intelligence among humans (Woolley et al. 2010), or one augmented by AI, it becomes important to consider the merits and shortcomings of AI, including rigidity of evaluation function. Goodhart's law (Goodhart 1975) states that "when a measure becomes a target, it ceases to be a good measure". Originally proposed in the context of monetary policy, it is increasingly perceived to be of general relevance when it comes to problems of decision making and choice (Manheim and Garrabrant 2021). Here we investigate the relevance of Goodhart's law in AI alignment with humans, and collective intelligence between humans. The key assumption is that the validity of Goodhart's law comes from interactions from multiple agents. The human society, to which Goodhart's rule was originally applied, is where interaction between multiple agents naturally occur. AI alignment is in a sense collective intelligence of AI and humans (Przegalinska 2023). Evaluation function defined for a human, or an AI, ceases to be a good measure when applied to the interaction between agents, human or AI, when the reinforcement loop becomes intersubjective. We give a coherent model of the interaction between humans and AI, and a group of humans. In the model, we show that,

under certain conditions, there always exists a Hebbian interaction matrix for prompt-response transactions given a posteriori. The interaction matrix representing Goodhart's rule is embedded in its resolution matrix. Therefore, AI alignment could be partially solved by an algorithm that finds "Goodhart transactions" through introspective simulations using AI-derived strategies. Based on the results, we discuss brain areas possibly involved in flexible alignment. Context-dependent value perception in the orbitofrontal cortex (Elliott et al. 2008, Vlaev et al. 2011) and related areas such as the medial prefrontal and cingulate cortex (Pischedda et al. 2020) and the hypothalamus (Ogawa et al. 2022) are analyzed.

Disclosures: **Y. Tamori:** None. **K. Mogi:** None.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR445.15/XX36

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

Support: R01AG075582
RF1NS128534

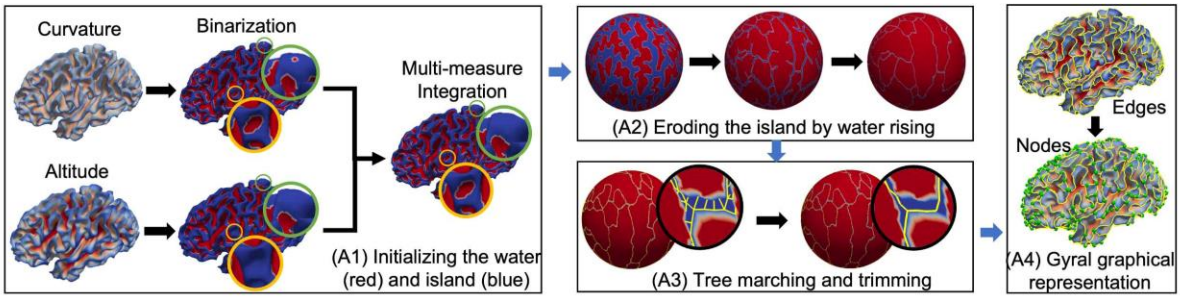
Title: Unveiling the Complexity of Cortical Folding: Introducing a Novel Graphical Representation Pipeline

Authors: ***L. ZHANG**, X. YU, Y. LYU, D. ZHU;
The Univ. of Texas at Arlington, Arlington, TX

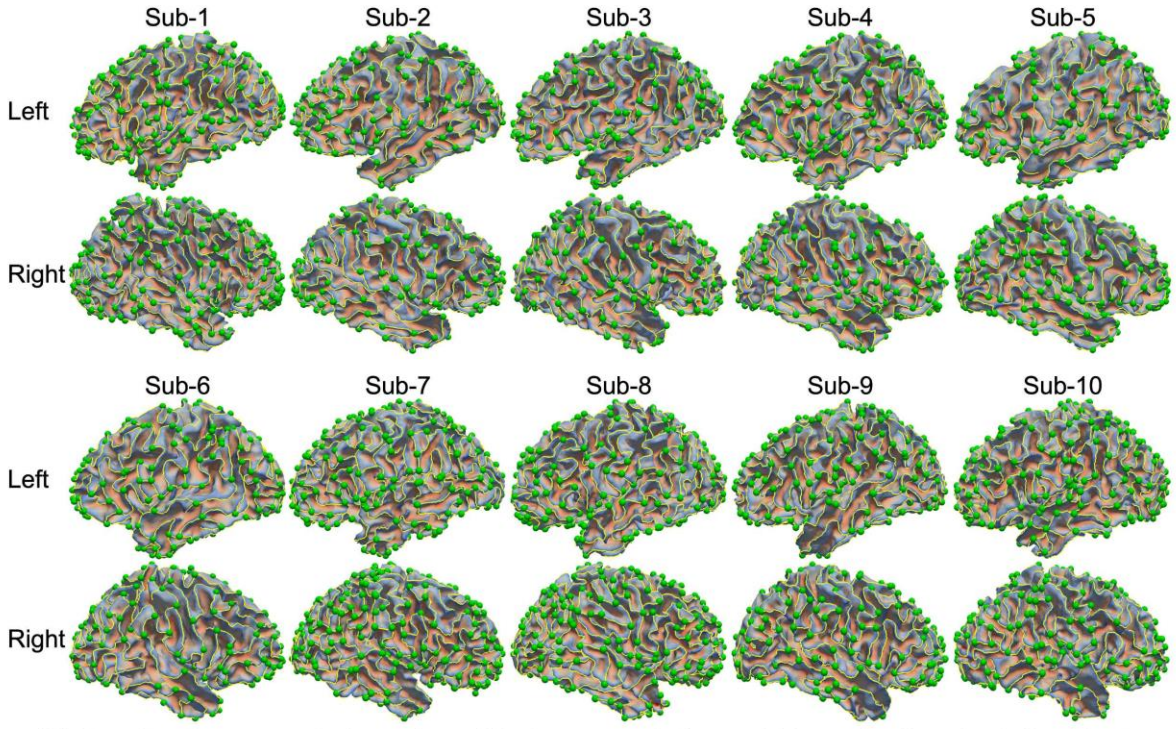
Abstract: The folding patterns of cerebral cortex, characterized by distinctive convex gyri and concave sulci, present challenges in their quantitative modeling due to their complex and variable nature. In a previous study by Chen et al. (2017), an attempt was made to address this gap by developing a computational framework called GyralNet. This framework employed the watershed algorithm (Bertrand (2005)) to model cortical architecture from a graph perspective. However, GyralNet has certain limitations. Firstly, it relies solely on gyral altitude, resulting in limited anatomical description capability. Secondly, the flooding process used in GyralNet stops prematurely after reaching a predefined threshold, leaving a significant portion of the gyral crest area, and making the identification of the major gyral skeleton more challenging. To overcome these limitations, we introduce a novel computational pipeline that offers two key contributions. Firstly, this pipeline integrates multiple measures of folding patterns, enabling a comprehensive description of the cerebral cortex. Secondly, instead of using a predefined threshold to terminate the flooding process, our pipeline adopts a completed flooding strategy, where the flooding process continues until different water sources meet. As a result, the remaining gyral crest area is significantly reduced, facilitating the identification of the gyral skeleton in these narrower regions. Moreover, by eliminating the reliance on a uniform threshold, our approach minimizes the impact of cross-subject variability. We evaluated the proposed pipeline using multiple

datasets, encompassing over 2,000+ brains. The results demonstrate the efficacy of our approach in accurately quantifying and analyzing cortical folding patterns. Fig. 1(A) illustrates the key steps of our proposed pipeline and Fig. 1(B) shows a randomly selected subset of obtained results.

Fig. 1. (A) Illustration of the proposed method and (B) Corresponding results.



(A) Methods: the proposed graphical representation pipeline of cortical folding



(B) Results: the generated gyral graphical representations of 10 randomly selected subjects

Disclosures: **L. Zhang:** None. **X. Yu:** None. **Y. Lyu:** None. **D. Zhu:** None.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR445.16/Web Only

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

Support: ISF 205501

Title: Handling missing MRI data in brain tumors classification tasks based on synthetic images by GAN model vs. conventional solutions

Authors: *Y. MOSHE[1], Y. BUCHSWEILER[2], M. TEICHER[3], M. ARTZI[4];
[1]Bar ilan Univ., Petak tikva, Israel; [2]Tel Aviv Univ., Tel Aviv, Israel; [3]Gonda Brain Res. Ctr., Ramat-Gan, Israel; [4]Tel Aviv Sourosky Med. Ctr., Tel Aviv, Israel

Abstract: Deep learning (DL) is widely used for lesion segmentation and classification based on multiparametric MRI. However, in the real-world, data often has missing values. In this study we evaluated the use of synthetic images generation vs. other solutions for handling missing MRI data in brain tumor classification tasks.The study include 224 patients: 37 with low grade glioma (LGG), and 187 with high grade gliomas (HGG). Three Pix2pix GAN models were trained separately on the local institutional dataset, to generate T1WI, T2WI and FLAIR images. Resnet152 was used for classification between LGG and HGG. Inference of the classification model was performed on the local and the public BraTS datasets, and on different scenarios of input data: replacing missing images by generated images; duplication of existent images; and usage of black images. The similarity between the generated and the original images were evaluated using the Peak-Signal-to-Noise-Ratio (PSNR), and the Structural-Similarity-Index-Measure, (SSIM). Classification results were evaluated using accuracy and F1 score. High similarity was obtained between the generated and the original images with mean PSNR and SSIM = 35.65 and 0.91. Regarding the inference of classification results on missing data, the classification model used synthetic images to produce the highest results with mean accuracy level of 0.91 for the generated images, 0.85 for duplication of existent ones, and 0.77 for use with black images. A similar hierarchy of results was obtained on the BraTS dataset. Using the generated images, we have demonstrated the feasibility for inference on newly acquired data with local institutional dataset and public dataset with cases of missing data. Additionally, we have demonstrated the stability and generalization ability of the model while remaining consistent with the public dataset.

Disclosures: **Y. Moshe:** None. **Y. Buchsweiler:** None. **M. Teicher:** None. **M. Artzi:** None.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR445.17/XX37

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

Support: NSF DBI 2015317 as part of the NSF/CIHR/DFG/FRQ/UKRI-MRC Next Generation Networks for Neuroscience Program.

Title: Communication, coordination, and control in neuromuscular systems: Synergistic interactions

Authors: *R. D. QUINN[1], H. J. CHIEL[2], V. WEBSTER-WOOD[3], G. SUTTON[4], A. J. HUNT[5], N. S. SZCZECINSKI[6];
[1]Mechanical and Aerospace Engin., Case Western Reserve Univ., Cleveland, OH; [2]Biol., Case Western Res. Univ., Cleveland, OH; [3]Mechanical Engin., Carnegie Mellon Univ., Pittsburgh, PA; [4]Univ. of Lincoln, LINCOLN, United Kingdom; [5]Mechanical Engin., Portland State Univ., Portland, OR; [6]Mechanical Engin., West Virginia Univ., Morgantown, WV

Abstract: Brains are embodied. Many fundamental questions remain unanswered: How is neural information encoded and communicated? How does the system correct for environmental perturbations? How do passive biomechanics affect the neuronal control of behavior? This leads to the foundational question: **How do nervous systems control and execute interactions with the environment?** Our international Network of interdisciplinary research groups (IRGs), which incorporates modelers, engineers, and experimentalists, is exploring the *Communication, Coordination, and Control of Neuromechanical Systems* (**C^{3}NS**).As an animal's length-scale and behavioral time-scale changes, the relative importance of forms of energy also changes. We have created a framework that quantifies the changes in these forms of energy and enables us to predict control and responses to perturbations. Thus, we have chosen to study three different groups of animals that exemplify these different dominant energy regimes.IRG1 is developing the framework as well as tools and technologies that are broadly applicable to neuromuscular modeling. IRG2, by focusing on legged locomotion in adult *Drosophila melanogaster*, investigates the effect of scale and speed in a system dominated by viscous forces. IRG3, by focusing on feeding in the soft-bodied marine mollusk *Aplysia californica*, investigates the effect of changing size in a system dominated by elastic forces. IRG4, by focusing on legged locomotion in mice, rats, cats, and dogs, investigates the effects of changing size and joint parameters on dynamic control in systems in which inertial forces increasingly dominate.

Disclosures: R.D. Quinn: None. **H.J. Chiel:** None. **V. Webster-Wood:** None. **G. Sutton:** None. **A.J. Hunt:** None. **N.S. Szczecinski:** None.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR445.18/XX38

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

Support: NIH EY026924
NIH NS113073

NIH EY014800
Research to Prevent Blindness

Title: Sensory and working memory information representation in neural systems near oscillatory instabilities

Authors: *W. NESSE[1], K. CLARK[2], B. NOUDOOST[2];
[1]Mathematics, [2]Opthamology, Univ. of Utah, Salt Lake Cty, UT

Abstract: We studied a dynamical neural model near an oscillatory instability to understand how the relative oscillatory phase and mean activity of the system work in tandem to represent and transmit information, as well as how such representations can be modulated by global top-down working memory-like signals. We establish that sensory input discrimination based on the relative oscillatory phase of neural field model (i.e. a phase code) is enhanced by working memory signals; whereas, concomitant rate coding of sensory input is reduced. While statistical discrimination via phase coding is far more effective than rate coding, we also study how information carried by a rate code may serve an important role in transmitting information beyond local sensory networks, as well as coordinating downstream computations. The goal is to address the dilemma of why rate-based coding exhibits only weak sensory discriminability and weak changes in response to working memory- or attention-like signals, and fails to account for observed behavioral benefits of these cognitive functions. We specifically propose a dual phase-plus-rate code that utilizes a phase code for maintaining the sensory representation, and rate code means of transmission. By segregating the role of phase versus rate code, our model reveals the utility of concomitant encoding of information by a dual code.

Disclosures: W. Nesse: None. **K. Clark:** None. **B. Noudoost:** None.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR445.19/XX39

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

Title: Genes regulating cytoskeleton organization identified as neuro-common drivers of blood NfL change rate and disease-specific clinical progression in AI driven digital twins

Authors: X. SHEN[1], D. SHOKEEN[1], O. ISACSON[2], ***R. HARRISON**[1], S.-Y. SHIN[1], J. LATOURELLE[1];
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Abstract: Elevated Neurofilament Light Chain (NfL) is commonly observed in neurodegenerative disorders (NDs), including AD and PD, and is widely recognized as a potential biomarker (Khalil et al. 2018). A recent study suggested that the rate of change in blood

NfL may be a better predictor of AD progression than the absolute NfL at baseline (Preishe et al. 2019). Here we use causal AI based digital twins to identify genes and pathways driving the rate of change in blood NfL robustly across AD and PD.
Two digital twins, each comprising of sets of Bayseian network models were built; one connecting 59k clinical and multi-omic variables measured in 317 subjects from the ADNI study, and the other connecting 38k variables in 514 subjects of the PPMI and Genetic cohort study. We performed ~18k counterfactual *in silico* experiments on digital twins and identified 20 overlapping genes driving the rate of change of blood NfL in both models. These 20 genes, quantified at the blood transcript level, explain 3% of the variance of the NfL change rate in the AD train dataset, adjusting for age, sex and APOE4 genotype ($p=0.07$); and 2% in the PD train dataset adjusting for age, sex, and pathogenic variants ($p=0.03$).
Pathway enrichment analyses found the 20 overlapping genes significantly overrepresented in gene sets known for the regulation of cytoskeleton organization ($p < 0.0002$). We next identified all additional drivers of NfL change rate in either PD or AD represented in these genes sets to create disease-specific cytoskeleton gene signatures and evaluate their effect on clinical disease progression. The 24 total PD-related cytoskeleton genes were shown to drive motor decline in the PD cohort, measured as rate of change in UPDRS III score ($p=0.04$), while the 28 AD-related cytoskeleton genes strongly affected cognitive decline, measured by the rate of change in CDR-SB score ($p=0.006$) in the AD train dataset. The AD related signature was validated in an independent out-of-sample AD dataset (ANMerge, $p=0.02$).
Causal network based digital twins provided evidence that AD and PD may share common genes and pathways causally affecting a neuro-common biomarker as well as disease-specific progression rates. Our finding that genes driving the NFL change rate are involved in the mechanism underlying the regulation of cytoskeleton organization is consistent with the fact that the cytoskeleton disorganization in neurons is a known early pathogenic event in multiple NDs and observed along with the excessive extraneuronal abundance of NfL. The findings of this study suggest further investigation of cytoskeleton genes for potential novel therapeutic strategies to slow disease progression may benefit multiple NDs

Disclosures: **X. Shen:** A. Employment/Salary (full or part-time):; Aitia. **D. Shokeen:** A. Employment/Salary (full or part-time):; Aitia. **O. Isacson:** F. Consulting Fees (e.g., advisory boards); Aitia. **R. Harrison:** A. Employment/Salary (full or part-time):; Aitia. **S. Shin:** A. Employment/Salary (full or part-time):; Aitia. **J. Latourelle:** A. Employment/Salary (full or part-time):; Aitia.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR445.20/XX40

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

Support: NIH RF1 DA055667
Simons-Emory International Consortium on Motor Control Accelerator Grant

Title: Interpreting Neural Computation via Sequential-Autoencoders with Injective, Nonlinear Readouts

Authors: *C. VERSTEEG[1], A. R. SEDLER[3,4], J. D. MCCART[3,4], C. PANDARINATH[5,2]; [1]Emory Univ., Decatur, GA; [2]Dept. of Neurosurg., Emory Univ., Atlanta, GA; [3]Wallace H. Coulter Dept. of Biomed. Engin., [4]Ctr. for Machine Learning, Georgia Inst. of Technol., Atlanta, GA; [5]Wallace H. Coulter Dept. of Biomed. Engin., Emory Univ. / Georgia Tech., Atlanta, GA

Abstract: In light of recent advances that have dramatically increased the number of neurons that can be simultaneously recorded from the brain, we need new computational tools that can mine these datasets for interpretable explanations of neural computation. One promising approach has emerged from the field of machine learning, where the dynamics of artificial neural networks - the rules that dictate how their activity develops over time - can give insight into how thel networks perform cognitive tasks. However, because the dynamics of biological neural circuits cannot be directly observed, we need computational models that can approximate them from recorded neural activity. Recently developed Sequential Auto-Encoders (SAEs) model neural activity with lower-dimensional latent dynamics that are embedded into higher-dimensional neural activity. Unfortunately, existing SAEs have shortcomings that limit their interpretability, leading to inferred dynamics that are unnecessarily complex or unrepresentative of the underlying system. In this work, we solve this problem with recently developed invertible neural network architectures, creating a new model called ODIN (Ordinary Differential equations auto-encoder with Injective Nonlinear readout). We first demonstrate that ODIN can extract more interpretable dynamics from realistic synthetic spiking datasets than alternative models, including more accurate recovery of latent activity (mean Latent R2 of 0.93, 0.70 for ODIN, Linear-readout model, respectively), and better robustness to hyperparameter choice. Next, we apply this model to real neural datasets recorded from behaving monkeys, including motor cortical activity from a reaching task with obstacles (Maze) and frontal cortical activity from an interval timing task (ITT). We show that ODIN fit to the Maze dataset can achieve state-of-the-art reconstruction performance while using far fewer dimensions than alternative models (~10 vs. >25 for ODIN, alternative model, respectively), enhancing the interpretability of the latent representations. We also demonstrate that ODIN confirms previous findings in the ITT dataset, namely, that precisely tuned neural dynamics build a timing circuit. However, unlike the approaches used to analyze these data previously, ODIN has an explicit dynamics model that can predict how the neural circuit will generalize to untested conditions. Overall, this new computational method will help us to translate large neural datasets into an understanding of how computation is built by the brain.

Disclosures: C. Versteeg: None. **A.R. Sedler:** None. **J.D. McCart:** None. **C. Pandarinath:** F. Consulting Fees (e.g., advisory boards); Synchron, Meta (Reality Labs).

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR445.21/XX41

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

Support: NICT (1940201)
Grants-in-Aid for Scientific Research (19H04999 / 21K19812)
ATLA (JPJ004596)
NEDO (P20006)

Title: Categorical Invariant Generative Model (CIGMO): Deep Generative Learning Inspired by Primate Higher Vision

Authors: *H. HOSOYA;
ATR, Kyoto, Japan

Abstract: In this study, we propose a novel learning model called the Categorical Invariant Generative Model (CIGMO), drawing inspiration from the modular architecture of the primate visual system, which employs multiple cortical regions to encode invariant features specific to diverse object categories. Current deep generative models capture either the view variation or the categorization of object images, with scarce research targeting the integration of both structures in a single framework. To address this gap, CIGMO was designed to learn and encode three latent factors from a dataset of general object images: category, shape, and view. Overcoming challenges associated with unsupervised learning, we employed group-based weakly supervised learning as an 'inductive bias', allowing the model to learn separate shape and view representations from object images. We further extended this approach by introducing multiple modules of shape representations and creating mechanisms to specialize each shape representation to a particular object category while disentangling it from the view representation. Empirical investigation of CIGMO demonstrates its representational advantages. The model successfully solved the invariant clustering problem, discovering categories of object shapes in an unsupervised manner despite significant variation in object views. Quantitative performance surpassed various previous methods, including state-of-the-art invariant clustering. Further, our category-specialization approach enhanced the learned shape representation's capacity for multiple tasks, including one-shot identification and shape-view disentanglement in multiple criteria. Thus, CIGMO offers a novel learning approach to represent object images, providing more precise information for downstream tasks than typical approaches. This enhanced understanding of object image representation not only enhances our understanding of neural encoding mechanisms but also informs the development of advanced object recognition systems.

Disclosures: H. Hosoya: None.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

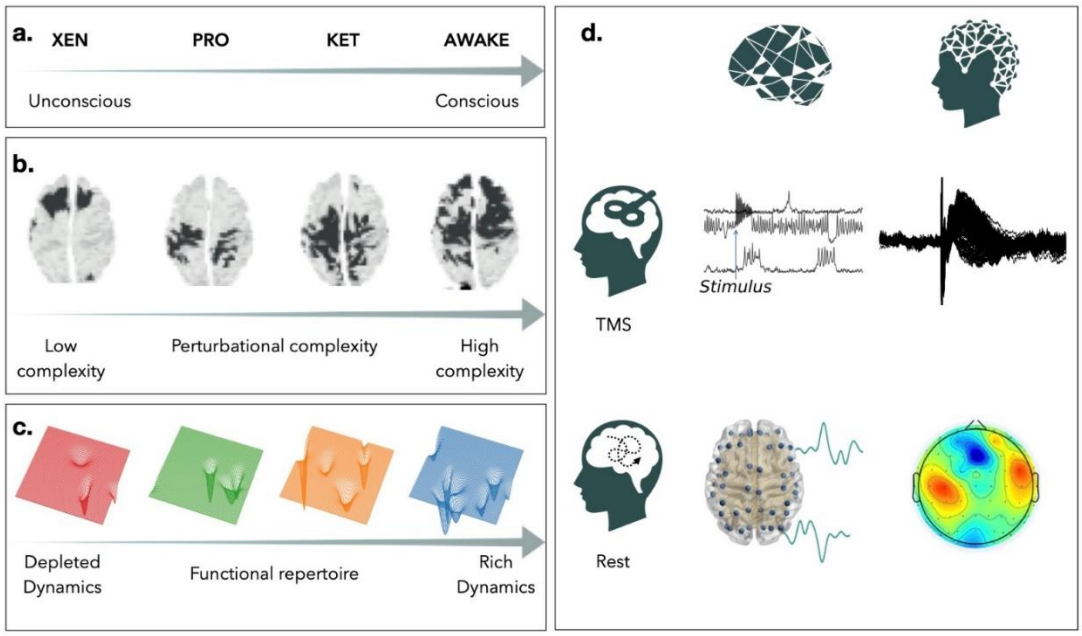
Program #/Poster #: PSTR445.22/XX42

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

Title: Large-scale brain signatures of fluid dynamics and responsiveness linked to consciousness

Authors: *M. BREYTON, J. FOUSEK, P. SORRENTINO, G. RABUFFO, L. KUSCH, S. PETKOSKI, V. JIRSA;
Aix-Marseille Univ., Marseille, France

Abstract: Neural bases of consciousness have been explored through many different paradigms and the notion of complexity emerged as a unifying framework to characterize conscious experience. To date, the perturbational complexity index (PCI) performs best to assess consciousness through brain stimulation. However, the mechanisms underpinning this complexity remain unclear and reliable metrics on spontaneous activity are still missing. In this study, we explore brain responsiveness and resting-state activity through large-scale brain modelling with The Virtual Brain (TVB) and prove that complexity and consciousness are directly associated with a fluid dynamical regime (Fig 1). This regime corresponds to a specific set of parameters in the model associated with non-trivial spontaneous activity. Fluidity is reflected in the dynamic functional connectivity, calculated as the variance of its upper triangular part. Other metrics: Lempel-Ziv (LZ) complexity, the size of the functional repertoire (SFR), and the busting potential (BP) can also capture this dynamic in synthetic data. We validated our findings on a cohort of 15 subjects (spontaneous EEG recordings and maximum PCI values) under anesthesia and wakefulness. In this empirical data, PCI is systematically higher during wakefulness than under anesthesia for Xenon (N=5) and Propofol (N=5) drugs, but not for Ketamine (N=5). Metrics on spontaneous activity revealed that *fluidity* is also systematically higher during wakefulness than anesthesia (not for Ketamine) with a classification accuracy of 100%. Results for LZ complexity, the SFR and BP are similar. We demonstrated that the symmetry breaking caused by the connectome is sufficient for setting the global working point of the brain, allowing the generation of complex behavior in different paradigms: rest and stimulation. In the future, the imperfect separation of groups for some of the metrics could be improved by personalized brain modelling and including more realistic parameters in the models such as neuromodulatory pathways to improve explanatory power.



Disclosures: **M. Breyton:** None. **J. Fousek:** None. **P. Sorrentino:** None. **G. Rabuffo:** None. **L. Kusch:** None. **S. Petkoski:** None. **V. Jirsa:** None.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR445.23/XX43

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

Title: Temporal variability of brain-behavior relationships in functional connectivity

Authors: *S. A. CUTTS, E. CHUMIN, R. F. BETZEL, O. SPORNS;
Indiana Univ., Bloomington, IN

Abstract: Title: Temporal variability of brain-behavior relationships in functional connectivity
Introduction: Most work on functional connectivity (FC) in neuroimaging data preference longer scan sessions or greater subject count to improve reliability of brain-behavior relationships or predictive models. Here, we investigate whether systematically isolating moments in time can improve brain-behavior relationships and outperform full scan data. We perform optimizations using a temporal filtering strategy to identify time points that improve brain-behavior relationships across 58 different behaviors.
Methods: We analyzed functional brain networks from resting state fMRI data of 352 healthy subjects from the Human Connectome Project. Templates were created to select time points with similar patterns of brain activity. Optimizations were performed to produce templates for each behavior that maximize brain-behavior relationships from reconstructed functional networks (Fig

1A).

Results: Results for the behavioral measure "working memory" are shown in Fig 1B-D. Optimization templates filtered time points with stronger behavioral relationships (Fig 1B) and brain-behavior correlation maps that transferred to held out test subjects (Fig 1C,D). With 10% of scan data, optimized templates of select behavioral measures (Fig 1E) achieved greater magnitude of brain-behavior correlations and greater transfer across groups of subjects than full FC.

Conclusions: We show that for a subset of behaviors, brain-behavior relations and reliability can be improved above full FC using only a fraction of neuroimaging data. Selectively filtering time points may allow for development of more targeted FC analyses and increased understanding of how specific moments in time contribute to behavioral prediction.

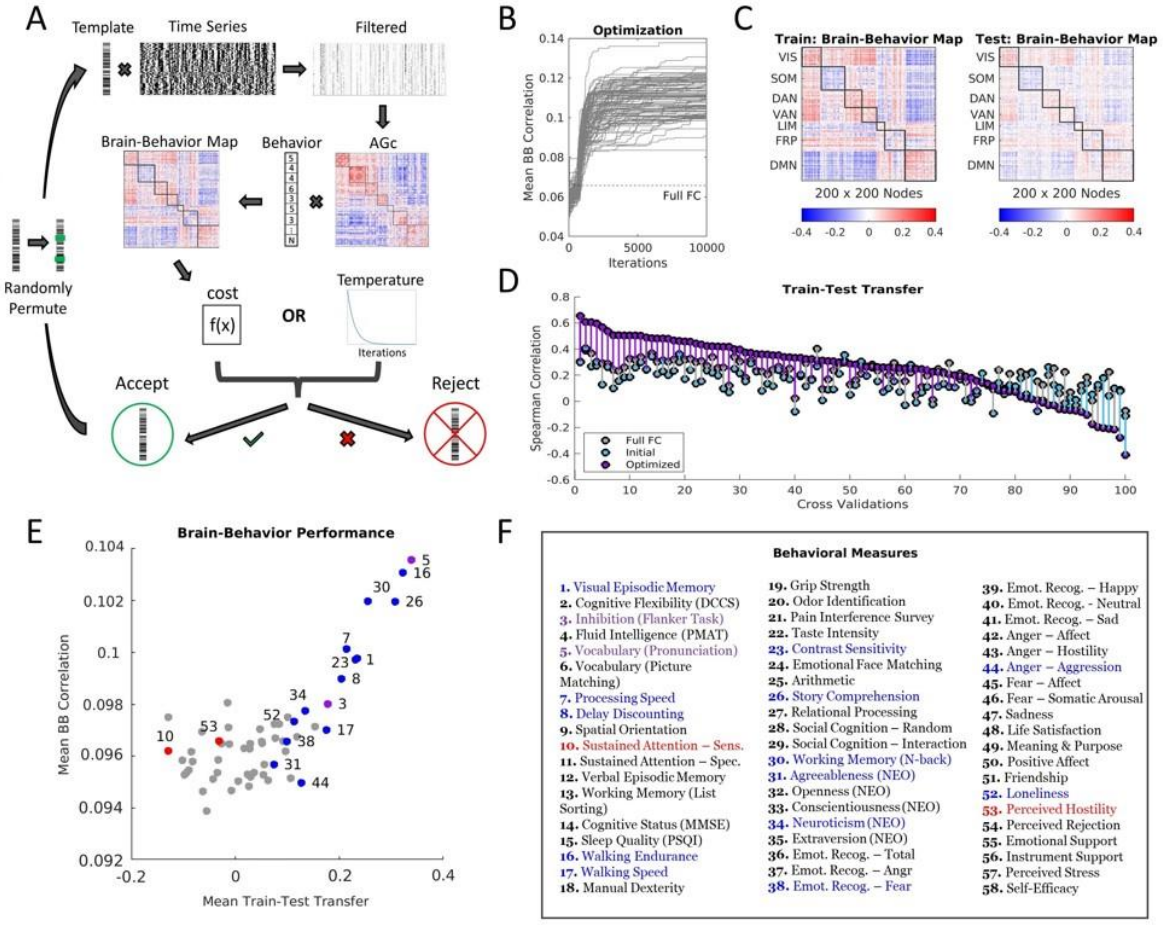


Figure 1. **(A)** Optimization pipeline utilizing filtering approach. The optimization is initialized with a random binary template and adjusted across 10,000 iterations to maximize behavioral relations. The agreement across binarized z-scored time series of brain regions is nearly identical to functional connectivity (FC; r = 0.96) and non-contiguous filtered frames produce a matrix similar in structure to FC, here referred as AG components (AGc). Normalized mutual information is used to compare the current template to each time step and filter the top 10% of frames with highest similarity. An AGc was created from the selected frames and each edge was correlated to behavioral scores across subjects. This produced a brain-behavior correlation map for the selected template that the optimization used to compare and improve the template pattern. **(B-D)** Example results for 'working memory'. **(B)** Improvement in behavioral correlations across each iteration of the optimizations for 'working memory' (computed on 100 separate cross validations) compared to average FC. **(C)** Average across the brain-behavior correlation maps for training (left) and testing (right) subjects. Black lines denote boundaries of functional networks. **(D)** Transfer of results across training and testing subjects of the cross validations were estimated based on Spearman correlation between train and test brain-behavior correlation maps. Optimized results (purple), initial randomized template (blue), full FC (gray). **(E)** Summary results across all behaviors showing behavior correlation magnitude and transfer between training and testing sets. Optimizations that significantly outperformed full FC ($p<0.01$) for train-test transfer (blue), magnitude of behavior correlations (red), both (purple). **(F)** List of behavioral measures.

Disclosures: **S.A. Cutts:** None. **E. Chumin:** None. **R.F. Betzel:** None. **O. Sporns:** None.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR445.24/XX44

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

Title: Low dimensional manifolds of whole brain activity at single neuron resolution that map activity to behavior

Authors: *G. M. PAO[1], J. PARK[2], K. TAKAHASHI[3], Z. WEI[4], M. B. AHRENS[5], T. J. SEJNOWSKI[6], S. SMIRNOV[7], H. NATSUKAWA[8], J. OGAWA[1];
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Abstract: Recent advances in large scale neuronal recordings have achieved recordings from thousands up to a million neurons simultaneously taking neuroscience to the Big Data world. The thinking has traditionally been that the dimensionality of brain activity is minimally as high as the number of neurons in the brain. This has led to the notion that a Zebrafish or fly brain will have a complexity of ~100,000 dimensions. Here in the present work we will show that using nonlinear approaches that are not based on correlation for the analysis of whole brain activity in zebrafish larvae and drosophila, one finds that the dimensionality of brain activity is much lower than thought <20 even when Takens embeddings tend to overestimate dimensionality by 2n+1 where n is the real dimensionality. Neuronal activity dynamics can be described well on the surfaces of low dimensional manifolds. This approach has allowed us to estimate the dimensionality of whole brain activity at single neuron resolution showing that the majority of neurons in the brain operate in the low dimensional regime. Using these Takens embedding theorem based methods has allowed us to perform causal inference across the brain of such model organisms up to single neuron resolution that allow the creation of predictive models of behavior based on brain activity and ultimately create models of brain activity that predict behavior at single neuron resolution as well as the accompanying motor behavior of the animals.

Disclosures: **G.M. Pao:** None. **J. Park:** None. **K. Takahashi:** None. **Z. Wei:** None. **M.B. Ahrens:** None. **T.J. Sejnowski:** None. **S. Smirnov:** None. **H. Natsukawa:** None. **J. Ogawa:** None.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR445.25/XX45

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

Title: A multi-scale robust coding system in biology: the grid cells

Authors: *A. WINN[1], I. DAUBECHIES[1], S. CUI[2], C. L. CHARIKER[1];
[1]Duke Univ., Durham, NC; [2]Univ. of Chicago, Chicago, IL

Abstract: The parametrization of spatial location within the brain has been studied extensively in several species; in rats and mice, researchers have observed dedicated neurons in the entorhinal cortex that use an implicit multiresolution parametrization. In particular, these grid neurons have the following special properties: 1) each neuron activates when the animal is located in a particular region of physical space, which is called the neuron's receptive field; each neuron's receptive field consists of circular regions that are arranged in a triangular lattice on the plane; 3) grid neurons can be grouped into modules where the receptive fields are translates of one another; 4) there are about 5-10 modules with geometrically increasing lattice spacing and a fixed orientation offset between subsequent modules. We study mathematically the corresponding location-encoder design, in terms of its resolution, coverage, and robustness. The values of the parameters that optimize for these criteria are remarkably close to the empirically observed ones. This presents a biological example of a multi-scale, robust encoding system with rotation.

Disclosures: **A. Winn:** None. **I. Daubechies:** None. **S. Cui:** None. **C.L. Chariker:** None.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR445.26/XX46

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

Title: Advancing Neuromorphic Design Through Biophysical Understanding of Neuronal Computational Complexity

Authors: *C. J. HARPER, A. KUMAR;
Univ. of California, Berkeley, Berkeley, CA

Abstract: How do we get computers to work like brains? Brains were optimized by evolution under space, weight and power (SWAP) constraints, similar to neuromorphic systems. The dominant paradigm in neuromorphic computing is use of simple neuron models. However, brains use complex neurons which are highly sophisticated computing elements. Our hypothesis is that

circuits composed of complex neurons are capable of performing sophisticated computations with fewer neurons than those containing simple neurons. However, our neuroscientific understanding of the biophysical determinants of single-neuron computational complexity is nascent. This gap impedes our design of neuromorphic systems and understanding of neurological disorders arising from disruption of those biophysics.
We leveraged natural occurring biophysical variability of 169 cortical neuron types, modeled by Blue Brain Project. We examined 'intrinsic complexity' by analyzing spatio-temporal Vm profiles in response to somatic current injection. We deployed PCA to determine dimensionality across neurons. This revealed that excitatory neurons were higher dimensional than inhibitory neurons, and that Ih currents, which are known to impart non-trivial Vm dynamics, were most important. We deployed Dynamical Components Analysis, which quantifies intrinsic dimensionality and computational complexity using predictive information (PI). Initial results indicate morphologically complex neurons have higher dimensionality. A more nuanced picture was observed when individual neuron PI was examined: simpler neurons could have more complex dynamics. Across excitatory neurons, PI increased monotonically with cortical layer (i.e., L2/3< L4<L5<L6). As neurons in different layers perform distinct computations, this suggests single neuron specializations for those computations. Finally, we modeled neurons as bilinear systems, facilitating explicit calculation of PI as a quantifier of I/O transformation complexity, which forms a pivotal aspect of our hypothesis. Together, our work provides a theoretically grounded measure of single neuron computational complexity, and leverages the naturally occuring design space to understand the biophysical determinants of computation towards SWAP efficient neuromorphic systems.

Disclosures: C.J. Harper: None. **A. Kumar:** None.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR445.27/XX47

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

Title: A characterization of brain modular structure based on higher-order functional interactions

Authors: *M. PUXEDDU[1], T. VARLEY[4], M. POPE[2], O. SPORNS[3];
[2]Indiana Univ., [3]Olaf Sporns, [1]Indiana Univ., Bloomington, IN; [4]Indiana University, Bloomington, Bloomington, IN

Abstract: Brain functional connectivity can be modeled as a network of brain regions and their interactions. Network analyses revealed key topological features of brain networks, including modular structure [1]. So far, modules in the brain have been identified through algorithms accounting only for pairwise interactions among brain regions, which provide modules of highly interconnected nodes. Recently, higher-order interactions and multivariate measures of information are receiving more attention as playing a key role in complex systems such as the

brain. Thus, network neuroscience is moving into the development of tools able to infer the topology of brain networks considering higher-order dependencies [2].
Here, we propose a new algorithm for community detection in brain functional networks that capitalizes on these developments. Known that the redundancy in a subset of nodes is the information that is carried by all the elements [3], we aim to find modules composed by nodes whose activity is maximally redundant. We developed an algorithm that uses Total Correlation (TC) as a measure of redundancy, and, starting from a random partition, iteratively reassigns nodes to modules to maximize the sum of the TC of nodes constituting individual modules (Figure). Thus, the brain is divided into optimally redundant modules.
Running the algorithm on a group-averaged network estimated from the HCP [4], we identified modules that only partially match those found with conventional algorithms that maximize an edge-based modularity function [5]. This result suggests the existence of unexplored topological properties of brain functional networks that are revealed by multivariate measures of information. Further analysis will elucidate the role that they play in human cognition and behavior.
[1] O. Sporns and R.F. Betzel, 2016, Annu. Rev. Psychol.[2] T.F. Varley, et al., 2023, Communications biology[3] G. Tononi, et al., 1994, PNAS[4] D.C. Van Essen, et al, 2013, Neuroimage[4] L.G.S. Jeub et al., 2018, Scientific Reports

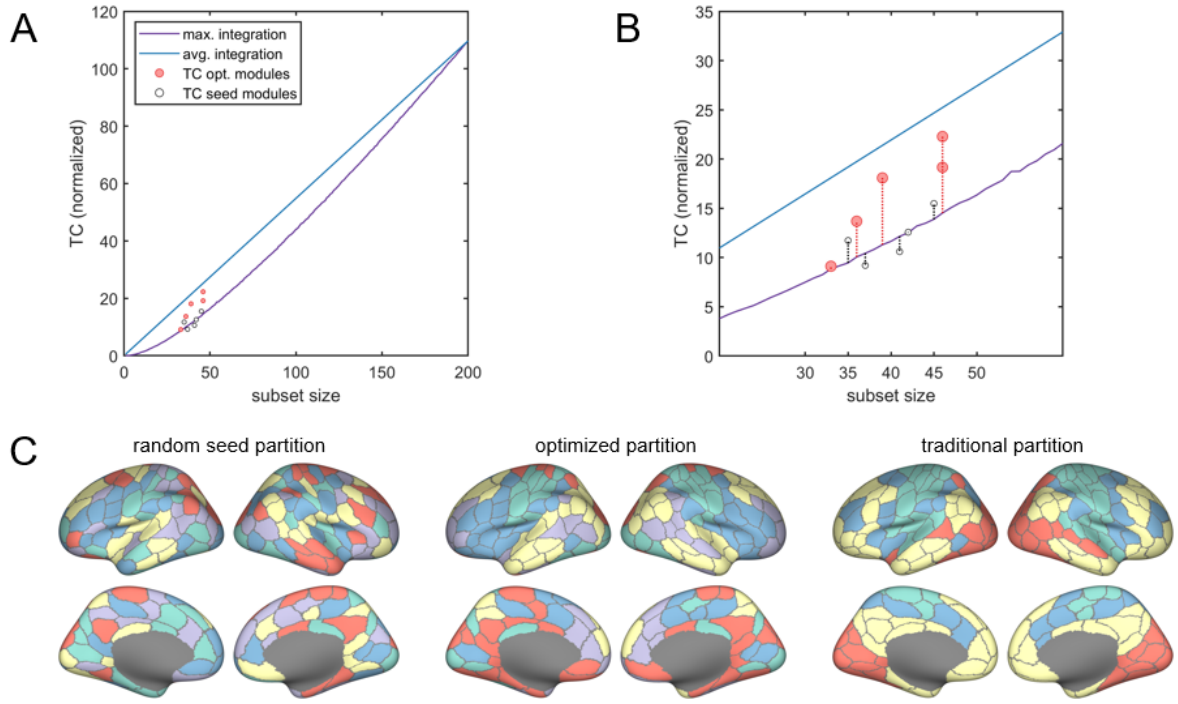

Figure. (A) TSE complexity graph [2]. Given a multivariate set of time series, like FC estimated on top of different brain areas, we can define at each scale (i.e. subset size) the average and maximum level of integration (Total Correlation) of the subset (purple and blue line, respectively.) Partitioning the brain regions into modules we can observe where their TC is located with respect to those curves. **(B)** Zoom in of the graph reported in panel A. Our proposed algorithm assigns nodes to modules optimizing the difference between the TC of modules and the average TC of an equal sized subset of nodes (dotted line). **(C)** Projection on the brain cortex of: a random modular structure (left); the modular structure obtained by optimizing within-modules TC (middle); the modular structure obtain with conventional modularity optimization (right). The Normalized Mutual Information between the last two partitions is equal to 0.20.

Disclosures: M. Puxeddu: None. **T. Varley:** None. **M. Pope:** None. **O. Sporns:** None.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR445.28/XX48

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

Support: PTDC/MED-NEU/4584/2021

Title: Optimal control of spiking neural networks

Authors: *T. COSTA, J. R. CASTIÑEIRAS, A. RENART;
Champalimaud Fndn., Lisboa, Portugal

Abstract: The ability to efficiently control a recurrent circuit would facilitate computation generically, but optimal control is technically difficult, so applications of optimal control to circuit dynamics have mostly been restricted to linear networks and quadratic costs. Here, building on our previous work on control-limited decision-making [Castiñeiras, J. R., & Renart, A., bioRxiv, (2022)], we developed a mathematical framework for the optimal control of activity in recurrent networks of spiking neurons with a stochastic spiking threshold under arbitrary costs. In our framework, which is fully event-based and operates in continuous time, the uncontrolled dynamics of the network imposed by the connectivity is viewed as the passive policy in a KL control framework [Todorov, E., PNAS, 106, 11478 (2009)]. We define a notion of reward over network states through low-dimensional projections of neural activity, as in brain-machine interface experiments, and find the policy that maximizes future expected reward subject to a KL control cost. The optimal control law takes the form of an additive synaptic input to the recurrent input from the uncontrolled network. This control input depends on the Value function of the problem, which is obtained by solving a Bellman Equation that can be expressed as a system of nonlinear ordinary differential equations, and is thus tractable. Our work opens the door to a principled understanding of how activity in recurrent circuits can be adequately shaped by inputs from other brain areas, or by experimental manipulations.

Disclosures: T. Costa: None. **J.R. Castiñeiras:** None. **A. Renart:** None.

Poster

PSTR445. Network Computations: Theory and Modeling I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR445.29/XX49

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

Title: Self-organized critical dynamics improve the performance of deep neural networks

Authors: *S. VOCK[1], C. MEISEL[2];
[1]Neurol., Charite Universtätsmedizin Berlin, Berlin, Germany; [2]Charité Universitätsmedizin, Berlin, Germany

Abstract: Deep Learning has made a large impact in industry and research. However, training a deep neural network (DNN) can be challenging due to instabilities in the training process, leading to slow learning and suboptimal results. A theory-driven understanding of the conditions and control of DNNs is still missing. Recent research has indicated that critical phase transitions play a crucial role in optimal information processing in biological and artificial neural networks. We apply this concept to DNNs using adaptive self-organized criticality (aSOC), which is considered a plausible mechanism by which networks can be autonomously organized towards criticality through adaptive changes of their synaptic weights. By identifying the links between dynamics and performance, we aim to improve the understanding of information processing in neural networks, and ultimately enhance the computational properties of deep learning systems.Specifically, we first characterize the dynamics of DNNs using the Lyapunov Exponents and the dynamic range of the network, two well-established indicators of critical phase-transitions in dynamical systems. Next, we introduce aSOC to DNNs, allowing us to study the impact of a simple, local rule-set: Quiet nodes grow links and active nodes lose links. The neurons thereby adaptively strengthen and weaken randomly selected incoming connections based on a preset threshold for activation. Finally, we apply aSOC alongside with gradient descent: While aSOC drives the system to criticality, gradient descent learns the representations. We here use the MNIST dataset, which is widely applied for training and testing in the field of machine learning.We demonstrate that the critical state in fully-connected DNNs is characterized by disappearing Lyapunov Exponents and a maximal dynamic range, which has been questioned in previous investigations. By using aSOC during training, the entire DNN exhibits robust self-organization towards criticality. To investigate performance improvements, we study the effect of gradient descent alongside aSOC on a state-of-the-art DNN from the literature trained on the MNIST dataset. The results show faster learning, improved resilience against random weight initializations, and performance increases of up to 90% accuracy.In conclusion, our work explores the use of aSOC in DNNs and its effect on dynamics and performance. The results provide general insights into the dynamics of neural networks and show promising improvements in training DNNs. While we focus on fully-connected DNNs, future work may extend investigations toward other networks and different machine learning approaches.

Disclosures: S. Vock: None. **C. Meisel:** None.

Poster

PSTR446. Software Tools: Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR446.01/XX50

Topic: I.07. Data Analysis and Statistics

Support: NIH BRAIN Initiative U01NS113252

Title: Creating spike sorting reference data using high-density electrode arrays

Authors: *J. COLONELL[1], S. CHEN[1], C. MANAGAN[1], R. ARRUDA[1], N. A. STEINMETZ[2], T. D. HARRIS[1];
[1]Janelia Res. Campus, Ashburn, VA; [2]Dept. of Biol. Structure, Univ. of Washington, Seattle, WA

Abstract: There is a critical need for spike sorting reference data to quantify and optimize spike sorter performance. Simultaneous recordings from extracellular probes and a juxtacellular or intracellular patch are the gold standard, but only provide data for a single cell in the ensemble of recorded units. In this study we performed multiple recordings across five brain regions in mouse using Neuropixels Ultra probes, which have 10X the site density of standard Neuropixels 1.0 probes. Higher site density improves spike sorting accuracy in two ways: (1) Detection on more sites creates higher signal to noise in template matching and (2) Denser sampling improves spike localization. This high accuracy sorting combined with manual curation to identify units with unimodal distributions in the high-density feature space provides a set of known spike times and labels for an average of 14 +/- 8 units/recording (N=33 recordings). Spatial resampling of the high-density data accurately reproduces the result that would be measured with a probe with standard site density, including real noise and background activity. The manually verified high-density sorting results serve as reference data to measure sorting accuracy of the standard density data, in which the spikes have on average lower SNR and poorer localization precision. Relative to simultaneous patch recordings, this method produces substantially higher yield of verified high-quality sorted spikes from simple and easily replicable data collection, while being limited by the accuracy of the initial sorting of the high-density data. We demonstrate the use of this reference data, which will be publicly available, in a parameter optimization study of Kilosort.

Disclosures: J. Colonell: None. **S. Chen:** None. **C. Managan:** None. **R. Arruda:** None. **N.A. Steinmetz:** None. **T.D. Harris:** None.

Poster

PSTR446. Software Tools: Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR446.02/XX51

Topic: I.07. Data Analysis and Statistics

Title: A new graphical user interface for the synaptic and cross-frequency analysis derived from brain signals

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Abstract: Using different computational techniques, it is possible to analyse neural oscillations in order to understand the relationship between brain activity and cognitive processes. Considering that processing those bio-signals is a complex and time-consuming task, we develop a new graphical user interface, named BOARD-FTD-PACC, using MATLAB (The Mathworks, Inc.). That software was designed to facilitate visualization and quantification of neurophysiological recordings in different set-ups (i.e. intracellular and extracellular recordings with or without stimulation). BOARD-FTD-PACC provides different methods to analyzing post-synaptic responses and neural oscillatory data, mainly cross-frequency analysis such as phase-amplitude coupling (PAC) and phase coherence. At difference of most programs for analyzing synaptic activity, BOARD-FTD-PACC includes three methods for PAC quantification and four methods for power spectral analysis. This interface is a flexible and user-friendly tool that can be used by users without advance background in programing or computer science. In the present work, we show recordings derived from in vivo experiments in the thalamus, cortex, and hippocampus, illustrating the use of this graphical user interface.

Disclosures: **M.O. Nava-Mesa:** None. **C. Gauthier-Umaña:** None. **M. Valderrama:** None. **A. Munera:** None.

Poster

PSTR446. Software Tools: Neurophysiology

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Program #/Poster #: PSTR446.03/XX52

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant P01DA047233
Friedman Brain Institute

Title: Deepregfinder: deep learning-based regulatory elements finder

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Abstract: DNA regulatory elements (DREs) are genomic regions that play a crucial role in controlling gene expression by interacting with chromatin and DNA binding proteins. Promoters are DREs located proximal to the transcriptional start sites (TSSs) facilitating transcription initiation. Enhancers are cell-type-specific DREs that can act over long distances to stimulate gene expression. They coordinate with promoters via formation of DNA loops to mediate gene expression. Identifying enhancers at a genomic scale is crucial as changes in enhancer activity can give rise to diseases. DREs exhibit unique histone mark binding patterns which can be captured by high-throughput ChIP-seq experiments. However, a simple rule-based method often fails to account for the variations and noises among all the DREs at different locations on the

genome, the total number of which is estimated to be in the order of millions. machine learning models can be trained on known enhancer/promoter sites using histone mark ChIP-seq data as input and predict enhancers/promoters at other genomic regions. Several machine learning methods have been developed to predict DREs across the genome. But existing methods are often difficult to use, especially in preprocessing the raw data to be ready for consumption by the models. To this end, we have developed a highly customizable program named DeepRegFinder, which automates the entire process of data processing, model training and prediction. We have employed two deep neural network architectures - convolutional and recurrent neural networks - for model training and prediction. Additionally, DeepRegFinder can categorize enhancers and promoters into active and poised states, making it a unique and valuable feature for researchers. We conducted a comparative analysis of DeepRegFinder against five established methods, namely RFECS, eHMM, PREPRINT, EP-DNN, and ChromHMM. Our method demonstrated consistently higher precision and recall in comparison to existing algorithms for enhancer and promoter prediction across multiple cell types. Moreover, our pipeline is modular and eliminates the tedious steps involved in preprocessing, making it easier for users to apply on their data quickly. Finally, we demonstrated that the deep learning models, without any explicit programming, can learn to discern characteristic patterns of histone marks associated with different types of DREs such as active enhancers, poised promoters, and genomic backgrounds. This was accomplished through exploiting the interpretability of the first convolutional layer.

Disclosures: **A. Ramakrishnan:** None. **G. Wangensteen:** None. **S. Kim:** None. **E.J. Nestler:** None. **L. Shen:** None.

Poster

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Program #/Poster #: PSTR446.04/XX53

Topic: I.07. Data Analysis and Statistics

Support: 5R01NS047293

Title: Recording the 3D locations of EEG scalp electrodes using an ordinary cell phone camera

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Abstract: Growing evidence supports the need for individual subject scalp electrode locations to perform source-resolved EEG brain imaging. Using 3D head surface scanning to record these locations reduces the acquisition time compared to sequential position-measuring systems, thereby maximizing participant engagement during the recording. However, current (photogrammetric) 3D scanner systems and attachments may be comparatively expensive (>$1000) and may require bright, uniform lighting conditions. Here we tested advanced light-based 3D scanning technologies that are more affordable and less sensitive to lighting

conditions: (1) using a currently recommended 3D scanner (*Occipital Structure.io*); (2) using an iPhone (> v10) 'Face unlock' 3D camera; (3) using a more affordable (~$250) smartphone 3D-scanning attachment (*Creality Ferret*), and (4) using an ordinary smartphone camera to capture 2D images of the participant head surface, then combining them using available software to reconstruct a 3D head surface mesh. **Method**. We used each method to scan a mannequin head wearing a BioSemi EEG scalp cap three or more times. To determine scan quality, we quantified the resolution and noise levels of the 3D scans. As a measure of ***spatial resolution***, we used normalized mean edge length, the mean length of the head mesh edges normalized by the cube root of the mesh volume. This measure captures the average granularity of the 3D scan while considering the object scale. To quantify a ***local mesh noise*** score for the 3D head meshes, we first identified each four mesh nodes adjacent to each other (i.e., mesh neighborhood). We then applied Principal Component Analysis (PCA) to find the best-fitting plane. The distance of each node in the neighborhood to the plane was used to quantify the degree of local mesh irregularity. The standard deviation of these values, normalized by the mean edge length, provided a local noise score for the scan. **Results**. For all four methods, both measures were stable across repeated scans (within-scanner variations < 10% of resolution or noise score), and local mesh noise scores were comparable (0.12-0.19). *Ferret* scanner meshes had the best resolution (0.006, iPhone: 0.011; *Structure*: 0.020; smartphone: 0.020). Reducing the room light level showed the *Ferret* scanner and ordinary smartphone camera to be the least sensitive to the light level. **Conclusions.** The results suggest that affordable, state-of-art scanning - including 3D scans composed from standard 2D camera images - is a viable approach to EEG electrode 'digitization', in particular where cost, space, and/or lighting conditions limit the use of more elaborate and expensive photogrammetric methods.

Disclosures: S. Shirazi: None. **S. Makeig:** None.

Poster

PSTR446. Software Tools: Neurophysiology

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Program #/Poster #: PSTR446.05/XX54

Topic: I.07. Data Analysis and Statistics

Support: ACHRI
NSERC CREATE
NIH Grant P41 EB018783
Nvidia Corporation

Title: The SSVEP toolbox: a GPU-accelerated toolbox for SSVEP feature extraction

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Abstract: We present the Steady-State Visual Evoked Potential (SSVEP) Toolbox, an open-source, Python-based, multithreaded, and graphical processing unit (GPU)-accelerated data analysis toolbox for SSVEP feature extraction and classification. SSVEPs, the brain's response to flashing lights, are widely used in brain-computer interfaces (BCIs) for communication and control. Over the past 25 years, many methods have been developed to extract SSVEP features from EEG. The SSVEP Toolbox provides easy-to-use implementations of three feature extraction methods—filter bank canonical correlation analysis (fbCCA), minimum energy combination (MEC), and multivariate synchronization index (MSI). The toolbox was developed in Python 3.9.16. Validation was done using a classification analysis on the SSVEPEXO dataset (3 targets, 12 participants, 8 EEG channels, 256 Hz sampling rate). Classification accuracies for were compared to the classifier suggested in the dataset website (i.e., Riemannian geometry (RG) with logistic regression). The classification results for fbCCA (81.3 +/- 10%), MEC (74.7 +/- 13.5%), and MSI (75 +/- 17.4%) are on par or exceeding the RG classifier (72 +/- 12%). The CPU (2.89 +/- 0.03 sec) and GPU (2.34 +/- 0.03 sec) implementations had similar runtimes for the complete dataset. To further test the timing of the tool, the first two subjects of the Wang2016 dataset were used. This dataset was selected as it has a higher target and electrode count (i.e., 40 targets, and 64 channels). With the Wang2016 dataset, the GPU (3.43 +/- 0.11 sec) version vastly outperformed the CPU (70 +/- 1.21 sec) implementation. The SSVEP Toolbox implements CPU and GPU-accelerated versions of three families of SSVEP feature extraction methods and demonstrated their use in a classification analysis. The GPU outperforms the CPU implementation for the Wang2016 dataset. With further development and release to an open-source repository (e.g., GitHub), the toolbox could accelerate the development of SSVEP-based BCIs. We will optimize the remaining feature extractions methods for the GPU, validate the toolbox using multiple other datasets, demonstrate the use of the toolbox in ensemble classifiers, and integrate the toolbox with BCI2000 to enable GPU-accelerated real-time feature extraction of SSVEPs. Acknowledgments: We thank ACHRI, NVIDIA Corporation, NIH Grant P41 EB018783, Resources at the Stratton VA Medical Center, and NSERC CREATE for the funding of this work.

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Poster

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Program #/Poster #: PSTR446.06/XX55

Topic: I.07. Data Analysis and Statistics

Support: NSF 1840218
NSF 2114202
NIH 3U24EB029005-04S1

Title: Enabling Integrity and Metadata Provenance for Neuroscience Artifacts Using Open Science Chain

Authors: *S. SIVAGNANAM[1,3], S. SAKAI[2], K. LIN[1], F. GARZON[1], S. YEU[1], K. YOSHIMOTO[1], K. PRANTZALOS[4], A. MAJUMDAR[5], S. SAHOO[4], W. LYTTON[3]; [1]UCSD, La Jolla, CA; [2]San Diego Supercomputer Ctr., UCSD, San Diego, CA; [3]SUNY Downstate Hlth. Sci. Univ., Brooklyn, NY; [4]Case Western Univ., Columbus, OH; [5]Univ. of California San Diego, LA JOLLA, CA

Abstract: Neuroscience generates vast amounts of experimental and imaging data in various formats, which play a crucial role in developing data-driven computational models to understand neuronal and network functions. Researchers working with these extensive multimodal datasets need techniques to maintain data integrity, especially when building upon prior research conducted by other scientists. When scientific datasets evolve or are reused to create derived datasets, it is crucial to securely preserve the integrity, metadata, and provenance to prevent unintended or malicious alterations during the process. Open Science Chain (OSC) provides a cyberinfrastructure platform where the integrity information about scientific dataset is stored and managed in a consortium blockchain. Other researchers can independently verify authenticity of scientific data using the information stored in the blockchain and provide feedback when the data cannot be validated. OSC allows researchers to store the cryptographic hash of the data as a manifest in the blockchain along with the metadata. When updates are made to a dataset or data collection in OSC, all metadata changes, including the SHA256 checksum for every file in that data collection, are tracked in the blockchain, enabling users to view a detailed, immutable history of that dataset over time. Neuroscience researchers, especially those involved in collaborative research will benefit from using the OSC to track the dataset that maybe generated and maintained at various locations. Members of research labs will benefit from tracking the provenance of data produced and referenced during different stages of research by various members. OSC aims to enhance data sharing and reproducibility in the neuroscience community by increasing confidence in the scientific results. API-based integrity services are being developed to generate and verify the integrity information of scientific data for data-driven research platforms and hubs. The poster presentation will discuss the integration of OSC with Neuroscience Gateway to capture the provenance metadata corresponding to the components for reproducibility.

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Poster

PSTR446. Software Tools: Neurophysiology

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Program #/Poster #: PSTR446.07/XX56

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant NS033310
NIH Grant NS100064
NIH Grant NS104116
NIH Grant NS131108

Title: An approach for reliably identifying high-frequency oscillations and reducing false positive detections

Authors: Y. ZHOU[1], J. YOU[1], U. KUMAR[2], X. TAO[1], S. A. WEISS[6], A. BRAGIN[2,3], J. ENGEL[2,4,3,5], C. PAPADELIS[7], ***L. LI**[1,2];
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Abstract: Aiming to improve the feasibility and reliability of using high-frequency oscillations (HFOs) for translational studies of epilepsy, we present a pipeline with features specifically designed to reject false positives for HFOs to improve the automatic HFO detector.We presented an integrated, multi-layered procedure capable of automatically rejecting HFOs from a variety of common false positives, such as motion, background signals, and sharp transients. This method utilizes a Time-Frequency Contour approach that embeds three different layers including peak constraints, power thresholds, and morphological identification to discard false positives. Four experts were involved in rating detected HFO events that were randomly selected from different posttraumatic epilepsy (PTE) animals for a comprehensive evaluation.The algorithm was run on 768 hours recordings of intracranial electrodes in 48 PTE animals. A total of 453,917 HFOs were identified by initial HFO detection, of which 450,917 were implemented for HFO refinement and 203,531 events were retained. Radom sampling was used to evaluate the performance of the detector. The HFO detection yielded an overall accuracy of 0.95±0.03, with precision, recall, and F1 scores of 0.92±0.05, 0.99±0.01, and 0.94±0.03, respectively. For the HFO classification, our algorithm obtained an accuracy of 0.97±0.02. For the inter-rater reliability of algorithm evaluation, the agreement among four experts was 0.94±0.03 for HFO detection and 0.85±0.04 for HFO classification.Our approach shows that a segregated pipeline design with a focus on false positives rejection can improve the detection efficiency and provide reliable results. This pipeline does not require customization and uses fixed parameters, making it highly feasible and translatable for basic and clinical applications of epilepsy.

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Poster

PSTR446. Software Tools: Neurophysiology

Location: WCC Halls A-C

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Program #/Poster #: PSTR446.08/XX57

Topic: I.07. Data Analysis and Statistics

Title: Solving the spike sorting problem with Kilosort

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Abstract: Spike sorting is the computational process of extracting the firing times of single neurons from recordings of local electrical fields. This is an important but hard problem in neuroscience, complicated by the nonstationarity of the recordings and the dense overlap in electrical fields between nearby neurons. To solve the spike sorting problem, we have continuously developed over the past eight years a framework known as Kilosort. This paper describes the various algorithmic steps introduced in different versions of Kilosort. We also report the development of Kilosort4, a new version with substantially improved performance due to new clustering algorithms inspired by graph-based approaches. To test the performance of Kilosort, we developed a realistic simulation framework which uses densely sampled electrical fields from real experiments to generate non-stationary spike waveforms and realistic noise. We find that nearly all versions of Kilosort outperform other algorithms on a variety of simulated conditions, and Kilosort4 performs best in all cases, correctly identifying even neurons with low amplitudes and small spatial extents in high drift conditions.

Disclosures: M. Pachitariu: None. **S. Sridhar:** None. **J. Pennington:** None. **C. Stringer:** None.

Poster

PSTR446. Software Tools: Neurophysiology

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Program #/Poster #: PSTR446.09/XX58

Topic: I.07. Data Analysis and Statistics

Support: Labex Cortex Grant

Title: Establishment of an optimized and automated workflow for whole brain probing of neuronal activity patterns in TRAP mice

Authors: ***S. CABRERA**[1,2,3,4], R. MARCIANO MACIEL[3], N. VACHOUD[2], S. DESMERCIERES[2], M. BREUILLY[5], G. MEYER-DILHET[6], S. ELLOUZE[2,6], J. COURCHET[6], P.-H. LUPPI[3], N. MANDAIRON[4], O. RAINETEAU[2];
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Abstract: Aims: Behaviors are encoded by neural circuits that are widespread within the brain and change with age and experience. Immunodetection of the immediate early gene c-fos has been successfully used for decades to reveal neural circuits active during specific tasks or conditions. Our aims here is to develop a workflow that circumvents classical temporal and spatial limitations associated to c-fos quantifications.
Method: We used a new genetic TRAP method combining Cre-dependent tdTomato expression under c-fos promoter with Fos immunohistochemistry, allowing visualization and direct comparison of neural circuits activated at different times or during different tasks. By using open-source softwares (i.e. Qupath and Abba), we established a workflow that optimize and automate cell detection, cell classification (e.g. Fos vs. Fos/tdTomato) and whole brain registration.
Results: We demonstrate that this automatic workflow, based on fully automatic script, allows accurate cell number quantification with minimal interindividual variability. Further, interrogation of brain atlases at different scales (from simplified to detailed) was achieved allowing gradually zooming on brain regions to explore spatial distribution of activated cells. Then, we illustrated the potential of this approach by comparing patterns of neuronal activation in various contexts (e. g. different vigilance states, complex behavioral tasks ⋯). Finally, we explored programs (e.g. BrainRender) for intuitive representation of obtained results.
Conclusion: Altogether, this automated workflow allows a fast and accurate analysis of whole brain activity pattern at the cellular level, in various contexts.

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Poster

PSTR446. Software Tools: Neurophysiology

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Program #/Poster #: PSTR446.10/XX59

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant 5R24MH117295-05

Title: Dandi: an archive and collaboration space for neurophysiology projects

Authors: S. GHOSH[1], C. BAKER[2], R. CHOUDHURY[3], B. DICHTER[2], D. JARECKA[1], ***N. DEHGHANI**[1], H. IOANAS[4], D. LAMANNA[3], J. NESBITT[3], M. VANDENBRUGH[3], J.

WODDER[4], Y. O. HALCHENKO[4];
[1]MIT, Cambridge, MA; [2]Catalyst Neuro, Benicia, CA; [3]Kitware, New York, NY; [4]Dept. of Psychological and Brain Sci., Dartmouth Col., Hanover, NH

Abstract: DANDI (http://dandiarchive.org) is a cutting-edge platform for neurophysiology research. By serving as a multi-species data archive, a collaboration space, and a computational platform, DANDI enables researchers to access the resources they need to support both computational and experimental neuroscience.DANDI datasets use a consistent layout based on the BIDS standard and common metadata structures to promote easier comprehension of included data. The infrastructure of the platform is built using open-source technologies and is hosted in the cloud, available to researchers who wish to use the platform to search, visualize, compute, collaborate, and coordinate their neurophysiology research projects while promoting FAIRness and efficiency.As of May 2023, DANDI boasts an impressive archive of over 500+TB of data from more than 293 datasets, spanning multiple species (C elegans, zebrafish, mice, rats, fruit flies, non-human primates, and humans) and from multiple recording modalities. The archive includes intracellular, extracellular, and behavioral timeseries from electrophysiology, optophysiology, and optogenetic experiments, as well as multimodal MRI, OCT, SPIM, ECOG, and immunostaining data from human ex vivo brain tissue samples. With over 6000 experiment participants and 890+ registered users worldwide, DANDI is the largest neurophysiology archive available.DANDI is dedicated to making data and software for neurophysiology research more FAIR (Findability, Accessibility, Interoperability, and Reusability). The platform provides user-friendly tools for data submission and access, a Jupyter-based computation hub for raw data introspection, data analyses, and integration with other analytic platforms and services. DANDI uses the BRAIN Initiative community data formats and standards such as NWB and BIDS and works closely with the community to expand these standards to neurophysiology and microscopy. The platform has an application programming interface server to attract scientists/developers to interact with the archive programmatically and has contributed to community efforts to provide efficient access to larger datasets in the cloud.With DANDI, researchers can now share, collaborate on, and publish citable datasets, which will significantly increase the rigor and reproducibility of cellular neurophysiology research. DANDI is a powerful resource that is transforming the field of cellular neurophysiology research, enabling researchers to work together more effectively and efficiently, and ultimately driving groundbreaking discoveries that will advance our understanding of the brain.

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Poster

PSTR446. Software Tools: Neurophysiology

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Program #/Poster #: PSTR446.11/XX60

Topic: I.07. Data Analysis and Statistics

Support: Washington Research Foundation Postdoctoral Fellowship
Wellcome Trust
Simons Foundation
NIH Grant U19NS123716

Title: Automating multi-probe insertions to improve the efficiency and reproducibility of electrophysiology experiments

Authors: *K. J. YANG[1], D. BIRMAN[1], I. BRAIN LABORATORY[2], N. A. STEINMETZ[1]; [1]Univ. of Washington, Seattle, WA; [2]Intl. Brain Lab., Seattle, WA

Abstract: Achieving consistent targeting of multiple simultaneous probes during electrophysiology experiments is a challenging and time-consuming process. Even with a planned insertion trajectory, experimenters still have to go through a lengthy process of positioning and inserting each probe. Electrophysiology experiments are increasingly focused on brain-wide coverage, requiring three or more simultaneous probes motivating researchers to accelerate their processes to reduce the duration of the experiment and the corresponding stress levels of their subjects. To improve the efficiency and reproducibility of multi-probe electrophysiology experiments, we developed two frameworks: a communication platform to allow software control of hardware micro-manipulators and an automation platform to perform multiple synchronous probe insertions. Each existing manipulator platform has proprietary software for programmatic control, which are rarely cross-platform and often expose inconsistent interfaces. To standardize manipulator communication, we developed a Python server that acts as a generic cross-platform application programming interface (API). This platform ensures that client applications only need to interface with one API to be compatible with many different manipulator platforms connected across various computer operating systems. Building on top of this communication platform and an existing trajectory planning tool, Pinpoint, we next developed a system that automates the insertion process for multiple probes, saving time. The automation system provides three guarantees for researchers: first, that probes will reach their intended targets without manually-introduced errors in targeting; second, that experiments can be repeated exactly to improve reproducibility; and third, that movement speeds are limited to low levels for reduced tissue damage. Because our software drives multiple probes simultaneously, complex multi-probe insertions are more manageable. Taken together, these open-source tools for communicating with hardware manipulators and automating multi-probe insertions enable the next generation of reproducible, high-efficiency, brain-wide electrophysiology data collection.

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Poster

PSTR446. Software Tools: Neurophysiology

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Program #/Poster #: PSTR446.12/XX61

Topic: I.07. Data Analysis and Statistics

Support: NIH NINDS U01NS117765

Title: Intraoperative refinement of human Neuropixels recordings

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Abstract: Recent work has demonstrated the feasibility of intraoperative use of Neuropixels probes to obtain high-density recordings of single-unit activity in human cortex. These data promise large scale studies of the fundamental computational units underlying complex human behaviors like speech and language processing. However, data acquisition in operating rooms poses a set of unique challenges due to the clinical constraints placed on the recording site and duration. Unlike the deployment of Neuropixels probes in model organisms, intraoperative recordings must be completed in a single session, within the span of 15-20 minutes. Furthermore, probes are placed within the pre-identified resection margins, and only a subset of this tissue may have intact function, let alone task-relevant activity. Accordingly, it is crucial to the yield of these experiments to be able to rapidly assess the quality of a recording site within the first few minutes of insertion so that a new site and/or task may be selected if the observed task-relevant activity in the tissue is poor. We present here a cross-platform, open-source application for real-time processing and visualization of data from Neuropixels probes using the latest version of the C++ API for SpikeGLX. With support for simultaneous recording from multiple probes, this toolset includes a map of all spiking activity over the length of each probe, spike-waveform displays for each channel, and event-triggered rasters and peristimulus time histograms for each channel that can be time-locked to a variety of custom event subtypes. Although development of this application was motivated by our group's intraoperative visualization needs, the functionality it offers is likely to be useful for guiding and troubleshooting recordings in more general contexts. The real-time spike map, in particular, will enable experimenters to make three valuable estimates at a glance: the overall level of activity in a region, the anatomical positioning (inferred from changes in spiking patterns along the shank), and the extent of tissue motion relative to the shank.

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Poster

PSTR446. Software Tools: Neurophysiology

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Topic: I.07. Data Analysis and Statistics

Support: NIH Grant R24MH120037
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Title: Sharing analysis-ready human electrophysiology data on NEMAR/OpenNeuro using Hierarchical Event Descriptors (HED)

Authors: *S. MAKEIG[1,2], A. MAJUMDAR[3], A. DELORME[4], D. TRUONG[4], K. ROBBINS[5]; [1]Swartz Ctr. for Computat. Neurosci., La Jolla, CA; [2]Inst. for Neural Computation, [3]San Diego Supercomputer Ctr., [4]Swartz Ctr. for Computat. Neurosci., Univ. of California San Diego, La Jolla, CA; [5]Univ. of Texas at San Antonio, San Antonio, TX

Abstract: As ML/AI tools increasingly show the potential of finding and exploiting hitherto hidden structure in large collections of diverse data, NIH and other research funding agencies are increasing requirements, standards, and infrastructure for freely sharing experiment data in facilities that allow (1) search for data of interest that is stored in an analysis-ready format, (2) tools for analyzing the search-identified data, and (3) ready access to sufficient compute power to perform advanced analyses of both small and large data collections. Further, (4) analysis of very large data collections should avoid the need to download the data. We propose the term 'Integrated Data, Tools, and Compute Resource' (datcor) for such facilities and here describe NEMAR.org, a portal to shared human 'neuroelectromagnetic' (NEM) brain data (EEG, MEG, iEEG) shared publicly through OpenNeuro.org. NEMAR stores a copy of the growing number of OpenNeuro NEM datasets (currently >200 and >20TB, with >10,000 participants) and now offers data search, quality estimates, and visualizations. It also enables search-identified data to be analyzed without need for download using the high-performance computing resources of the Neuroscience Gateway (nsgportal.org). NEMAR/OpenNeuro datasets are stored in BIDS formats. Analysis-ready data requires detailed descriptions of experiment events using a common language. NEMAR and BIDS use the system of Hierarchical Event Descriptors (HED) under development since 2011 (hedtags.org). Here we demonstrate use of NEMAR and HED for creating, sharing, discovering, and analyzing NEM data.

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Poster

PSTR446. Software Tools: Neurophysiology

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Topic: I.07. Data Analysis and Statistics

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Title: Automating the modeling of single cell neurons

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Abstract: Neurons perform synaptic integration of the stimuli they receive on their synapses using a complex set of intrinsic conductances that seemingly work in coordinated groups. However, prevalent single cell modeling techniques do not consider such potential interactions. A recent paper suggesting possible grouping of currents into distinct modules associated with specific neurocomputational properties suggests that the process of selection of model parameters could be simplified (Alturki et al., 2016). That hypothesis led to the development of design methodology that simplified the biophysical conductance-based cell design process by allowing the designer to focus on specific attributes of the cell one at a time: starting with passive properties, moving to baseline firing dynamics, then adding features such as burst firing and low- and high-threshold oscillations. By separating the cell design process into these stages, parameter selection can be simplified due to decreased interaction between the distinct current modules. The present study uses these findings to build an automatic optimization pipeline termed Automatic Cell Tuner (ACT) that generates conductance estimates from neurons' biological data. The pipeline is simulation-based; it trains a predictive model on voltage data simulated by a black-box simulator to estimate conductance values which could have generated external target data. Designed with flexibility in mind, it allows for usage of arbitrary models and feature generation. Our default implementation incorporates a shallow neural network to take advantage of fast gradient-based optimization. We embed the segregation approach by applying the ACT to voltage regions specified in the approach. We utilize the hierarchical current structure of the regions, i.e., that each new region introduces a new current set active for all further regions, to sequentially estimate conductance values in a compartmentalized manner. Preliminary results on regular spiking and bursting cells showed the segregation method outperforms estimation on the full voltage trace in terms of our composite quality metric. We are presently extending the methodology to complex multicompartment cells.

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Poster

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Topic: I.07. Data Analysis and Statistics

Support: NIMH Grant 5R24MH120037-04

Title: Nemar: an open access data, tools and compute resource operating on neuroelectromagnetic data

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Abstract: To preserve scientific data created by publicly and/or philanthropically funded research projects and to make it ready for exploitation using recent and ongoing advances in advanced and large-scale computational modeling methods, publicly available data must use in common, now-evolving standards for formatting, identifying and annotating should share data. The OpenNeuro.org archive, built first as a repository for magnetic resonance imaging data based on the Brain Imaging Data Structure formatting standards, aims to house and share all types of human neuroimaging data. Here, we present NEMAR.org, a web gateway to OpenNeuro data for human neuroelectromagnetic data. NEMAR allows users to search through, visually explore and assess the quality of shared electroencephalography (EEG), magnetoencephalography and intracranial EEG data and then to directly process selected data using high-performance computing resources of the San Diego Supercomputer Center via the Neuroscience Gateway (nsgportal.org, NSG), a freely available web portal to high-performance computing serving a variety of neuroscientific analysis environments and tools. Combined, OpenNeuro, NEMAR and NSG form an efficient, integrated data, tools and compute resource for human neuroimaging data analysis and meta-analysis.

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Poster

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Title: Robust registration of high-density electrophysiology data with DREDge

Authors: *C. WINDOLF[1], A. PAULK[5], Y. KFIR[6], E. TRAUTMANN[2], S. GARCIA[7], D. MESZENA[8], W. MUÑOZ[9], R. HARDSTONE[10], I. CAPRARA[12], M. JAMALI[13], J. BOUSSARD[3], Z. WILLIAMS[14], S. S. CASH[11], L. PANINSKI[4], E. VAROL[15];
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Abstract: High-density electrophysiology probes have opened new possibilities for systems neuroscience in human and non-human animals, but probe motion (or drift) while recording poses a challenge for downstream analyses, particularly in human recordings. In the action potential (AP) band, probe motion can cause neurons to drift across channels, and similar drift occurs for the potentials studied in the local field potential (LFP) band, so that motion estimation is a critical preprocessing step which enables downstream analyses to account for and control the possible confounding effects of the relative motion of the probe and brain tissue. Here, we improve on the state of the art for tracking this drift with an algorithm termed DREDGe (Decentralized Registration of Electrophysiology Data), with four major contributions. First, we extend previous decentralized methods to operate within and across frequency bands, leveraging the LFP in addition to spikes detected in the AP band. Second, we show that the LFP-based approach enables registration at sub-second temporal resolution. Third, we introduce a faster than real-time online motion tracking algorithm, allowing the method to scale up to longer and higher resolution recordings, which could facilitate real-time applications. Finally, we improve the robustness of the approach by accounting for the nonstationarities and artifacts that occur in real data and by automating parameter selection. These findings are robustly validated in large scale experiments in humans, mice, and non-human primates (NHPs), including repeated validation and comparison against competing algorithms in tens of mouse and human datasets and a proof-of-concept demonstration in NHP of a new method enabled by DREDge: localization of the probe during insertion.

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Poster

PSTR446. Software Tools: Neurophysiology

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NIH 1R01HL144071 (LFS)
NIH 1OT2OD026580 (LFS)
NIH 1K01NS124828 (TNG)

Title: Sanpy: an open-source whole-cell electrophysiology analysis pipeline

Authors: *R. H. CUDMORE, L. GUARINA, J. T. LE, T. N. GRIFFITH, L. F. SANTANA; Physiol. and Membrane Biol., Univ. of California - Davis, Davis, CA

Abstract: Whole-cell recording is a gold-standard technique for interrogating the biophysical properties and mechanisms of single cell function. With continued advances in genetic, pharmacological, and computational tools, the utility of this technique will continue to grow well into the future. A major bottleneck in the interpretation of this critical data is the lack of community based open-source software tools for its analysis. Here, we present SanPy, a Python-based open-source and freely available software pipeline for the analysis and exploration of whole-cell current- and voltage- clamp recordings. SanPy provides a computational engine with an application programming interface allowing it to be programmatically scripted. Using this engine, we have developed a cross-platform desktop graphical user interface that does not require programming experience. A key benefit of SanPy is that it provides one-click downloads of this desktop application. SanPy is designed to extract parameters from current-clamp recordings of action potentials including threshold time and voltage, peak, half-width, and interval statistics. In addition, SanPy extracts parameters from a range of voltage-clamp protocols. SanPy is built to be fully extensible by providing frameworks for the addition of custom file loaders, analysis, and graphical user interface plugins. A key feature of SanPy is its focus on quality control and data exploration. In the desktop interface, all plots of the data and analysis are linked allowing simultaneous data visualization from different dimensions with the goal of obtaining ground truth analysis. We provide documentation for all aspects of SanPy including several use cases and examples. To test SanPy, we have performed analysis on current- and voltage- clamp recordings from neurons and cardiac myocytes. Taken together, SanPy is a powerful tool for whole-cell analysis and effectively lays the foundation for future extension by the scientific community.

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Poster

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Support: NIH National Institute of General Medical Sciences grant R01GM134363-01
The Tiny Blue Dot Foundation, Templeton World Charity Foundation, Allen Institute

Title: Novel parameterization of event-related potentials: a step towards characterizing the biophysical origins

Authors: *M. PRESTON[1], D. CELLIER[2], E. KOSIK[2], P. SEYFOURIAN[5], L. CLAAR[6], L. MARKS[6], C. KOCH[6], I. REMBADO[6], B. VOYTEK[2,1,3,4];
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Abstract: Event-related potentials (ERPs) are foundational in linking human electroencephalography (EEG) to cognition and disease. For decades, systematic variation in ERP timing and amplitude have been linked to various perceptual and cognitive states, and have been leveraged in clinical applications ranging from anesthesia to brain-computer interfaces. Despite their wide adoption and utility, the biophysiological origins of ERPs remain largely unknown. Here, we test the hypothesis that early visual evoked potentials are driven by thalamic excitatory projections to input layers of primary visual cortex (V1). We make use of large scale single-unit and local field potentials (LFP) recordings using Neuropixels probes in head-fixed mice targeting the visual thalamic nuclei and V1, in conjunction with biophysically informed models. We also introduce a novel ERP parameterization approach that quantifies each ERP waveform in terms of its onset, amplitude, time-to-peak, duration, and peak-sharpness. Using simulated LFP and our novel ERP parameterization approach, we demonstrate that visual evoked potentials are driven by thalamocortical excitatory postsynaptic currents (EPSPs). These currents, which have a sharp temporal onset and slower decay, integrate such that when thalamic spikes are temporally aligned, the resulting V1 ERP is sharper and has a larger magnitude. When thalamic spikes are more temporally variable, the resulting EPSPs in V1 integrate into a slightly smoother ERP. These results further our understanding of the ERP origins and their biophysical interpretability.

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Poster

PSTR446. Software Tools: Neurophysiology

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Topic: I.07. Data Analysis and Statistics

Support: NSF Neuronex
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Title: Decodanda: a Python toolbox for decoding and geometrical analysis of neural data.

Authors: *L. POSANI, S. FUSI;
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Abstract: Neural decoding is a powerful tool to obtain insight into which variables of the external world are represented in the activity of a population of neurons, with broad applicability that spans from basic research to clinical applications such as brain-computer interfaces. Additionally, neural decoding has recently emerged as a tool to unveil the geometrical properties of neural representations, offering computational insights into the collective activity of neurons. Here we introduce Decodanda (https://www.github.com/lposani/decodanda), a Python toolbox for geometrical decoding of population activity. Decodanda exposes a series of functions for decoding and geometrical analysis of neural data, such as CCGP and parallel score, and automates essential data management and balancing practices such as trial-based cross-validation, a null model for statistical significance, pseudo-population pooling, and cross-variable balancing. Decodanda is agnostic to the specific classifier used for decoding, and its APIs are designed to expose a user-friendly and highly customizable syntax that allows researchers to build custom analysis pipelines using its functions as building blocks. Here, we give an overview of the design principles of Decodanda and its use cases in neuroscience research.

Disclosures: L. Posani: None. **S. Fusi:** None.

Poster

PSTR446. Software Tools: Neurophysiology

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Topic: I.07. Data Analysis and Statistics

Support: NIMH Grant MH114678

Title: Neuroscience Data Interface calculators: a motif for writing small analysis applets that are easy to learn, reuse, and test

Authors: *A. LEPSKY, R. WANG, X. CHENG, N. LASKY-NIELSON, R. GONG, C. JOHNSON, R. RODRIGUEZ, S. D. VAN HOOSER;
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Abstract: Advancements in data-driven fields such as neuroscience depend critically on small custom analysis programs. Many of these programs are developed in individual labs and suffer from minimal and narrowly scoped testing, poor documentation, and a dependency on particular file formats or data organization. Even when they are shared openly on sites such as GitHub, these programs can be difficult to reuse due to the issues above. To address this challenge, we have developed an applet motif for the Neuroscience Data Interface (NDI) called calculators. NDI provides a platform-independent, searchable database of documents containing all raw data and analyzed data for an experiment. Calculators access these databases and search for document types that they can operate on, eliminating the need to wire or pipe calculators together. In order to make them intuitive for new users, calculators produce only one type of output document, contain documentation in specific places, and override only 4 functions while leaving the rest as is. Importantly, calculators also must be able to generate several self-test cases, evaluate that the results produced are within tolerance of the expected results, and, if the user requests, show the actual and expected results graphically. The calculator motif allows users to quickly understand and trust calculators written by labs or groups. Users can then integrate these community-tested programs into analysis pipelines - sets of calculator objects along with any modifying parameters of calculator operations. We show examples of calculators for the visual system that compute tuning curves for orientation, direction, contrast, spatial, temporal frequency, and speed.

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Poster

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Title: Universal Renderer for Neuroscience: interactive 3D rendering for electrophysiology and neuroimaging in the browser, on desktops, and in virtual reality

Authors: *D. BIRMAN[1], J. SCHOCH[1], I. BRAIN LABORATORY[2], N. A. STEINMETZ[1]; [1]Univ. of Washington, Seattle, WA; [2]Virtual Entity, Virtual, United Kingdom

Abstract: Modern large-scale electrophysiology and neuroimaging techniques are generating unprecedented amounts of data. Exploring these data in their original anatomical context depends on the existence of easy-to-use and powerful 3D visualization tools. To solve this problem, we have developed an open-source software package, the Universal Renderer Creating

Helpful Images for Neuroscience (Urchin), that can visualize anatomically registered data from a variety of input sources in the space of common reference atlases. Our rendering package is platform-agnostic, working equally well as a standalone desktop application, in a virtual reality headset, or as a website. When running in a web browser, the renderer requires no installation and allows users to build complex 3D renderings in seconds. Users send data to the renderer through an application programming interface (API), using simple commands to pass information about their 2D or 3D scene. The visuals that the renderer can create include: brain regions rendered as opaque or transparent 3D objects, simple or complex 3D models such as spheres to represent neurons, probes, or neuron morphology, videos of neuroimaging data projected onto 3D surfaces, volumetric data such as MRI images, and 2D accents such as lines and text. Visualizations can be exported as high quality static images or videos from one or more camera angles. Unlike existing rendering packages intended for neuroscience, our software is interactive and allows users to explore their data in 3D space using keyboard and mouse interactions, or in virtual reality. This interactivity is not limited to simple scenes: by taking advantage of a powerful existing video game engine (Unity), our 3D renderer can support extraordinarily complex interactive scenes and at 60 hz we are able to display real-time firing rate data from up to 100,000 neurons overlaid on hundreds of individual brain regions. To demonstrate the power of Urchin we have developed several applications using the renderer, including a virtual reality experience in which participants explore a 30,000 neuron electrophysiology dataset, an online data viewer where users can explore 2D and 3D views of the mouse brain with per-region analysis results overlaid, and other interactive data viewers. Urchin makes it possible for neuroscientists to build powerful interactive explorations of their three-dimensional datasets with minimal effort and to share these easily with colleagues over the internet.

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Poster

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Title: Methods for semi-automated parcellation of the globus pallidus internus for localizing neurophysiological activity and deep brain stimulation leads

Authors: *B. POBIEL[1], K. J. O'NEILL, III[2], R. PATRIAT[3], T. PALNITKAR[2], H. BRAUN[4], O. SOLOMON[2], S. ALBERICO[2], M. HILL[2], B. MOHANTY[3], D. L. BAUER[3], B. PARKS[5], J. VITEK[2], N. HAREL[6], J. E. AMAN[3];
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Abstract: Background: Historically, atlas-based stereotactic coordinates or qualitative 2D brain slices have been used to localize sites of neurophysiological activity or deep brain stimulation (DBS) leads within the internal segment of the globus pallidus (GPi). These approaches have limited spatial resolution and lack patient specificity. This project creates a reproducible, semi-automated method for parcellating the GPi in Cartesian 3D space using models previously generated by our team. This approach allows comparison across patients and can be used to generalize findings relating to localizing DBS leads and pathophysiological biomarkers in Parkinson's disease (PD) and dystonia patients.
Methods: In 3DSlicer™ an axial AC/PC plane is constructed from the anterior/posterior commissure (AC/PC). The segmented GPi volume is exported, and a C++ script generates a least-volume, axis-agnostic bounding box around GPi. The GPi volume, bounding box, AC/PC plane, and reconstructed lead and/or microelectrode positions are imported into Unity™. The AC/PC plane is fixed to an XY plane of a new coordinate system. The GPi bounding box is used to determine a longitudinal axis of the GPi and the axis is used to transform the GPi to the XY and ZY planes. A new plane-aligned bounding box is created and divided into dorsal/ventral aspects and anterior/central/posterior thirds. A series of planes are generated along the longitudinal axis and the centroid of the GPi is found in each plane to divide the GPi into mesial/lateral halves, creating 12 total subsections (e.g., posterior dorsolateral). Using collision detection, the intersections of lead contact positions and microelectrode sites are determined algorithmically and compared with patient-specific neurophysiology and/or clinical outcomes.
Results: One patient has been studied using this method. The segmented lead position was determined quantitatively to fall within the central portion of the GPi, with the therapeutic stimulation contact in the dorsal region spanning the mesial-lateral midline, facing in the posterior direction. The patient showed a 59% improvement in their total UPDRS-III score from this active contact location.
Conclusion: This methodology will be expanded to a larger study designed to localize DBS leads and distribution of neurophysiological activity in the GPi. Correlating these data with clinical outcomes in PD and dystonia will help determine optimal locations for lead placement and for localizing phenotype-specific biomarkers.

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Poster

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Topic: I.07. Data Analysis and Statistics

Support: 1R24MH117295-01A1
Kavli Foundation

Title: Simplifying the conversion of neurophysiology data to NeurodataWithoutBorders format

Authors: G. FLYNN[1], ***C. BAKER**[2], R. LY[3], O. RUEBEL[4], B. DICHTER[5];
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Abstract: Neurodata Without Borders (NWB) is a data standard that packages neurophysiology data with the metadata necessary for reanalysis. The NWB format allows data to be human- and machine-readable, and will enable data to be aggregated across many labs. The neuroscientists who want to use NWB have diverse data formats, and many of them have limited programming experience. To approach this problem, we have developed the NWB Graphical User Interface for Data Entry (GUIDE) to provide a simple entrypoint to the NWB ecosystem for any lab interested in adopting the standard.
Researchers must often convert their data from common proprietary formats such as Intan, SpikeGLX, TIFF, ABF, etc. It can be challenging to map these different data formats, each with its own unique structure, to NWB. This challenge has been overcome with the use of NeuroConv, a library for automatically handling the data mapping for 40+ proprietary formats spanning the modalities of intra- and extra-cellular electrophysiology, optical imaging and behavior. However, the use of NeuroConv requires experience with Python and is poorly suited for potential users that are unfamiliar with programming.
This initial release of NWB GUIDE is a cross-platform desktop application that walks users through all the requirements for converting their data to the NWB format and uploading datasets to the DANDI Archive. NWB GUIDE streamlines the data conversion experience, walking users through input file specification, metadata extraction and curation, efficient handling of large datasets, and finally uploading to the DANDI Archive. We look forward to working with members of the NWB community to test and improve the platform based on user needs and feedback.

Disclosures: G. Flynn: None. **C. Baker:** None. **R. Ly:** None. **O. Ruebel:** None. **B. Dichter:** None.

Poster

PSTR446. Software Tools: Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR446.24/XX73

Topic: I.07. Data Analysis and Statistics

Support: NIH U24NS120057
The Kavli Foundation

Title: Advancements in the Neurodata Without Borders Software Ecosystem for Neurophysiology

Authors: *R. LY[1], C. BAKER[2], M. AVAYLON[1], G. FLYNN[2], B. DICHTER[2], O. RUEBEL[1]; [1]Lawrence Berkeley Natl. Lab., Berkeley, CA; [2]CatalystNeuro, Benicia, CA

Abstract: The Neurodata Without Borders (NWB) format standardizes how neurophysiology data and associated metadata are stored, and the NWB software enables researchers to easily access and save data in NWB. By standardizing neurophysiology data, NWB enables researchers to more fully extract return-on-investment from neurophysiology experiments. To lower the barrier of adopting NWB, we have made significant enhancements to the core NWB software tools, supported the integration of NWB with a wide array of powerful tools and technologies, and expanded NWB training efforts to reach a broader international neuroscience community.
We have enhanced PyNWB to allow users to read and write NWB data in Zarr, a modern, cloud-efficient storage format for chunked, compressed, N-dimensional arrays. To ensure FAIR data use, we have added support in NWB for linking neurophysiology metadata with persistent identifiers in external resources, such as ontologies and online databases. We have made enhancements to NeuroConv, a software suite for converting neurophysiology data from 40 popular raw acquisition data formats to NWB, and to NWB Inspector, a tool for assessing the quality of NWB files based on compliance with NWB Best Practices. Most recently, we have developed the NWB Graphical User Interface for Data Entry (GUIDE), an application that walks users through the process of converting raw data to NWB, without writing any code.
The NWB software ecosystem has grown to include support for NWB in tools for pose estimation and tracking (DeepLabCut, SLEAP), intracellular electrophysiology (PatchView), optical physiology (EXTRACT), data analysis (Pynapple, Spyglass), and data acquisition (OpenEphys). In addition, the DANDI Archive for neurophysiology now contains over 100 open datasets from a diversity of data modalities, model animals, and labs in the NWB format. We will describe several projects that make use of published NWB data on DANDI and our efforts to train and support neuroscientists from around the world to reuse NWB data.

Disclosures: R. Ly: None. **C. Baker:** None. **M. Avaylon:** None. **G. Flynn:** None. **B. Dichter:** None. **O. Ruebel:** None.

Poster

PSTR446. Software Tools: Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR446.25/XX74

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

Support: NIH R00MH116100

Title: Cortical gamma-beta rhythms perform predictive routing via stochastic reinforcement learning

Authors: *H. NEJAT[1], J. SHERFEY[2], A. BASTOS[1];
[1]Psychology, Vanderbilt Univ., Nashville, TN; [2]Psychological and Brain Sci., Boston Univ., Boston, MA

Abstract: Predictive coding is a key theoretical framework in neuroscience. Deficits in prediction are associated with mental disorders such as schizophrenia and major depressive disorder. Recent studies have revealed a dynamic shift between beta and gamma bands in cortical circuits during predictable and unpredictable tasks. This suggests a gamma [40-90Hz]/beta[10-30Hz] push-pull mechanism for suppressing unpredictable stimuli. We call this mechanism predictive routing. Current neuronal models of predictive coding/routing are incomplete. One class of models can be trained to perform computations but are not biophysically realistic. Another class of models focus on dynamics but do not perform computations. One solution to this limitation is to implement biophysically detailed neuronal models capable of learning. However, current models lack detailed neurobiological constraints and rely on manual tuning. To address these limitations, we introduce DynaLearn, an extension of DynaSim, which utilizes reinforcement learning to discover neurobiologically constrained solutions for optimization problems. In this study, we employed the semi-supervised learning algorithm known as the "Generalized Stochastic Delta Rule" to train DynaLearn on resting membrane potential, Pyramidal Interneuron Network Gamma/Beta (PING/PINB) network frequency tuning, and gamma-beta push-pull. Our findings demonstrate that learned top-down connection weights effectively reduce gamma strength while enhancing beta strength, enabling predictive routing. Therefore, computational modeling using DynaLearn/DynaSim can be used to understand both neuronal circuit mechanisms as well as the computations they perform.

Disclosures: H. Nejat: A. Employment/Salary (full or part-time):; Vanderbilt University. **J. Sherfey:** None. **A. Bastos:** A. Employment/Salary (full or part-time):; Vanderbilt University.

Poster

PSTR446. Software Tools: Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR446.26/XX75

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

Support: National Institute Of Neurological Disorders And Stroke, NIH, Award Number U24NS124001

Title: Tools for large-scale modeling, simulation and visualization of realistic biological networks: The SONATA Data format and integrated software

Authors: *K. DAI[1], B. ISRALEWITZ[2], X.-P. LIU[1], M. SPIVAK[2], S. ITO[1], N. REN[1], D. HAUFLER[1], E. TAJKHORSHID[2], A. ARKHIPOV[1];
[1]Allen Inst., Seattle, WA; [2]Beckman Inst. for Advanced Sci. and Technol., Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: With the emergence of large-scale experimental multi-area connectomic and extracellular electrophysiology recordings, there is an increasing need for detailed in-silico modeling and simulation on a matching scale. Yet much of the current research into neural dynamics of computation and behavior is still limited to the scale of individual cells or very small circuits. Biologically realistic simulatable network models ranging from thousands to billions of cells can elucidate the fundamental working of large-scale networks and drive future research. But for most neuroscientists working in network and systems neuroscience, trying to create, run, analyze, and share such large scale models can be an intractable technological hurdle. Here we present a set of tools and formats that can help scientists with the efforts in building and simulating large-scale biologically realistic network models. At the core of this effort is the SONATA data format, a multi-organization, open-source set of standards for describing and sharing large heterogeneous network models. With SONATA a scientist can describe their model, simulation parameters and results in a way that can be readily shared and reproduced. It is highly modular, easy to read and write, and can be used across a variety of model types ranging from multi-compartment, to point-neurons, and even rate models. SONATA has already been implemented across a range of software simulation and analysis software tools, including the Brain Modeling Toolkit (BMTK) and Visual Neuronal Dynamics (VND).
BMTK is an open-source python API for building and simulating large network models across different levels-of-resolutions. It provides an intuitive workflow including automated parallelization and an array of built-in features. VND is a C++ network visualization tool, with advanced data querying and rendering techniques to visualize and analyze large network models and simulations ranging from synaptic activity to whole-network spiking dynamics. By utilizing SONATA, both pieces of software can be used seamlessly for building, simulating, and analyzing large network models in an intuitive workflow.

Disclosures: K. Dai: None. **B. Isralewitz:** None. **X. Liu:** None. **M. Spivak:** None. **S. Ito:** None. **N. Ren:** None. **D. Haufler:** None. **E. Tajkhorshid:** None. **A. Arkhipov:** None.

Poster

PSTR446. Software Tools: Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR446.27/XX76

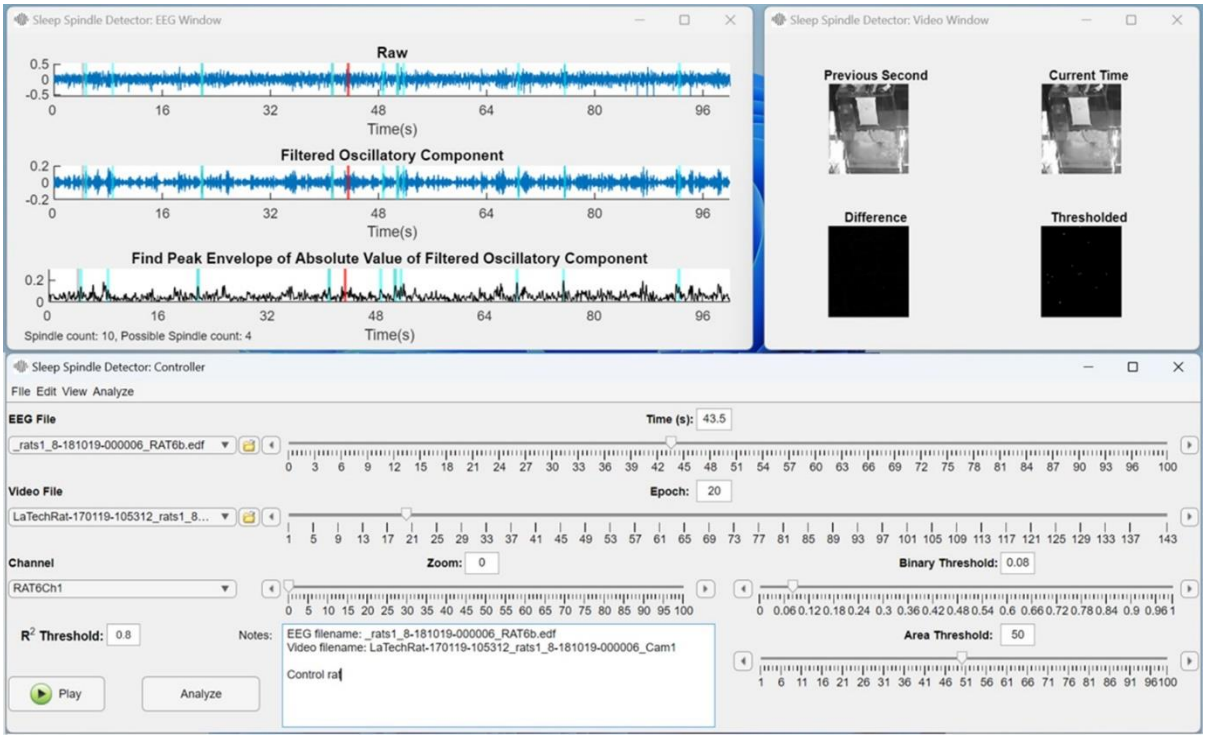
Topic: I.06. Computation, Modeling, and Simulation

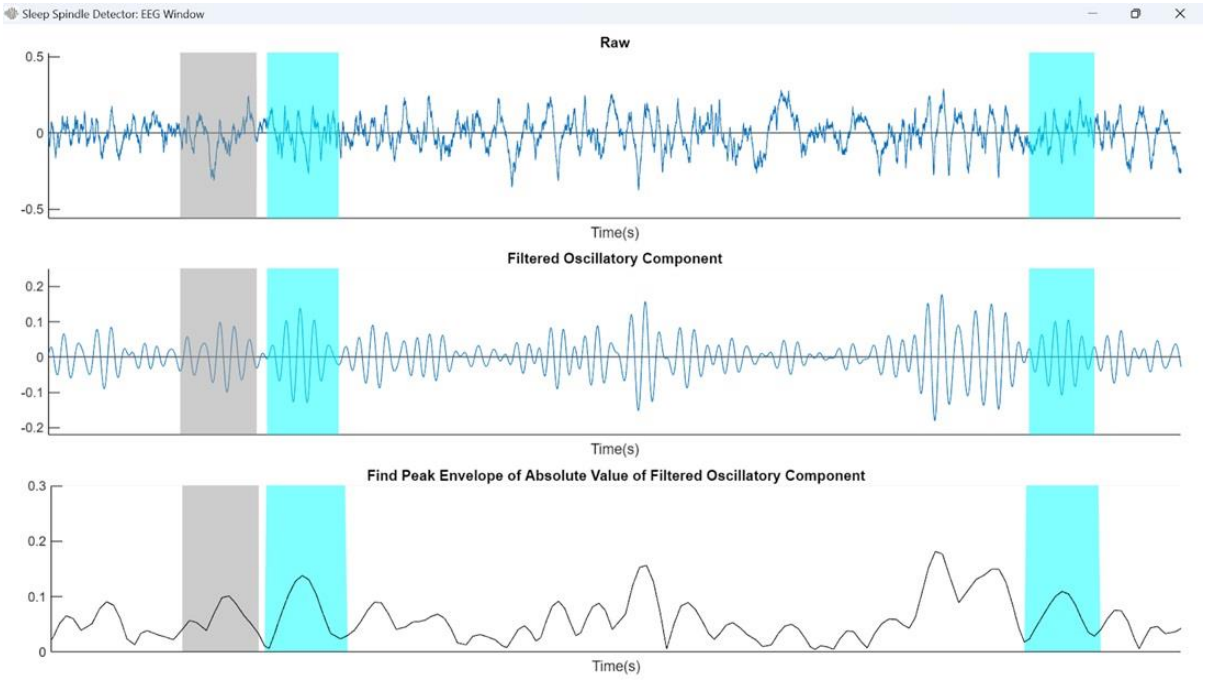
Support: NSF (EPSCoR RII-2 FEC OIA1632891)

Title: Automated rodent sleep spindle detector app

Authors: *K. HOLLY[1], A. KUMLER[2], P. DHUNGEL[2], S. RUDRASHETTY[2], S. VILLARRUBIA[2], J. MERTEN[3], A. KEMP[4], L. J. LARSON-PRIOR[5], T. A. MURRAY[2]; [1]MathWorks, Natick, MA; [2]Biomed. Engin., Louisiana Tech. Univ., Ruston, LA; [3]Col. of Med., [4]Departments of Psychiatry and Biomed. Informatics, Univ. of Arkansas for Med. Sci., Little Rock, AR; [5]Neurobio. and Developmental Sci., Univ. of Arkansas For Med. Sci., Little Rock, AR

Abstract:





A Rodent Sleep Spindle Detector application was developed to assist researchers working with high volume studies examining the impact of sleep on neurological function. Our Rodent Sleep Spindle Detector app is a MATLAB-based program with a user interface that automatically identifies sleep spindles within intracranial EEG (iEEG) recordings of rodents using two novel yet complementary algorithmic approaches. To validate the program, 6,000 real spindles of 5 different variations ranging from 11-17 Hz with a duration of at least 0.3 seconds were randomly placed within a noisy simulated prefrontal cortex iEEG signal with a duration of 50,000 seconds. When compared to the ground truth on a datapoint-by-datapoint basis, the program had an accuracy of 98.40±5.62% (mean±SD) with 95% C.I.[91.93,104.89] and 96.90±4.34% (mean±SD) with 95% C.I.[91.91, 101.90] for the primary and secondary algorithmic approach, respectively. On a holistic evaluation, the program had an accuracy of 93.68±13.66% (mean±SD) with 95% C.I.[81.71, 105.66] and 99.85±0.12% (mean±SD) with 95% C.I.[99.71, 99.96], for the primary and secondary algorithmic approach, respectively. The robustness of the sleep spindle detection was evaluated in additional validation tests by embedding artificial spindles at various durations, amplitudes, and frequencies in lieu of real spindles. Optionally, the app can process video recordings to identify periods of quiescence to improve sleep spindle detection.

Disclosures: **K. Holly:** A. Employment/Salary (full or part-time):; MathWorks. **A. Kumler:** None. **P. Dhungel:** None. **S. Rudrashetty:** None. **S. Villarrubia:** None. **J. Merten:** None. **A. Kemp:** None. **L.J. Larson-Prior:** None. **T.A. Murray:** None.

Poster

PSTR446. Software Tools: Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR446.28/XX77

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

Support: NINDS U24NS109043

Title: The Open Ephys GUI: A collaboratively developed platform for high-channel-count electrophysiology data acquisition

Authors: *A. DOSHI[1], P. KULIK[1], A. MUNK[2], A. LÓPEZ[3], J. VOIGTS[4], J. H. SIEGLE[1]; [1]Allen Inst. for Neural Dynamics, Seattle, WA; [2]Integrated Electronics and Biointerfaces Laboratory, UCSD, La Jolla, CA; [3]Open Ephys Production Site, Lisbon, Portugal; [4]HHMI Janelia Res. Campus, Ashburn, VA

Abstract: The Open Ephys GUI (https://open-ephys.org/gui) is an open-source, cross-platform application for acquiring data from multichannel implanted electrodes, a technique that has fostered countless discoveries in systems neuroscience. Unlike most other software in this domain, the GUI was designed from the ground up with collaborative development in mind.

Modules for real-time analysis and visualization are encapsulated within plugins that adhere to a standardized interface, providing a straightforward way for users to write their own extensions without needing to understand the entire code base. A growing number of scientists have developed their own plugins for the GUI, which had previously been stored in a variety of locations, typically in source-code form. With funding from a BRAIN Initiative U24 award, we have created a centralized repository for plugins, which can be downloaded and installed via a single click. Over the course of this award, we have curated, polished, and documented additional community-developed plugins in order to expand the capabilities of the software. Some examples of these plugins include Falcon Output, which streams high-channel-count data with low latency, Phase Calculator, which can be used to deliver closed-loop feedback at specific phases of an ongoing oscillation, and Ripple Detector, which emits events at the onset of hippocampal sharp-wave ripples. Priority has been given to plugins that can be used with Neuropixels, a new type of silicon probe capable of recording single units across dozens of cortical and subcortical structures simultaneously. Providing free, flexible, user-friendly, and performant software for recording spiking activity makes extracellular electrophysiology experiments more accessible to labs around the globe.

Disclosures: **A. Doshi:** None. **P. Kulik:** None. **A. Munk:** None. **A. López:** A. Employment/Salary (full or part-time):; Open Ephys Production Site. **J. Voigts:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Open Ephys, Incorporated. **J.H. Siegle:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Open Ephys, Incorporated.

Poster

PSTR446. Software Tools: Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR446.29/XX78

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

Support: NIH grant U24EB029005
NSF grant 1935771
NSF grant 1935749
NIH grant R24MH120037

Title: Neuroscience Gateway enabling software dissemination and large scale modeling and data processing

Authors: *A. MAJUMDAR[1], S. SIVAGNANAM[1], K. YOSHIMOTO[2], N. CARNEVALE[3], M. KANDES[2], S. YEU[2], D. CHOI[2];
[1]Univ. of California San Diego, LA JOLLA, CA; [2]Univ. of California San Diego, La Jolla, CA; [3]Yale Univ., New Haven, CT

Abstract: For about a decade the Neuroscience Gateway (NSG) has been enabling the broader neuroscience community of computational neuroscientists, experimental neuroscientists, cognitive neuroscientists, psychologists, biomedical researchers, and clinical researchers to easily utilize high performance computing (HPC), high throughput computing (HTC) and cloud computing resources for large scale modeling, data processing, and Artificial Intelligence/Machine Learning (AI/ML) work. NSG provides close to 20 neuroscience modeling, data processing and AI/ML software and tools optimally made available on supercomputer resources at national supercomputer centers in the US. It currently has over 1,550 users and has acquired 27 million supercomputer-time allocation for 2023 for its users. Via NSG's easy to use web-based and programmatic interface neuroscientists can upload input file or data to the NSG, choose the appropriate tool, specify the tool and computing related parameters and submit the computing workload to a supercomputer. Upon completion of the processing, users can download the output results. NSG eliminates administrative and technical barriers to utilize supercomputing resources for modeling, data processing and data analytics work in neuroscience. NSG also provides a software developer platform which neuroscience software developers can use to test, benchmark and scale their software and then release the software on supercomputers where NSG acquires yearly allocation for the neuroscience community. NSG provides a platform for dissemination of neuroscience software. NSG is free and open to any neuroscientist researchers and students from US and other countries. Yearly training and workshops are hosted by the NSG team where NSG users and software developers discuss their research results and software. NSG is utilized by other neuroscience projects, such as the NeuroElectroMagnetic data Archive and tools Resource (NEMAR), the Open Source Brain, as the computational engine for processing of data and simulations. NSG is used in classroom teaching for undergraduate and graduate students. NSG has special training and classroom teaching sessions for Minority Serving Institutions. The presentation at SfN will describe the tools that NSG provides, how they are utilized on supercomputers, statistics of NSG's growth, research enabled by NSG and external projects that are utilizing NSG as the compute engine.

Disclosures: **A. Majumdar:** None. **S. Sivagnanam:** None. **K. Yoshimoto:** None. **N. Carnevale:** None. **M. Kandes:** None. **S. Yeu:** None. **D. Choi:** None.